See Special Notices on p. 327

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The Reduction of Tin by Metallic Antimony and by Certain Other Metals in Presence of Antimony and Copper Salts*

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THE vast majority of tin determinations carried out involve its reduction to the stannous form and its subsequent titration. There seems to be no doubt that the general method of achieving this reduction is by boiling the hydrochloric acid solution with some reducing metal; the choice of metals varies considerably and antimony, lead,[1] iron, zinc,[2] aluminium[3] and nickel all have their advocates. The literature of the subject is very extensive, and we have no wish to enlarge it or to reopen it in a general way; there have, however, been certain statements made in the scientific press which appear to be largely ignored and which, therefore, require re-examination and either affirming or denying. Much of this examination concerns attempts to determine the tin volumetrically in the presence of ions (notably copper^{II} and antimony^{III}) which are precipitable by the reducing metal. As, however, metallic antimony ppts. neither of these ions, it falls into a different category as a reductant and is therefore dealt with first.

ANTIMONY—Okell said in a recent review[4]: "The method . . . of reduction by means of metallic antimony, as a preliminary to titration by iodine, has been abandoned by most routine workers as unreliable, probably on account of after-reduction or action of iodine on the remaining antimony powder."

Clarke,[5] in a somewhat casual footnote to a paper dealing with a different matter, stated that the reducing power of antimony varied widely according to the fineness of the grinding, and that results either too high or too low might readily be obtained. In spite of these pronouncements it seems plain that the method is used widely and enthusiastically by a large number of chemists. The reason for this is obvious; the method is so clean, the end-point is so stable, and there are no complicating metal precipitates.

The results we give here were obtained with a number of grades of powder from a single sample of antimony. The attempt was first made to grade the antimony by means of sieves this immediately gave rise to discordant and unreproducible results which merely exhibited a general trend. Microscopic examination of the particles revealed the cause of this; the very fine particles seem to adhere strongly to the larger ones, so the separation is more apparent than real. As there seemed a possibility that elutriation might effect a more complete grading, a simple elutriator was devised and, by its help, an easy, rapid, and very fairly complete separation into 13 grades was obtained. Each grade was allowed to settle in its elutriator water, which was then decanted off, and the powder was washed with alcohol and ether and finally rapidly dried in a current of air. Each sample was examined under a microscope with a micrometer eyepiece, a number of measurements of the dimensions of the particles were taken and an approximate mean diameter was calculated. The gradings proved to be remarkably clean in appearance and each one to be confined to a fairly limited range of sizes; naturally there was overlapping, but it was not serious. The estimation of size was of course very approximate and, owing to the usually pyramidal form of the grains, the calculated diameter has no absolute significance; it provided, however, some sort of indication of the relationship of surface to weight, which was all that was here needed. Grinding was carried out by hand in an agate mortar, a small quantity at a time, and each portion was ground as fine as was reasonably possible, the separate portions being subsequently mixed and the whole elutriated. Mechanical grinding in an agate mortar was also attempted but the results were considerably less satisfactory (*vide infra*).

* Communication from the Armament Research Department (formerly the Research Department, Woolwich).

The reducing power of the various fractions obtained and also of the mixed unfractionated powder was tested in the following manner. Portions, 2·0 g in weight, of electrolytic iron were dissolved in 20-ml quantities of conc. hydrochloric acid under carbon dioxide; when solution was complete the liquid was cooled with the carbon dioxide still passing. A solution of 0·010 g of tin in the stannic condition was added to each followed by dilute (1 : 1) hydrochloric acid to a total vol. of 180 ml and then by 0·5 g of the antimony powder under examination. The solns. so treated were gently boiled under carbon dioxide for 30 minutes and cooled under pressure of the carbon dioxide Kipp's apparatus. The liquid was next diluted to 250 ml with boiled-out distilled water, 5 ml of potassium iodide solution (4%) and 2 ml of starch solution (0·1%) were added, and the titration with $N/100$ iodine was carried out. The whole operation was performed in the apparatus (Fig. 1) previously described by one of

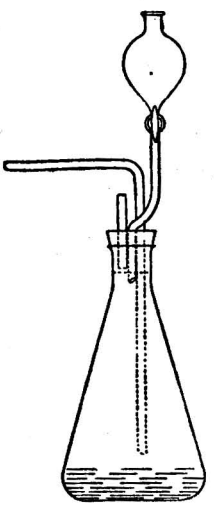

Fig. 1

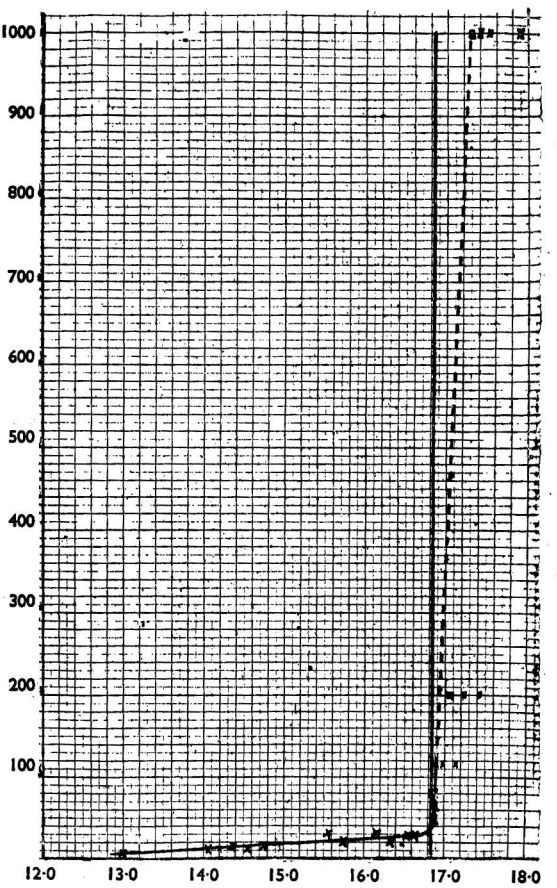

Fig. 2

the authors[6] and, after the addition of the antimony, the soln. did not come in contact with the air at all. Iron was added in each test because the investigation was originally undertaken to test the validity of the use of antimony as a reducing agent in the direct determination of tin in steel; it made no difference whatever to the findings. Results obtained are given in Table I.

These results, plotted on a graph against the reciprocal of the approx. mean radius of the particles, are shown in Fig. 2. From this it is at once evident that Clarke's statement is substantiated and that below a certain particle radius there is a continued reducing action by the powdered antimony in the cold. As a matter of fact, below this limiting radius the character of the titration changes, and it becomes increasingly difficult to see the end-point. As the time factor had obviously become involved, the end-point showing a marked tendency to fade, it was thought advisable to carry out a series of titrations with the finer grades,

varying and accurately recording the time taken for titration. In this series the first result for each grade represents the quickest possible titration consonant with accuracy. Results are given in Table II and are marked as outlying points on the curve (Fig. 2).

TABLE I

Elutriate	Weight obtained g	Per cent. of total weight	Approx. mean diam. of particles mm	1/mean radius	Titration: Actual	Titration: Theoretical
Unelutriated	47 (approx.)	100	—	—	16·83 16·88	16·84
(*a*)	3·688	8·2	0·002	1000	17·30	16·84
(*b*)	3·075	6·8	0·010	200	17·07	16·84
(*c*)	8·174	18·08	0·017	117·6	17·14	16·84
(*d*)	2·560	5·67	0·025	80·0	16·88	16·84
(*e*)	1·947	4·31	0·031	64·5	16·88	16·84
(*f*)	0·671	1·5	0·041	49·4	16·88	16·84
(*g*)	2·185	4·83	0·066	30·3	16·15	16·84
(*h*)	3·637	8·1	0·072	28·0	16·54	16·84
(*i*)	4·490	10·0	0·099	20·1	16·33	16·84
(*j*)	8·106	18·0	0·144	14·0	14·75	16·84
(*k*)	1·767	3·9	0·188	10·6	14·55	16·84
(*l*)	4·815	10·7	0·294	6·9	12·97	16·84

TABLE II

Sn taken in each case 0·0100 g

Approx. mean diameter mm	Time of titration secs.	Titration (ml of *N*/100 I): Actual	Titration (ml of *N*/100 I): Theoretical
0·002	105	17·28	16·84
	156	17·40	16·84
	225	17·52	16·84
	294	17·92	16·84
0·010	50	17·04	16·84
	129	17·08	16·84
	230	17·24	16·84
	286	17·43	16·84
0·017	78	16·89	16·84
	122	16·89	16·84
	215	16·98	16·84

Examination of the curve shows that all the coarser grades give low results, the reduction being apparently proportional to the surface exposed and the curve going up in a straight line practically to the point where complete reduction is obtained. Here the curve turns sharply and over a very limited range (0·025–0·040 mm) theoretical figures are obtained; re-elutriation of one of the grades in this range gave a finer fractionation, and results of these fractions all lay upon the original curve. Beyond this range the phenomenon studied in the results of Table II begins to appear, and grows more marked as the particle size diminishes. It seems obvious that even with the coarser grades reduction should be complete and the titration accurate if sufficient powder is used to provide the necessary surface,* but that it is necessary to eliminate powder of more than a certain degree of fineness. The accurate results undoubtedly and frequently obtained by mere grinding of antimony in an agate mortar and using the resulting powder without treatment would seem to depend both on chance and on a balance of errors.

MECHANICAL GRINDING—In order to study the properties of the finer grades more closely, resort was had to prolonged mechanical grinding in an agate mortar. A very much greater proportion of the finer grades was thus obtained, but the results were perplexing. Instead of greater reduction being obtained the results were, almost invariably, too low. This effect has not been explained, but was presumably due to oxidation; it introduces a new factor of uncertainty into the reduction.

* It would seem that simple calculation of the weight necessary to give the required surface is not quite sufficient, as a repetition with grade *j*, using 2·0 g instead of 0·5 g, which should bring the surface within the accurate range, gave a result of 16·14 ml (16·84 theor.) as against the 14·75 ml for 0·5 g.

REDUCTION BY OTHER METALS—The accuracy of reduction by lead, iron, aluminium and zinc is well established.[1,3,2] That by nickel has been criticised, it having been stated that it may give only 80% reduction.[7] On the other hand, according to a recent publication,[9] used with certain precautions (*e.g.*, preliminary etching with hydrochloric acid) it gives the best results of any. It was therefore necessary to examine nickel reduction in somewhat greater detail.

NICKEL—*Method*—As before, 0·010 g of tin in the form of Sn^{IV} was used in each instance. Reduction was carried out by boiling with nickel (previously etched by boiling for 10 min. in a mixture of equal parts of 1 : 1 HCl and 20% NaCl; area 32 sq.in., where available) for 40 min. in a soln. of volume 250 ml and containing 50 ml of concentrated hydrochloric acid. All reductions were done in an atmosphere of carbon dioxide when cold, 2 ml of 0·1% starch soln. and 5 ml of 4% potassium iodide soln. were added and the titration was carried out with *N*/100 iodine under carbon dioxide. The apparatus used was that shown in Fig. 1. The following results were obtained.

TABLE III

Nickel used	Approx. area sq.in.	Tin added g	Titration: Actual ml of *N*/100	Titration: Theoretical ml of *N*/100	Tin found g	Error g
Shot	32	0·0100	16·92	16·84	0·01004	+0·00004
Foil	32	0·0100	16·92	16·84	0·01004	+0·00004
Material from nickel crucible	32	0·0100	16·96	16·84	0·01007	+0·00007
Spectroscopically pure	13	0·0100	16·81	16·84	0·00998	−0·00002

From these figures it is evident that by this method (for the details of which we are indebted to the Bragg Laboratory, Sheffield) reduction by nickel gives accurate results, and no symptoms of incomplete reduction were manifest.

REDUCTION OF STANNIC SALTS BY METALS IN PRESENCE OF ANTIMONY AND COPPER SALTS—Work has been published showing that antimony pptd. by iron[8] and copper and antimony pptd. by lead[5] withdraw quite appreciable amounts of tin from solution by co-precipitation or some such phenomenon. So far as we are aware the behaviour of nickel, zinc and aluminium in these circumstances has not been investigated. Our object here is merely to show whether such co-pptn. does or does not take place and not whether under certain conditions any co-pptn. that occurs is likely to be of such magnitude as to vitiate a titration of the tin. Our technique was to boil a solution of tin and of an equal weight of either copper or antimony of definite acid concn. for a specified time with the metal under investigation, to filter hot and to wash very thoroughly with hot 10% hydrochloric acid and subsequently to test the metallic ppt. for tin and, if it is present, to determine its proportion in the bulk of the ppt. For lead, iron and nickel the soln. was boiled for 30 min. For zinc and aluminium a different technique was required, as in these instances reduction of tin takes place direct to the metal, which then has to be dissolved in the stannous condition after the reducing metal has all disappeared. In these circumstances and with free access of air the copper and antimony also dissolved leaving no ppt. to investigate; consequently for these metals reduction was carried out under carbon dioxide, the acid concn. was 25 ml of conc. hydrochloric acid per 250 ml, and the boiling was continued for 30 min. after the reducing metal had all disappeared. Acid conditions in all tests are shown in the table. The following results were obtained. (See Table IV.)

It is noteworthy in these results that antimony is pptd. by all these metals, and copper by all except nickel, apparently completely. The pptn. of copper by nickel appears to be very slight and often nil. The expts. were repeated with the nickel in both the cold-worked and the annealed state with the same result; the most remarkable feature was that it was the purest samples of nickel, *i.e.*, the "spectroscopic" samples, which in both instances caused what pptn. of copper there was.

CONCLUSIONS—The conclusions to be drawn from the foregoing appear to be:

(*a*) That, although antimony powder will give accurate results and a very clean titration, this is over a very limited range of particle size. Accurate results with ordinary powder would seem therefore to be due either to chance or to a balance of errors, and to put the process on a sound theoretical basis would require some method of grading the powder before use.

(*b*) The metals lead, iron, zinc and aluminium, whilst giving accurate reduction with tin alone, cause co-pptn. of tin with any antimony or copper they precipitate.

(*c*) Only nickel causes no co-pptn. of tin with either copper or antimony. It appears to reduce tin completely and accurately.

TABLE IV

Reducing metal	HCl, % (ml of conc. acid per 100 ml of solution)	Sn taken g	Sb added g	Cu added g	Sn found in pptd. metal g	Ratio in ppt. Sb : Sn
Lead	10	0·20	0·20	—	0·0025	80 : 1
,,	10	0·20	0·20	—	0·0019	105 : 1
,,	10	0·20	0·20	—	0·0012	167 : 1
,,	20	0·20	0·20	—	0·0020	100 : 1
,,	35	0·20	0·20	—	0·0012	167 : 1
,,	50	0·20	0·20	—	0·0013	154 : 1
Iron	20	0·20	0·20	—	0·0016	125 : 1
,,	20	0·20	0·20	—	0·0016	125 : 1
,,	20	0·20	0·20	—	0·0054	37 : 1
Nickel	20	0·20	0·20		nil	
,,	20	0·20	0·20	—	nil	—
,,	20	0·20	0·20	—	nil	—
,,	20	0·20	0·20	—	nil	—
,,	50	0·20	0·20	—	nil	—
Aluminium	10	0·20	0·20	—	0·0162	12·3 : 1
,,	10	0·20	0·20	—	0·0104	19·2 : 1
,,	10	0·20	0·20	—	0·0171	11·7 : 1
Zinc	10	0·20	0·20	—	0·0479	4·2 : 1
,,	10	0·20	0·20	—	0·0210	9·5 : 1
,,	10	0·20	0·20	—	0·0151	13·2 : 1
						Cu : Sn
Lead	20	0·10	—	0·10	0·0288	3·5 : 1
,,	20	0·20	—	0·20	0·0265	6·4 : 1
,,	20	0·20	—	0·20	0·0304	4·7 : 1
Iron	20	0·10	—	0·10	0·0026	38·5 : 1
,,	20	0·20	—	0·20	0·0071	22·4 : 1
,,	20	0·20	—	0·20	0·0145	11·8 : 1
Aluminium	10	0·20	—	0·10	0·1254	1·0 : 1·3
,,	10	0·20	—	0·10	0·1183	1·0 : 1·2
Zinc	10	0·20	—	0·10	0·1409	1·0 : 1·4
,,	10	0·20	—	0·10	0·1542	1·0 : 1·5
Nickel "spectroscopic"	20	0·10	—	0·10	nil	—
,, ,,	20	0·10	—	0·10	nil	—
Nickel from crucible	20	0·10	—	0·10	nil	—
,, shot	20	0·10	—	0·10	nil	—
,, foil	20	0·10	—	0·10	nil	—

SUMMARY—(1) The reducing power of antimony powder has been examined and shown (for a weight of 0·5 g) to be accurate only over a limited range of particle diameter. Above this diameter reduction is incomplete, below it a back reaction comes into play giving too high results.

(2) The reducing power of nickel has been tested by the method of the Bragg Laboratory, Sheffield, and found to be satisfactory for nickel from several different sources.

(3) The question of the co-pptn. of tin with antimony and copper pptd. by lead, iron, nickel, zinc and aluminium has been investigated. It has been found that co-pptn. occurs with both metals pptd. by lead, iron, zinc or aluminium, but with neither pptd. by nickel.

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May, 1944

Determination of Small Additions of Milk Powder to Flour

BY E. C. DAWSON, M.Sc., A.R.I.C.

FROM time to time dried skim milk powder has been added to National flour at the rate of 2 lbs. per sack (0·71%); it is therefore desirable to have a method of checking this addition.

Several methods of determining milk products in materials containing a mixture of sugars have been published, but excepting polarimetric methods they are dependent upon the resistance of lactose to fermentation by ordinary bakers' yeast (*S. Cerevisiae*).[1] Polarimetric methods are inapplicable for the present purpose because of the low concentration of sugar. Further, none of the fermentation procedures could be applied satisfactorily to National flour because it contains non-fermentable reducing substances in addition to the lactose; a differential fermentation method has therefore been developed.

An aqueous extract is prepared, one portion is fermented with *S. cerevisiae* and a second with *S. cerevisiae* and *S. fragilis* concurrently. Of the sugars present, lactose only is resistant to *S. cerevisiae* but is destroyed by *S. fragilis* in the mixed yeasts. The lactose content of the second soln. is then restored to about its original level by means of a standard lactose soln. (thus increasing the accuracy of the determination of the non-fermentable reducing substances still remaining) and the reducing powers of both are determined with Fehling's solution. The second, corrected for the added lactose, gives the "blank" (*i.e.*, non-fermentable reducing substances); and subtraction of this from the apparent lactose content of the first soln. gives the true lactose content of the flour. From this figure the quantity of milk powder present can be calculated.

Complete aqueous extraction of soluble substances from flour is not practicable in a routine method, owing to the formation of a dough which resists further treatment. The flour is therefore shaken with one portion of water and centrifuged, and the assumption is made that the lactose is distributed uniformly through the whole of the added water, that is, in the part retained in the dough as well as in the extract.* This assumption has been shown to be justifiable. The Lane–Eynon method of determination has been satisfactorily adapted for the low concns. of sugar in the extracts.

The blank increases on standing under acid conditions, and therefore increases slowly during the fermentation, which takes place at *p*H 4·5. In about 4 days the rate of destruction of reducing sugars falls below the rate of increase of the blank, and the total reducing power reaches a minimum. However, for consistent results the fermentation must be completed, for which 7 to 9 days are required (see *Nature of Blank*).

It is necessary to maintain pure cultures of both yeasts on "slopes" of malt extract, maize extract and agar. For use, each yeast may be grown in a medium of dextrose 5%, peptone 1%, KH_2PO_4 0·3%, $MgSO_47H_2O$ 0·2%, in distilled water, and separated by centrifuging under sterile conditions. Although flour contains considerable amounts of magnesium potassium, sulphur and phosphate, it is necessary to add magnesium sulphate and potassium dihydrogen phosphate to the extracts for satisfactory yeast growth.

The lactose content of milk powder may generally be taken as 50%, although variations of ±5% are not uncommon. When available, a sample of skimmed milk powder may be assayed by using a solution of about 0·2% for the titration described below, without previous treatment.

* The bulk of the water naturally present in the flour (approx. 14%, *i.e.*, 0·16 g of water per g of solids) is almost certainly of the nature of bound water and therefore not free to dissolve lactose.

METHOD—Add 80 g of flour to 240 ml of distilled water in a 500-ml conical flask and shake thoroughly. Leave for a few min. then centrifuge the suspension at 2500 r.p.m. for 5 min., and pour off the liquid.* Place 60 ml in each of two 250-ml conical flasks, and to each add 1 ml of a soln. containing 15% of KH_2PO_4 and 10% of $MgSO_4,7H_2O$. Bring the *p*H of each soln. to 4·4–4·6 by means of a few drops of *N* hydrochloric acid, using bromocresol green as external indicator. Sterilise the solns. by heating to b.p., and stopper the flasks with cotton wool. Cool both flasks (A and B) and inoculate with a little *S. cerevisiae* on the end of a platinum wire; also inoculate flask B with *S. fragilis*. Allow fermentation to proceed for 7–9 days at 28° C.

At the end of fermentation add 3 ml of 10% sodium tungstate soln. and 0·35 ml of conc. sulphuric acid to each soln. with shaking, and filter. Collect the filtrate in a 100-ml graduated flask, containing a slight excess (1 ml of 20%) of sodium hydroxide soln., using a conical, not fluted, paper, and continue washing to give a total vol. of 90 ml. Make solution A up to 100 ml, and determine its sugar content (x) as described below under *Titration*. Add enough lactose (y) as standard solution to soln. B to bring the sugar content to approx. that of A. For this purpose y should be approximately $x-1$ if it has reference to lb. of milk powder (50% lactose) per sack, or $x-0{\cdot}035$ for g of lactose per 100 ml of soln. For the latter purpose the standard soln. may be 2%, or for the former 1·79%, of which 1 ml per 100 ml equals 0·5 lb. of milk powder per sack. Make soln. B up to volume and determine its sugar content (z). Lactose (g per 20 g) or milk powder (lb./280 lb.) is $x + y - z$.

Titration—The Lane-Eynon standard method is used.[2] It must usually be preceded, at least for soln. A, by a titration by their incremental method, but the incremental method alone is not adequate. However, only 1 ml of copper sulphate soln. is used, together with 1·0 to 1·2 ml of alkaline tartrate soln., and the whole is diluted to 60 ml in a 250-ml conical flask. Two drops of 1% methylene blue soln. should be used, and it is important not to add this until almost the end of the 2 min. ebullition. Table I relates vol. of soln. required with

TABLE I

For 1 ml of copper sulphate soln., with alkaline tartrate soln. and water

Sugar soln. required ml	Sugar content as lactose in g per 100 ml	Milk powder (50% lactose) lb. per sack
10·0	0·166	4·65
11·0	0·151	4·23
12·0	0·139	3·89
13·0	0·129	3·61
14·0	0·120	3·36
15·0	0·112	3·14
16·0	0·105	2·94
17·0	0·100	2·80
18·0	0·094	2·63
19·0	0·090	2·51
20·0	0·085	2·38
22·0	0·078	2·18
24·0	0·072	2·02
26·0	0·066	1·85
28·0	0·062	1·73
30·0	0·058	1·62
32·0	0·055	1·54
34·0	0·052	1·46
36·0	0·049	1·37
38·0	0·047	1·31
40·0	0·045	1·26
42·0	0·043	1·20
44·0	0·041	1·15
46·0	0·039	1·09
48·0	0·038	1·06
50·0	0·037	1·04

reducing sugar expressed as lactose, in g per 100 ml of soln., *i.e.*, in g per 20 g of flour, and also with milk powder (at 50% lactose content) in lb. per sack. If the sugar concn. is higher than the range of this table, the unmodified Lane–Eynon method should be used with the Lane–Eynon tables (1 mg of lactose per 100 ml = 0·028 lb. of milk powder per sack).

* Gallenkamp's Technico Universal Centrifuge: Capacity of each tube, 100 ml; level of each tube, 10 cm; distance from centre to the cup mounting pivots, 7 cm.

In Table II are shown results for commercial and laboratory prepared flours. The accuracy of the method is about ±10%.

TABLE II

Sample Prepared in laboratory	Reducing power of soln. A (x) (lb. of milk powder per sack)	Lactose added to soln. B (y) (lb. of milk powder per sack)	Reducing power of soln. B (z) (lb. of milk powder per sack)	Blank ($z-y$) (lb. of milk powder per sack)	Milk powder ($x+y-z$) (lb./sack) found
Flour (1) no milk powder added ..	0·72	0·00	0·75	0·75	−0·03
,, (1) 2·02 lb. m.p./sack	2·72	2·05	2·74	0·69	2·03
,, (1) 2·05 lb. m.p./sack	2·77	2·09	2·83	0·74	2·03
,, (2) 1·84 lb. m.p./sack	2·66	1·68	2·58	0·90	1·76
,, (2) 2·59 lb. m.p./sack	3·44	2·24	3·16	0·92	2·52
,, (2) 1·09 lb. m.p./sack	1·88	1·12	2·05	0·93	0·95
,, (3) 2·02 lb. m.p./sack	2·70	1·68	2·55	0·87	1·83
,, (4) 1·89 lb. m.p./sack	3·33	1·96	3·61	1·65	1·68
,, (5) Commercial	2·41	1·68	2·49	0·81	1·60
,, (6) ,, ...	3·47	2·52	3·41	0·89	2·58
,, (7) ,, ...	3·44	2·52	3·36	0·84	2·60

Nature of Blank—The nature of the substances causing the blank was not ascertained, but the presence of several different compounds was demonstrated. No separation from lactose could be obtained by extraction of the flour with either abs. or aqueous alcohol. Pentoses or pentosans were shown to be present, but in amounts unrelated to the total reducing power.[3] Part of the material was resistant to boiling with dil. (1 : 2) hydrochloric acid, whilst another part, in addition to the pentoses, was destroyed by such treatment. Prolonged standing with 1% sulphuric acid increased the reducing power, sometimes even to 160%, of its original value, after which further fermentation reduced it to between 15% and 105% of the original figure, according to the sample. Reducing dextrins resistant to yeast amylase were shown to be absent, since addition of wheat amylase had no effect.[4]

The distribution of the non-fermentable reducing substances in the wheat berry was investigated, with the results shown in Table III. The high figure for germ is of interest.

TABLE III

Sample	Apparent lactose content, %
White flour, 70% of grist (A)	0·08
Fine offal, 16·5% ,, ...	0·66
Coarse offal, 13·5% ,, ...	0·575
Weighted average, grist (A) *calculated* ..	0·24
Grist A *found*	0·245
C-roll flour	0·06
Bran, commercial	0·30
Germ, commercial (1)	4·25
,, ,, (2)	4·90

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CEREALS RESEARCH STATION
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July, 1944

The Determination of the Mineral Content of Foods by Wet Oxidation and Absorptiometric Methods

By R. MILTON, B.Sc., F.R.I.C., J. L. HOSKINS and W. H. F. JACKMAN, B.Sc., A.R.I.C.

In the assessment of the mineral content of foodstuffs, as required in investigations of serial individual diets, it is often advantageous to make a number of analyses on one sample. Analysis of residual matter after ashing is not permissible if volatile elements, such as sodium and potassium, are to be determined; wet oxidation methods are preferable. Methods involving the use of sulphuric acid as an oxidising agent leave unchanged acid in the residue, and this complicates subsequent determination of mineral matter and precludes determination of sulphur. The more recent use of perchloric acid has some disadvantages; it is liable to cause deflagration and does not give quantitative recovery of sulphur. It also tends to the formation of somewhat intractable metaphosphoric acid compounds that inhibit the pptn. of other elements, which may thus escape determination.

The method here described enables calcium, magnesium, sodium, potassium and sulphur to be determined, all on one sample, after destruction of organic matter by means of nitric acid and ammonium nitrate. Its advantages are: steady and rapid oxidation, formation of a residue which can be readily freed from excess of oxidising agent by volatilisation, and the obtaining of the elements in a form suitable for determination.

When individual foodstuffs are to be examined, a sample (*ca.* 100 g) is dried to constant weight and thoroughly ground, and 1 g is taken for oxidation. When information as to the actual content of a mixed diet is required the procedure is as follows. Weigh each article to be ingested and take a representative aliquot portion, *e.g.*, one-tenth, as sample. At the end of the investigation period mix the samples in a common receptacle, so as to represent a reconstruction of the total diet on an aliquot scale. Then autoclave this mixture at 30 lb. pressure for *ca.* 4 hr. This allows thorough digestion and enables the sample to be completely homogenised. Cool and thoroughly grind with pestle and mortar. Then transfer a known weight to a tared dish and dry in an oven at 100° C. until constant in weight. Powder the dried sample and take a portion, *e.g.*, 1 g, for analysis.

Oxidation—Transfer the sample to a 250-ml Kjeldahl flask and add 10 ml of conc. nitric acid. Gently warm the flask until solution is complete and then add 10 ml of oxidising reagent (50% ammonium nitrate soln. in 25% nitric acid), and heat gently to expel water, so that oxidation (indicated by effervescence) proceeds in a melt of ammonium nitrate. From time to time add more reagent, if necessary, and continue until no browning of the soln. is observed and a clear melt is obtained. In presence of much fat oxidation is slower and more reagent may be required, and there may be excessive initial frothing if much carbohydrate is present. When oxidation is complete, heat the clear melt more strongly to volatilise the excess of ammonium nitrate, holding the flask over a free flame to expel salt subliming on the side. Then dissolve the residue in 2 ml of conc. hydrochloric acid, evaporate to dryness, fuse the residue in the flask, so as to remove all nitric acid, dissolve it in *ca.* 10 ml of *N* hydrochloric acid, and evaporate the soln. to a small bulk to ensure conversion of any metaphosphate into orthophosphate. Evaporate to dryness in a stream of warm air, dissolve the residue in a few ml of warm water, add a few drops of *N* hydrochloric acid, and make up to 10 ml with water.

Determination of Constituents—*Calcium*[1]—Transfer 2 ml of the soln., obtained as described above, to a centrifuge, buffer it to *p*H 5·0 with 10% ammonia soln. and 10% acetic acid, add 2 ml of saturated ammonium oxalate soln., mix and leave for 30 min. Separate the ppt. by centrifuging, wash twice with water (2 ml each time), passing the washings through filter-paper, and reserve the supernatant liquid and washings for the determination of magnesium. Tap the ppt. free from the bottom of the tube, dissolve it (and any residue on the filter-paper) in 2 ml of 2 *N* sulphuric acid, and titrate at 70° C. with *N*/100 permanganate.

$$100\ (\text{ml of } N/100\ KMnO_4 - 0{\cdot}04) \doteq \text{mg of Ca in 100 g of dried food.}$$

Magnesium—Transfer the supernatant liquid and washings from the calcium determination to a centrifuge tube, and add 2 ml of 25% ammonia soln. and 1 ml of 10% ammonium phosphate soln. Stir vigorously with a glass rod and leave for at least 4 hr. Then centrifuge and wash the ppt. twice with 2 ml of 10% ammonia soln. and once with 2 ml of a mixture of

ammonia soln. and alcohol (20 : 80). Loosen the ppt. by tapping, and dry it thoroughly on the water-bath. Add 1 ml of $N/10$ hydrochloric acid to dissolve the ppt., transfer the soln. to a 50-ml graduated flask, and make up to the mark. Transfer 5 ml of this soln. to a tube and add 2 ml of water, 1 ml of 10 N sulphuric acid and 1 ml of 7·5% sodium molybdate soln. Mix and add 1 ml of dil. stannous chloride soln. (1 part of a 40% soln. of $SnCl_2$ in conc. hydrochloric acid diluted to 200 with water). Read the blue colour on a Spekker absorptiometer and compare the result with a standard calibration graph prepared as follows. Dissolve 2·264 g of pure potassium dihydrogen phosphate in 1 litre of water and dilute 10 ml of this soln. to 1 litre. Prepare a series of tubes containing 1 ml, 2 ml, and so on, up to 5 ml of the dil. soln., make up each tube to 7 ml with water, and add 1 ml of 10 N sulphuric acid, 1 ml of 7·5% sodium molybdate soln. and 1 ml of dil. stannous chloride soln. Read the blue colours on the Spekker absorptiometer (10-ml cells; No. 1 red filter). This gives a curve equiv. to quantities of magnesium ranging from 0·004 to 0·02 mg.

Magnesium, mg ..	0	0·004	0·008	0·012	0·016	0·020
Spekker reading ..	0·06	0·28	0·50	0·72	0·94	1·16

Multiply the figure given on the graph by 5000 to obtain the result as mg per 100 g of dried food.

Sodium[3]—Transfer 2 ml of the original oxidised soln. to a centrifuge tube and add 1 ml of 5% barium chloride soln. and, after mixing, 1 ml of 1% ammonia soln. Leave the tube for a few min. and then centrifuge to separate the barium phosphate. Transfer 2 ml of the supernatant liquid to another centrifuge tube and add 1 ml of 10% acetic acid and 7 ml of sodium precipitant, prepared by grinding 7 g of uranium acetate in 100 ml of 21% w/v anhydrous magnesium acetate soln., leaving overnight and filtering (it keeps indefinitely). Set the tube aside for 1 hr. and then centrifuge, drain well, wash the ppt. twice with 2 ml of 95% alcohol, drain again and dissolve in 10 ml of water. Transfer 1 ml of this soln. to a tube and add 4 ml of water and 5 ml of ferrocyanide reagent, prepared by dissolving 1 g of potassium ferrocyanide in 100 ml of 0·2% gum ghatti soln. Measure the colour of the uranium ferrocyanide on a Spekker absorptiometer fitted with green filter No. 5 and compare the result with a calibration curve.

To prepare the curve, dissolve 254 mg of sodium chloride in 100 ml of water, add 10 ml of sodium precipitant (*supra*) and leave for 1 hr. Filter through a glass Gooch crucible and wash the ppt. twice with a few ml of 95% alcohol. Remove the excess of alcohol by suction, dissolve the ppt. in 100 ml of water, and dilute 10 ml to 500 ml (1 ml $\equiv$ 0·02 mg of Na). Into a series of tubes put 1 ml, 2 ml and so on, up to 5 ml, of this soln., make up each to 5 ml with water, and add 5 ml of ferrocyanide reagent (*supra*). Read the red colour on a Spekker absorptiometer and plot the readings against concns.

Sodium, mg ..	0	0·02	0·04	0·06	0·08	0·10
Spekker reading ..	0	0·36	0·70	1·00	1·28	1·50

Multiply the figure shown on the graph by 10,000 to obtain the result in mg of sodium per 100 g of dried food.

Potassium[4]—Transfer 0·5 ml of the oxidised soln. to a centrifuge tube and add 0·5 ml of saturated sodium nitrite soln. and 0·1 ml of glacial acetic acid. Place the tube in a boiling water-bath for about 10 min., in order to remove any ammonium salts as gaseous nitrogen. Cool and add 4 ml of sodium cobaltinitrite soln. (25 mg of the salt in 100 ml of $N/10$ acetic acid), drop by drop, with shaking. After 30 min. centrifuge and wash the ppt. twice with 2 ml of water, with careful draining after each washing. Loosen the ppt. from the bottom of the tube by tapping, add excess (*e.g.*, 5 ml) of $N/100$ permanganate and *ca.* 2 ml of 2 N sulphuric acid, and continue the addition of known quantities of permanganate until a faint pink colour persists after 1½ min. heating. Then add a measured excess of $N/100$ oxalic acid and titrate back with permanganate.

Subtract from the total ml of $N/100$ potassium permanganate used, less the vol. of $N/100$ oxalic acid added, 0·04 ml and multiply the remainder by the potassium factor to obtain the concn. in the sample. Then multiply the result by 2000 to obtain the potassium content as mg in 100 g of dried food.

To find the potassium factor submit a standard soln. of a potassium salt (*e.g.*, 0·2 mg) to exactly the same technique. It is of the order of 0·07 mg per ml of $N/100$ permanganate. This factor must be standardised by each worker, since it varies widely with the conditions of precipitation. The factor given here is applicable to the technique described.

Phosphorus[5]—Transfer 1 ml of the oxidised soln. to a 100-ml graduated flask and make up

to the mark with water. Transfer 5 ml of this dilution to a test-tube and add 2 ml of water, 1 ml of 10 *N* sulphuric acid and 1 ml of 7·5% sodium molybdate soln. Mix and add 1 ml of dil. stannous chloride soln. (1 of 40% $SnCl_2$ soln. in conc. HCl diluted to 200 with water) Read the blue colour on a Spekker absorptiometer and compare the result with a standard graph.

To prepare the graph, dissolve 3·51 g of dihydrogen potassium phosphate in 1 litre of water and dilute 10 ml of this soln. to 1 litre. Prepare a series of tubes containing 1, 2 and so on, up to 5 ml of this dilution, and make each up to 7 ml with water. Add to each 1 ml of 10 *N* sulphuric acid, 1 ml of 7·5% sodium molybdate soln. and finally dil. stannous chloride soln. to develop the colour. Take the readings on the Spekker absorptiometer.

Phosphorus, mg ..	0	0·008	0·016	0·024	0·032	0·040
Spekker reading ..	0·06	0·40	0·74	1·06	1·42	1·76

Multiply the phosphorus content of the sample, as found from the calibration curve, by 20,000 to give the percentage of phosphorus in the dried food.

Sulphur[6]—Transfer 1 ml of the oxidised soln. to a test-tube, add 4 ml of water, 3 ml of *N* hydrochloric acid and 2 ml of sulphate precipitant, and leave for 30 min. Then gently shake the barium sulphate complex into suspension, measure the turbidity on a Spekker absorptiometer, and compare the result with a calibration curve.

To prepare the sulphate precipitant, dissolve 20 mg of bacteriological beef peptone in 5 ml of 1% barium chloride soln., buffer to *p*H 5·0 with *N*/50 hydrochloric acid, add 1 g of sodium chloride and make up to 10 ml. Heat in a boiling water-bath for 10 min., cool and add a few drops of chloroform. Store in a cool place. Dilute 1 ml of this soln., as required, with 50 ml of 1% barium chloride in 0·2% gum ghatti soln.

To prepare the calibration curve, make a soln. of sulphate containing 0·02 % of SO_3 (*e.g.*, *N*/200 H_2SO_4). Put 1, 2 and so on, up to 5 ml of this soln. into a series of tubes, make up each to 5 ml with water, and add 3 ml of *N* hydrochloric acid and 2 ml of precipitating reagent (*supra*). Mix, leave for 30 min. and read on the Spekker absorptiometer (No. 1 red filter).

Sulphate (SO_3), mg..	0	0·20	0·40	0·60	0·80	1·00
Spekker reading ..	0	0·16	0·29	0·39	0·48	0·56

Multiply the figure obtained from the graph by 1000 to find the SO_3 content in mg per 100 g of dried food.

Although determinations of nitrogen, chlorine and fat cannot be made on the oxidised sample, satisfactory analyses for these constituents may be made on the dried sample of mixed foods.

Chlorine[7]—The only satisfactory method is the classical Carius technique. It is convenient to use 1 g of the mixed food for the analysis.

Nitrogen[8]—This is determined by the semi-Kjeldahl procedure. Oxidise 0·2 g of the dried mixed food in a micro-Kjeldahl flask. Add *ca.* 4 ml of conc. sulphuric acid, 0·1 g of anhydrous copper sulphate and 1 g of sodium sulphate and heat until oxidation is complete. Then steam-distil the cooled residue with caustic soda in a micro-Kjeldahl distillation apparatus, collecting the liberated ammonia in excess of *N*/10 acid. Titrate the excess of acid with *N*/10 alkali, using screened methylene blue as indicator.

"Fat"[9]—Extract 0·5 g of the finely powdered mixed material with ether in a Soxhlet apparatus, distil the ether from the clear extract, and weigh the residue, which is taken as fat in metabolism experiments.

The technique described above has been used to estimate the ingestion of food in many hundreds of diets, with satisfactory accuracy and with a minimum of labour. Only the barest minimum of laboratory apparatus is necessary and, since so many of the analyses are made in test-tubes, quite small accommodation is required. In practice it is convenient to carry on six or eight series of analyses at the same time. In this way the entire procedure may be carried out in 2 working days by one chemist, *i.e.*, about 60 individual analyses in 16 working hours.

The technique is also applicable to individual foodstuffs without any modification and results of analyses carried out by this procedure on some food fish are given in Table I. The

results are expressed in mg per 100 g, except those for moisture, solids, protein and fat, which are percentages.

TABLE I

Fish	Moisture	Solids	Protein	Fat	Ca	Mg	Na	K	S	P	Cl
Halibut	73·5	26·5	22·7	0·5	0·068	0·030	0·127	0·350	0·173	0·240	0·200
Herring	56·0	44·0	23·0	20·1	0·075	0·044	0·143	0·375	0·226	0·260	0·320
Kipper	57·4	42·6	26·4	10·0	0·098	0·047	1·280	0·380	0·330	0·320	1·960
Cod	72·4	27·6	24·6	1·1	0·025	0·022	0·140	0·350	0·220	0·220	0·214
Haddock ..	71·5	28·5	23·2	1·1	0·030	0·026	1·320	0·296	0·245	0·190	2·020
Salmon	69·7	30·3	25·0	4·7	0·017	0·032	0·120	0·340	0·152	0·230	0·190
Sole	75·2	24·8	21·2	2·2	0·057	0·025	0·070	0·130	0·120	0·130	0·110
Turbot	75·5	24·5	19·5	1·1	0·067	0·028	0·360	0·220	0·190	0·220	0·550
Plaice	77·4	22·6	18·7	1·7	0·032	0·026	0·113	0·320	0·163	0·175	0·172
Shrimps (skinned)	64·0	36·0	23·0	0·5	0·380	0·066	1·300	0·150	0·235	0·140	2·200
Prawns (skinned)	65·5	34·5	24·5	2·8	0·200	0·061	1·770	0·200	0·270	0·160	2·700

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CLATFORD OAKCUTTS, NR. ANDOVER, HANTS. *March*, 1944

The Estimation of the Freshness of Canned Herring from Determinations of the Acid Value of the Oil

BY F. CHARNLEY AND F. R. E. DAVIES

As shown in a previous communication,[3] the *p*H of cooked fish muscle tissue, or the aqueous liquid derived therefrom, may in certain circumstances, be associated with organoleptic criteria of freshness. Further work with this test, however, shows that in estimating freshness of canned salmon both the *p*H value of the aqueous liquid and examiners' ratings are greatly influenced by the seasonal condition of the fish. This circumstance makes the test unsuitable for testing post-mortem spoilage in canned salmon. Likewise, owing to the addition of tomato sauce in the packing of canned herring, the *p*H value, trimethylamine value[1] and other similar tests are of little or no use for measuring spoilage in canned herring. The only simple test, therefore, which appears to be suitable for estimating the freshness of canned herring, is the acid value of the oil.

As is well known, the acid value has been widely used in evaluating the quality of fish and other marine oils. It has also been suggested as a measure of incipient decomposition.[2] So far as we are aware, however, no attempt has hitherto been made to determine the validity of this test by comparison with the ratings of experienced examiners using organoleptic methods. The object of the investigation reported in this paper, therefore, was to determine the relation between the acid value of the oil in canned herring and the criterion just mentioned, *i.e.*, the subjective ratings of experienced examiners.

METHODS—The methods employed were analogous to those described in determining the relation between the *p*H value and the examiners' rating for freshness of canned Chum salmon. The examiners' ratings were based on the odour and other evidences of incipient decomposition of the samples. These were determined immediately before the acid values of the samples were determined, and were recorded as follows:—1 = Good, 2 = Better than average, 3 = Average, 4 = Poorer than average, 5 = Poor, 6 = Stale, 7 = Tainted.

In order to obtain an estimate of the errors of individual examiners, however, three examiners were employed in the experiments. Each examiner worked independently of the others and recorded his ratings on a separate sheet. No discussion between examiners took place until all three had recorded their respective ratings for the particular set of samples

under examination. Two of the examiners, *B* and *C*, had no information regarding the origin of the samples. The third examiner, *D*, who prepared the samples for inspection, had knowledge of the codes and the companies that had packed the samples. The entire expt. extended over a number of weeks.

The acid values of the oil in the individual samples were determined with slight modifications by the method described by Brocklesby, using bromothymol blue as indicator. In order to save time, the analyses were carried out directly on the settled oil, *i.e.*, when the heated mixture of oil and liquid had separated out and become clear. Although this procedure gives acid values slightly lower than the true values owing to the presence of moisture in the oil, the relative error in the method is small. For example, five determinations of the acid value on the same sample gave 0·97, 1·02, 0·98, 1·01 and 1·01. The mean of these five values is 0·9980 with an estimated variance of 0·0004700. Hence the standard error of the method as carried out by the writers was of the order of 0·022.

TABLE I

Group	Mean acid val.	Mean of examiner's rating		
		B	*C*	*D*
I	0·8974	3·274	2·855	3·032
II	2·2661	5·039	5·137	5·000
III	3·0379	6·500	6·958	6·667

RESULTS—The results of the experiment are summarised in Table I, which shows the sample sizes and the respective means corresponding to the 24 different codes represented by the samples. As will be observed from an inspection of Table II the 24 codes, when classified according to mean acid value and mean examiners' ratings may be conveniently segregated into three groups, namely, group I consisting of codes 1 to 20 inclusive, group II consisting of codes 21, 22, 23 and group III consisting of code 24.

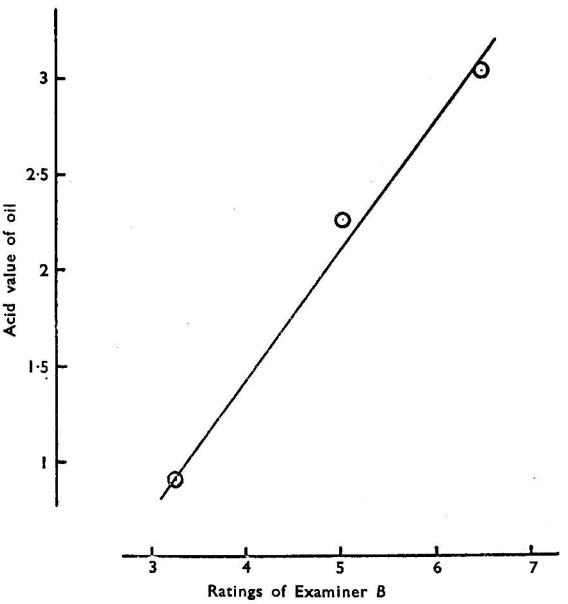

Fig. 1

Figs. 1, 2 and 3 show the resulting relations connecting mean acid value and mean examiners' rating obtained on plotting the corresponding means of these three groups. From the figures it will be seen that the relation between the means of the two characteristics, acid value of the oil and examiners' rating, is very closely linear. We do not require a statistical test to see this. In fact, owing to the lack of homogeneity in the variances of certain of the codes, and to the broad categories in examiners' rating, the plotted means afford far more reliable evidence of the essential linearity between mean acid value and mean examiners' rating than any evidence that might be deduced from a statistical examination of the data.

This result is also in harmony with what might be expected from simple chemical considerations. If, for example, it is assumed that incipient post-mortem spoilage of herring prior to canning is mainly the result of enzyme action, then inasmuch as the rate of hydrolysis over the first stage of most enzyme actions is constant under constant conditions, and further, since examiners' ratings are largely influenced by the disagreeable odour of certain hydrolytic products, the relation connecting the means of the above variates should be approximately linear.

The mean ratings of the three examiners *B*, *C* and *D* were, respectively, 4·49, 4·42 and 4·40. The corresponding values of the slope of the line of means were 0·677, 0·535 and 0·618, while the sums of the squares of odour rating for the three examiners calculated from the 24 codes listed in Table II were respectively 50·21, 53·00 and 35·05.

Similarly, it may be mentioned that the mean acid value of the samples listed in Table I was 1·779 and the variance estimated from 113 degrees of freedom was 0·0923, so that the standard deviation of acid value in the individual codes was about 0·3.

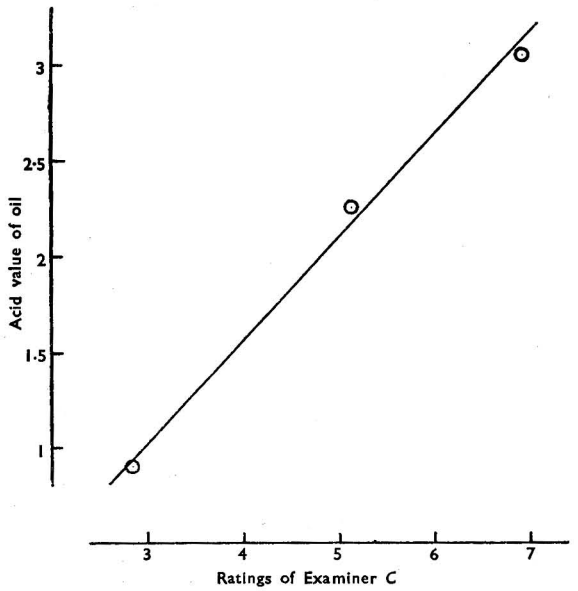

Fig. 2

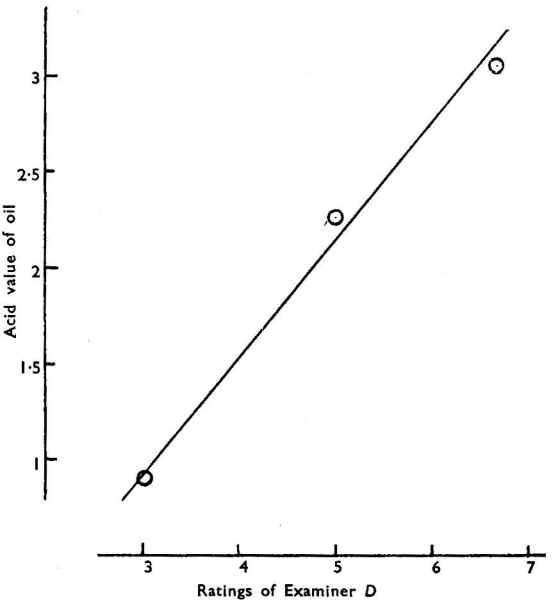

Fig. 3

TABLE II—SUMMARY OF RESULTS OF EXPERIMENTS RELATING MEAN ACID VALUE OF CANNED HERRING AND EXAMINERS' RATING FOR FRESHNESS

Code	Sample size	Mean acid value	Mean examiners' rating		
			B	*C*	*D*
1	4	0·53	3	3	3
2	1	0·62	4	2	3
3	1	0·69	3	2	3
4	3	0·71	3	3	3
5	3	0·71	2·67	3	3
6	3	0·74	3	3	3
7	2	0·78	4	3	3
8	1	0·80	2	3	2
9	10	0·819	3·60	3	3
10	8	0·820	3·38	3	3·12
11	4	0·90	3	2·75	3
12	3	0·93	3	3	3·33
13	2	0·94	5	2	2·5
14	3	1·06	3	3	3
15	2	1·08	4	2	2·5
16	1	1·12	4	3	3
17	4	1·16	2·75	2·75	3·5
18	2	1·19	4	3	3·5
19	4	1·33	3	2·67	2·67
20	2	1·53	2·5	3	3·5
21	9	1·956	5·33	5·67	4·89
22	33	2·290	5·24	5·39	5·24
23	9	2·488	4	3·67	4·22
24	24	3·038	6·46	6·96	6·67

TOLERANCES FOR FRESHNESS OF CANNED HERRING—Lastly, it should be noted that it is the line of means and not a regression line that is needed for practical grading purposes. From the line of means in Fig. 3 (or by calculation) it will be observed that in codes of canned herring in which the average examiner's rating is 6, *i.e.*, Stale, the average acid value is approximately 2·5, and for codes in which the average examiners' rating is 7, or Tainted, the

average acid value is about 3·0. On the basis of these data the following scale has been set up by this laboratory for grading freshness of suspected codes of canned herring:

Average acid value of oil	Grade	Size of sample examined
Less than 2·25 ..	A	6
2·25 to 2·75 ..	B	12
Greater than 2·75 ..	condemned	12

This scale, it should be observed, is based on the assumption that this product has been packed by the usual procedure followed by the industry and that any incipient decomposition present in the canned product has arisen prior to the processing of the sealed tins. In samples in which the decomposition has occurred subsequent to the sealing and processing of the tins, due, *e.g.*, to lack of sterility, insufficient cooking, etc., other tests are applied. Also it should be mentioned that after completion of the above investigation early in 1942 a much more rapid and convenient procedure than that described in this paper for determining acid values of the oil of canned herring was developed.

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May, 1944

Notes

THE ANALYSIS OF VULCANISED RUBBER, WITH SPECIAL REFERENCE TO SYNTHETIC MATERIALS

SINCE publishing the note[1] on this subject my attention has been drawn by the Research Association of British Rubber Manufacturers (R.A.B.R.M.) to two recent papers[2,3] in which various methods are discussed. The following are selected as being the most important features.

(1) A qualitative test for the identification of natural rubber, originally due to Kirchhof[4] and Pauly,[5] consists in warming the brominated rubber with phenol, a blue to violet colour being produced. This reaction is not given by rubber reclaimed by the alkali process.

(2) A valuable indication of the nature of a synthetic elastomer is obtained by determining the swelling of the sample in benzene, in light petroleum, and in aniline and plotting the ratios (*a*) benzene: aniline against (*b*) benzene: light petroleum; the results for the various materials fall in well-defined areas.

(3) Rubber hydrocarbon may be determined by wet oxidation. Kuhn and L'Orsa[6] found that compounds containing the grouping $-CH_2.C(CH_3) = CH-$ yield acetic acid with chromic-sulphuric acid, and the method has been applied to rubber analysis by Burger, Donaldson and Baty,[7] who obtained 0·75 mol. of acetic acid per isoprene unit. The R.A.B.R.M. (private communication) has found correction factors to be necessary with increasing proportions of rubber-combined sulphur; also caution is necessary in interpreting the results obtained with mixtures of natural and synthetic rubbers, particularly if the latter should be GR–S (butadiene-styrene copolymer).

Erratum—Thiokol-RD is not invariably soluble in acetone, as was stated previously.[1]

Thanks are due to the Ministry of Supply for permission to quote from the Memorandum,[3] and to the R.A.B.R.M. for helpful discussions.

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L.M.S. RAILWAY CO., RESEARCH DEPARTMENT
CHEMICAL LABORATORY, STONEBRIDGE PARK, WEMBLEY, MIDDLESEX
G. H. WYATT

August, 1944

ABSORPTIOMETRIC DETERMINATION OF PHOSPHORUS BY THE MOLYBDENUM BLUE METHOD

THE determination of small quantities of phosphorus by the reduction of phosphomolybdate to molybdenum blue by the Denigès colorimetric method has been described by Truog and Meyer,[1] and has been further studied by Woods and Mellon,[2] using a General Electric photoelectric spectrophotometer.

The reaction has also been investigated by Farber and Youngburg,[3] but, under the conditions they employed, an intense blue colour is produced in absence of phosphorus, which renders the test useless for

the determination of small quantities of this element. The development of a blue colour in the blank test was observed by Vaughan[4] in the determination of phosphorus in steels, but the intensity of the colour was small and a correction was applied for it. The formation of molybdenum blue in absence of phosphorus is attributed by Truog and Meyer[1] to the reduction of ammonium molybdate by stannous chloride and may be avoided by carefully controlling the amounts and acidity of the reagents used without impairing the sensitivity of the test.

A previous note[5] on the colorimetric determination of phosphorus, using the Truog and Meyer method, recorded that addition of 1 ml of a 4% soln. of potassium chlorate prevented the gradual change in colour from blue to green in test solutions and standards corresponding with the development of a yellow colour in the blank. This yellow colour is presumably produced by the reduction of the molybdate reagent by stannous chloride. Further experiment with the photo-electric absorptiometer confirms that potassium chlorate prevents the excess of stannous chloride from reducing the molybdate reagent without interfering with the reduction of the phosphomolybdate. Under the conditions of the test the reaction between stannous chloride and ammonium phosphomolybdate appears therefore to be relatively more rapid than that between stannous chloride and potassium chlorate.

Analyses of acetylene and hydrogen for traces of phosphine have been carried out, using reagents of strengths recommended by Truog and Meyer with addition of potassium chlorate. The stannous chloride reagent was freshly prepared from a stock soln. of ten times the strength. The phosphine was absorbed in sodium hypochlorite soln., the excess of which was decomposed by boiling with acid; the soln. after neutralisation was tested for phosphorus.

Comparison of the colour produced was made on a Hilger Spekker photoelectric absorptiometer, using 4-cm cells and spectrum red filters No. 608. By this means 0·000001 g of phosphorus in 100 ml of soln. was readily determined. For concns. from 0·0000005 g to 0·000006 g of phosphorus in 50 ml, standard curves have been prepared both with and without the addition of 1 ml of 4% potassium chlorate soln., with an initial setting on the drum of the instrument of 0·5 and distilled water in the cells both on the left and right of the light source. In presence of potassium chlorate the calibration curve, on extrapolation, cuts the nil phosphorus axis at a point approximately corresponding to the water/water setting of the instrument; in absence of potassium chlorate the colour due to a given amount of added phosphorus, as measured on the absorptiometer, is greater, since the instrument records not only the colour due to phosphorus but also that due to the blank. This is illustrated in the following figures on the absorptiometer for two standard solns. of phosphorus.

Comparison of readings with and without addition of potassium chlorate

	Setting water/water 0·5	
	with $KClO_3$	without $KClO_3$
Drum reading for 0·000005 g P/100 ml	0·34	0·31
Drum reading for 0·00001 g P/100 ml	0·205	0·16

Further, it has been shown that in presence of potassium chlorate the amount of stannous chloride added between 0·15 ml and 0·5 ml does not influence the colour produced, whereas in absence of potassium chlorate a marked effect has been detected. The colour of the blank is dependent on the total amount of stannous chloride added when chlorate is absent. This effect of stannous chloride is shown by the following readings on the absorptiometer for 0·000005 g of P/100 ml.

Effect of stannous chloride with and without addition of potassium chlorate

	Setting water/water 0·5					
$SnCl_2$ added, ml	0·1	0·15	0·2	0·3	0·4	0·5
Drum reading with $KClO_3$ ..	—	0·34	—	0·335	—	0·34
Drum reading without $KClO_3$ ..	0·33	—	0·30	0·28	0·245	0·225

The colour reaches its max. intensity in *ca.* 2 min. after addition of the stannous chloride and fading does not occur for *ca.* 20 min. with a concn. of phosphorus of the order of 0·000005 g/100 ml. The rate of fading of the blue colour is, however, somewhat slower if potassium chlorate has not been used. The colour may be regenerated by adding a further drop of stannous chloride soln. Within the limits of experimental error the original intensity of colour is recorded if the test has been carried out in presence of potassium chlorate; otherwise the drum reading indicates a higher absorption as reflected by the production of the yellow colour.

The elimination of disturbing colours in the molybdenum blue method for the determination of phosphorus makes the visual comparison of test solns. and standards much easier. When working with the absorptiometer the addition of potassium chlorate does not increase the sensitivity of the test, but the production of water white blanks and stable blue colours representative of the amount of phosphorus present, irrespective of the amount of stannous chloride added within limits, is an obvious advantage with very small quantities of phosphorus.

We wish to thank Sir John Fox, F.R.S., the Government Chemist, for permission to publish this note.

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August 21, 1944

THE DETERMINATION OF COBALT IN ANIMAL TISSUES: MODIFICATION OF THE METHOD

SINCE the publication of my paper,[1] work carried out by Kidson and Askew[2] has shown that under certain conditions low results may be obtained when the nitroso-R-salt reagent is added after, instead of before, "neutralisation" in the process of development of the cobalt colour. For this reason, and because of apparently conflicting evidence from work at the two New Zealand Laboratories, I made a careful revision of the whole procedure with the assistance of G. W. Paul.

As a result of evidence submitted for information, and later published by Marston and Dewey,[3] particular attention was paid to the effect of the reaction of the solns. on the development of the cobalt colour, and it was found that with sodium acetate buffer full development of the cobalt colour was obtained over a *p*H range from about 4 to 8. As completely satisfactory results can be obtained on the acid side of neutrality, the step involving "neutralisation" to phenolphthalein with caustic alkali has been eliminated. Instead, the amounts of acid and of sodium acetate are regulated to give solns. directly of the desired *p*H (for convenience of manipulation 5 to 6), the procedure being as follows.

After separation of copper as sulphide (a quicker method than dithizone extraction[4] and equally efficient) evaporate the soln. to dryness on the steam-bath, add 1 to 2 ml of conc. nitric acid, and again evaporate to dryness on a low-temperature hot-plate. Take up the residue with 10 ml of water containing 0·5 ml of hydrochloric acid of constant b.p. and 0·5 ml of 10% nitric acid (1 vol. of HNO_3 of sp.gr. 1·42 with 9 vols. of water). Boil for a few min. to effect complete solution and evaporate in the reaction vessel to *ca.* 7 ml. Add 2 ml of a 0·1% soln. of nitroso-R-salt and then 2 g (±0·1 g) of hydrated sodium acetate crystals. The *p*H of the soln. will usually be close to 5·5 (greenish-blue or blue to bromocresol green indicator; if yellow, the soln. is too acid). Boil for 45 sec., add 2 ml of conc. nitric acid and continue the boiling for 45 sec. Cool in a dark box away from light, and match the colour as described in the original paper. To exclude extraneous light and assist matching, put rubber tubing round the length of the matching tubes. Follow this identical procedure for standard and unknown sample.

NOTES—(*a*) Section (2) of the original paper should now be omitted, as neutralisation with caustic alkali is eliminated, and the comments in the sentence immediately following section (5) should be withdrawn.

(*b*) Marston and Dewey[3] recommend the use of sodium citrate as buffer in their method, but this does *not* give satisfactory results with the New Zealand methods.

(*c*) Marston and Dewey's criticism (*Id.*, p. 349) of the use of phenolphthalein internal indicator, based on the fact that this gives a slight residual orange colour, implies the indiscriminate use of variable amounts of the indicator. In work carried out with the New Zealand methods exactly the same amount of phenolphthalein was of course used in both unknown and standard. The present method, however, does not require an internal indicator.

(*d*) The modified procedure described above can also be recommended for the determination of cobalt in pastures, soils and various minerals. For pastures, ashing after treatment with sulphuric acid,[5] or better still nitric acid,[2] is preferable to wet digestion to destroy organic matter, while ashing without previous treatment is suitable for soils and minerals. With these the separation of copper is usually unnecessary.

(*e*) It is inadvisable to use more than 10 g of dried liver or pasture, or the equivalent of more than about 0·5 g of soil or mineral in any determination. With larger amounts full development of the cobalt colours may not always be obtained.

(*f*) With materials of relatively high cobalt content aliquot parts of soln. should be taken to ensure that the absolute amounts of cobalt estimated do not exceed about 0·02 mg.

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July, 1944

ACETYL METHYL CARBINOL IN CIDER

THE amount of acetyl methyl carbinol in cider depends on the degree of acetification that has taken place. Bottled ciders contain from 1 to 5 mg per 100 ml. Pricked ciders, with 0·3 to 1·5% of acetic acid, contain from 5 to 45 mg per 100 ml. For the determination, 100 ml of cider are distilled with 1 g of ferric chloride, and the distillate is made up to the original volume. Diacetyl can now be readily identified as nickel dimethyl glyoxime or aliquot parts may be tested colorimetrically with α-naphthol and creatine by the method of Eggleton, Elsden and Gough (*cf.* ANALYST, 1944, **69**, 130).

W. GAYMER & SON, LTD.
ATTLEBOROUGH

D. W. STEUART

September, 1944

"ESSENCE OF LEMON"

ON May 23rd last, in the Wakefield City Court, proceedings were instituted by the Corporation under Secs. 3 and 6 of the Food and Drugs Act, 1938, in respect of a product sold as "Essence of Lemon."

Under Sec. 83 of the Act, a London firm of manufacturers was brought in as the persons ultimately responsible for the offences committed. The defendants pleaded guilty, and were discharged under the Probation of Offenders Act, 1907, on payment of £27 4s. costs.

The product consisted of a yellow solution containing approx. 9% of *iso*propyl alcohol (IPA) and possessing a faint odour of oil of lemon. No actual oil of lemon was present. The absence of oil of lemon was proved (*a*) by none separating on treatment with water or brine, (*b*) by a negative reaction with Schiff's reagent (using controls), which incidentally proved also the absence of citral. As regards the citral, expt. showed that it was impossible to prepare even a 0·1% soln. in 9% spirit, and that at least a 50% spirit was necessary to make a 0·5% soln. equivalent to a 1% soln. of terpeneless oil of lemon.

It subsequently appeared that the manufacturers had been mixing 8 fl.oz. of citral and 16 fl.oz. of oil of lemon with 1 gallon of IPA, adding water and a yellow dye, making up to 10 gallons, and filtering the lower layer which separated on standing. The quantities used corresponded to 0·5% of citral, 1·0% of oil of lemon and 10% of IPA in the unfiltered mixture.

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C. H. MANLEY
K. L. NICHOLSON

July, 1944

Ministry of Food

STATUTORY RULES AND ORDERS

1944—No. 865. The Pickles and Sauces Order. Dated July 20, 1944.* Price 3d.

This is a consolidating Order, replacing and in substance repeating the provisions of the Pickles and Sauces (Control and Maximum Prices) Order, 1942, and its amendments. A provision requiring the keeping of records has been added (Art. 9).

Art. 7 requires "Tomato Ketchup" and "Tomato Catsup" to contain no fruit other than tomatoes (excepting only onions, garlic or spices added for flavouring purposes).

To conform with the Labelling of Food Order, 1944, a statement of the minimum contents of containers instead of a declaration of the size or capacity of containers is required. The old and new forms of label will be legal until Jan. 1, 1945. By Art. 12 in the event of proceedings for infringement of this Order the certificate of the Government Chemist or of a Public Analyst shall be sufficient evidence of the facts stated therein, unless the other party requires the attendance as a witness of the person making the analysis.

Proposed Standard for Coffee Essences†

THE Ministry of Food has under consideration the issue of an Order under Regulation 2 of the Defence (Sale of Food) Regulations, 1943, prescribing standards for coffee essences and coffee and chicory essences.

The Inter-departmental Committee on Food Standards has made the following recommendations as to the standard:—(1) Liquid coffee essences should be required to contain not less than 0·5% weight in volume of caffeine derived from coffee. (2) Liquid coffee essences should not be permitted to contain vegetable extractives other than extractives derived from coffee. (3) Liquid coffee and chicory essences should be required to contain not less than 0·25% w/v of caffeine derived from coffee. (4) Liquid coffee and chicory essences should not be permitted to contain vegetable extractives other than extractives derived from coffee or chicory. (5) In any proceedings in respect of an alleged infringement of the standard for coffee essences or for coffee and chicory essences, it should be a defence for the defendant to prove that the essence was prepared with not less than 4 lb. of roasted coffee per gall. in the case of coffee essences or 2 lb. per gall. in the case of coffee and chicory essences. (6) It should be made a condition of the grant of a licence under the Coffee Essence (Control) Order, 1942, for the manufacture of a coffee and chicory essence, that the product should be prepared with not less than 4 lb. of roasted coffee and chicory per gall.

In a précis of the Committee's report which has been issued by the Ministry, it is stated that in certain Dominions coffee essences are required to contain not less than 0·5% w/w of caffeine, and coffee and chicory essences not less than 0·25% w/w. In this country a minimum standard of 4% w/w of dry coffee extractives has been proposed for coffee and chicory essences. Assuming that coffee contains 1·25% of caffeine, that it yields 25% of dry extractives, and that these standards relate to essences that have a sp.gr. of 1·2, 0·5% of caffeine corresponds to about 4¾ lb. of coffee per gall, and 4% of extractives corresponds to slightly under 2 lb. per gall.

If the standards are expressed as a percentage weight in weight, manufacturers of products having a lower gravity would be able to use less coffee per unit volume of product than manufacturers of products of higher gravity. For this and other reasons the Committee consider it preferable to express the standard as a percentage weight in volume.

In view of the above standards and the proportions of coffee used both pre-war and at present in most of the coffee and coffee and chicory essences on the market, the Committee consider that a product should not be sold as coffee essence unless prepared with at least 4 lb. of roasted coffee per gall., and that no compounded coffee product for producing a beverage should be sold as derived from coffee, unless it contains at least 2 lb. of roasted coffee per gall. In absence of a direct method of determining the proportion of coffee used, and since part of the value of the essences must be ascribed to the stimulating effect of the caffeine content, the Committee recommend that the standard be expressed as a minimum percentage of caffeine. A coffee essence prepared with 4 lb. of coffee per gall. would, if the coffee contained 1·25%, which is regarded as a fair average, contain 0·5% w/v of caffeine, and coffee and chicory essences not less than 0·25% w/v of caffeine.

The defence suggested in recommendation 5 above is to provide for the possibility that the caffeine content of the coffee used may be below the average.

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* Obtainable from H.M. Stationery Office. Italics indicate changed wording.

† Press Notice. PN. 3321*a*, Aug. 16, 1944.

Reference is made in the recommendations to preparations containing alternatives to chicory, which may be regarded as falling within the broad definition of "Coffee Essence," adopted for the particular purposes of the Coffee Essence (Control) Order, 1942. The Committee consider that the sale of these preparations under the description "coffee essence" or "coffee and chicory essence" without qualification would, in general, be misleading. In addition, a requirement that they should contain not less than 2 lb. of coffee per gall. might prove embarrassing to the manufacturers. It is therefore suggested that products sold under these descriptions should not be permitted to contain vegetable extractives, other than extractives of coffee or coffee and chicory respectively. Under the Food Standards Order, 1944, it will then be obligatory to describe products containing alternatives to chicory in such a way as not to lead an intending purchaser to believe that he is purchasing either coffee essence or coffee and chicory essence. Traders and the public will thus be able clearly to distinguish products containing only coffee and chicory from those which contain alternatives either in addition to, or in place of, chicory.

The Committee suggest that it is unncecessary to fetter the discretion of manufacturers to the extent of fixing the relative proportions of coffee and chicory, provided that the combined weights of coffee and chicory are satisfactory as suggested in recommendation 6.

Fruit and Vegetable Preservation Research Station, Campden

ANNUAL REPORT FOR 1943

As in the previous year, much of the scientific work of the Station outlined by the Director, Mr. F. Hirst, has consisted in investigations of problems submitted by members of the Association, and in routine work for the Ministry of Food. Research work has therefore been restricted. The problems studied included the following.

CANS WITH BLACKPLATE ENDS—To obtain an indication of the risks run in substituting blackplate for tinplate in the ends of cans, a series of tests was made to ascertain the effects of various factors. The tests on vegetables indicated that cold-rolled plate gave better results than hot-rolled plate unless the lacquering was exceptionally efficient. The natural corrosive properties of some vegetables showed great variations. Thus with beetroot stored at 95° F. the rate of the loss of vacuum may range from 0·2 to 15 or 20 in. per month. The rate of corrosion with stringless beans was rather high, whereas potatoes were generally satisfactory. Carrots, processed peas and fresh peas proved the least corrosive of any vegetables tested. The flavour was good unless very severe corrosion had gone on for a long time, *e.g.*, in canned beetroot. Solution of iron from the ends was not serious except in canned beetroot, in which it caused pronounced discoloration. The highest concns. (p.p.m.) found in other packs after 1 year's storage at 95° F. were: processed peas 32, carrots 21, parnips 11 and beans in tomato sauce 21. These quantities did not produce a metallic flavour but provide an indication of the types of electrochemical reactions involved. The colour, except with beetroots and possibly green peas in cans with single lacquered ends, was not affected. Small black specks of iron sulphide were sometimes present in cans of peas, swedes and carrots with single lacquered blackplate ends.

Fruits—Cans with a double roller lacquer on blackplate ends were most unsuitable for fruits requiring lacquered bodies (*e.g.*, all red and purple fruits), and cannot be recommended even for fruits packed in cans with plain bodies. Formation of hydrogen was reduced to approximately the same as in tinplate cans by protecting the blacked plate ends with a dipped coating of lacquer, but a serious rise in the rate of corrosion was caused by imperfections in the dipped coating. The iron content in cans with roller-coated blackplate ends tended to rise very rapidly and to cause marked discoloration. In cans of loganberries and damsons stored for 3 to 6 months at 95° F. the iron content reached 200–400 p.p.m.

USE OF THE SPHEROMETER IN TESTS ON HYDROGEN SWELLS—The method depends on the measurement of the average rate of loss of vacuum in cans by determining the alteration in the degree of depression at the ends, by means of a specially designed spherometer. A representative sample (say, 8 to 12 cans) selected at random, is used throughout as a single unit, and the average rate of change of depression is determined.

The instrument, designed by Adam and Stanworth, has 3 legs adjustable so as to travel accurately round the countersink of any of the common sizes of cans. The scale on the head of the spherometer is graduated in thousandths of an inch; on a glass surface the instrument will give a reading accurate to 0·0002 in. The readings on the cans give the depression of the centre of the end below the level of the horizontal channel of the countersink. For comparative tests the average change in depression of the ends is sufficient, but it may readily be converted into the average loss of vacuum. For the purpose of most calculations it is safe to assume that each 0·001 inch change in depression corresponds to a change of 0·8 in. vacuum. A correction of 0·00005 in. depression for each mm difference from the normal barometric pressure is made—added for pressures below and subtracted for pressures above 760 mm.

The fact that in most canned vegetables the rate of change is constant suggests that the rate of formation of hydrogen is also virtually constant.

INTERNAL CORROSION OF CANS—The laboratory methods used in the tests (by D. Dickinson) are described. It was found that the use of hot-rolled and cold-rolled plates for different parts of a can does not lead to increased production of hydrogen swells; also that cans with cold-rolled steel ends and hot-rolled steel bodies gave much better results than cans entirely of hot-rolled steel when filled with loganberries and Victoria plums. The juice from Victoria plums in lacquered cans became more corrosive on storage at 95° F. when the fruit had been packed in syrup but not when packed in water.

TREATMENT OF POTATOES FOR CANNING—Soaking for 1 hr. in 2·6% hydrated calcium chloride soln. greatly improved the texture of potatoes from silt, skirt and fen soils; the texture of potatoes from the highland soils of Huntingdonshire was poor, even after the treatment. The colour of the treated samples was slightly inferior to that of untreated samples, but there was no detectable deterioration in natural flavour and no appreciable foreign flavour as a result of the treatment.

FRUIT GUMMING OF PLUMS—Positive evidence has been obtained that gumming is associated with rainfall in the later months of growth. Boron also has some effect if taken up by the trees. These two factors may be interconnected, and tests on these lines are in progress.

British Standards Institution

THE first revision of the following standard has been issued.

NO. 757—1944. BRITISH STANDARD METHODS FOR SAMPLING AND TESTING GELATINS.*

Rules, agreed by representatives of the manufacturing and consuming trade, for guidance in bulk sampling are given, and the preparation of the laboratory sample is described.

The tests comprise: (A) Determination of Moisture. (B) Determination of Jelly Strength by reference to an agreed standard. (C) Determination of Jelly Strength with the Bloom Gelometer (*Ind. Eng. Chem., Anal. Ed.*, **1930**, **2**, 348). (D) Viscosity, for which the B.S. U-tube viscometer (B.S., 188—1940) is recommended. (E) Melting-point, by Cambon's method (*Fabrication des Colles Animales*, 1907, 59). (F) Foam. (G) Water absorption. (H) Solubility of partially swollen Sheet. (J) Keeping Quality. (K) *p*H Value. (L) Grease. (M) Ash. (N) Sulphur dioxide (Monier-Williams's method is recommended). (O) Chlorides. (P) Colour of Jelly and its Solution (by means of the Lovibond Tintometer). (Q) Clarity (by photoelectric measurement of light transmitted through a 6·66% w/w soln. in a 2-in. glass trough, as compared with that transmitted by water). (R) Arsenic—the S.P.A. Gutzeit procedure is recommended. (S) Heavy Metals—a method based on that of Hamence (ANALYST, 1937, **62**, 18) is recommended.

The Appendix contains notes on the apparatus referred to in the text.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Detection of Glacial Acetic Acid in Vinegar. R. E. O'Neill and A. M. Henry (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 263–271)—The method of Edwards and Nanji (ANALYST, 1938, **63**, 410) for distinguishing artificial from brewed vinegar has the disadvantage that the reaction proceeds at a diminishing rate greatly influenced by temp., time and changing concn. The reaction was studied closely to discover the optimum conditions for the method. Temp. fluctuations cause relatively large changes in titre, and room temp. was found to be somewhat less critical than higher ones, which are however difficult to maintain. Therefore 25° C. was selected as the temp. at which the reaction should be carried out. The time factor and the concn. are interdependent, and the highest practicable concn. of potassium permanganate acting for 1 hr. gave the best results. Presence of acid is necessary for the oxidation, but a concn. of 3 *N* sulphuric acid causes reaction between the permanganate and the acetic acid, and *N* concn. was finally selected. To maintain a uniform rate of reaction the concn. of potassium permanganate must not fall below 0·15 *N* at the end of the titration. The conditions finally decided upon were adjustment of the vinegar to 4% acetic acid by dilution with water, use of 50 ml of the adjusted vinegar, addition of 25 ml of *N* potassium permanganate and addition of 10 ml of dil. sulphuric acid (1+1). The initial concn. of potassium permanganate in the reaction mixture is 0·29 *N*, and, if half the permanganate is used up in the oxidation, the concn. at the end is 0·15 *N*. Vinegars taking more than 12·5 ml of *N* potassium permanganate must be diluted so that the titre is less than 12·5 ml. Any method of distillation may be used, but steam distillation gives the best results. Corks and rubber stoppers should be wrapped in aluminium or tin foil. *Method*—Adjust the vinegar to 4% of acetic acid by dilution with water, steam distil 50 ml and maintain the vol. so as to have a residue of 45 ml with 50 ml of distillate. Treat the distillate in a glass-stoppered vessel with 10 ml of dil. sulphuric acid (1+1) and 25 ml of *N* potassium permanganate and keep the mixture at 25° C. for 1 hr., preferably in a water-bath. Immediately add 20 ml of 30% potassium iodide soln., mix and titrate the liberated iodine with 0·5 *N* sodium thiosulphate. Correct the result by means of a blank determination made with 50 ml of 4% acetic acid in place of the vinegar. To obtain the oxidation number, divide the number of ml of 0·5 *N* sodium thiosulphate by 2 and subtract the result from 25 ml minus the blank figure. If the oxidation number exceeds 15, repeat the determination with half the original amount of vinegar, halving the concn. again if necessary until the amount of *N* permanganate used is less than 15 ml and finally multiplying by the appropriate factor to obtain the oxidation number for 50 ml of the sample. Additional information is obtained by determining the oxidation number of 50 ml of the adjusted vinegar without distillation and also of the residue from the distillation made up to 50 ml with 4% acetic acid. Vinegars made by diluting acetic acid, coloured or uncoloured, gave oxidation numbers of the distillate of less than 0·5; distilled vinegars, molasses vinegar and cider vinegar gave oxidation numbers of more than 3·0. A. O. J.

Determination of [Butter] Fat in Cream and Ice Cream by Dry Extraction with Chloroform. G. R. Kinsley (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 337–338)—Determination of butter fat in ice cream is made more difficult by the presence of cocoa products, dextrose, agar, gelatin, egg powder, etc., which increase the tendency to char in the Babcock method and cause emulsions in the Röse–Gottlieb method. The following procedure removes most of the difficulties. Warm the sample of cream or ice cream to room temp. and mix thoroughly. Shake 4 g vigorously for 2 min. in a 50-ml glass-stoppered flask with 40 ml of chloroform, add 10 g of anhydrous sodium carbonate and shake immediately and vigorously for 2 min. or until the sides of the flask are free from adhering cream. Filter the chloroform extract through a medium retentive 9-cm filter paper into a tared 120-ml thin Pyrex evaporating dish. Again extract the residue in the flask with 20 ml of chloroform and repeat the extraction three more times and wash down the sides of the filter. Remove the chloroform by gentle heat and dry the fat to constant wt. Make a blank expt. with the reagents. Results are in satisfactory agreement with those obtained by the Röse–Gottlieb method. A. O. J.

* Pp. 36. Obtainable from British Standards Institution, Publications Dept., 28, Victoria Street, London, S.W.1. Price 3s. 6d. net.

Oils of the Seeds of *Ocimum kilimandscharicum, Euphorbia calycina, E. erythraeae, Sterculia tomentosa,* and *Trichilia emetica.* **A. J. Henry and D. N. Grindley** (*J. Soc. Chem. Ind.*, 1944, **63**, 188–190)—The principal constants of the oils are shown in the table below. The oils in the seeds of the first three plants have strong drying properties and are satisfactory substitutes for linseed oil in paints. The kernel and husk of the seeds of *T. emetica* contain an oil which has a high proportion of sat. fatty acids and is excellent for soap-making. The oil from *T. emetica* contains a bitter principle which is removed by caustic soda refining, leaving an oil free from objectionable taste. The oils in the seeds of *S. tomentosa* closely resemble that of *S. foetida* of the East Indies.

	Ocimum kilimandscharicum	*Euphorbia calycina*	*Euphorbia erythraeae*	*Sterculia tomentosa*			*Trichilia emetica*		
				Whole seed	Kernel	Husk	Whole seed	Kernel	Husk
Weight of 100 seeds, g	0·049	0·74	1·22	26·8	—	—	52·0	—	—
Oil content, % ..	16·2	20·8	33·0	33·0	43·3	21·7	59·5	61·8	53·5
Colour of oil ..	pale yellow	golden	golden	brown	pale yellow	dark brown	brown	pale yellow	brown
Constants of oils									
Sap. val. ..	193	189	190	193	193	200	199	198	199
Iodine val. ..	195·6	192·3	179·2	84·8	94·7	63·1	49·2	42·1	71·8
Thiocyanogen val.	122·5	121·0	105·7	66·3	69·1	59·0	42·1	39·9	49·8
n_D^{40}	1·4767	1·4759	1·4735	1·4637	1·4659	1·4612	1·4599	1·4592	1·4622
Insol. bromides, %	54·0	50·3	35·7	—	—	—	—	—	—
Sat. fatty acids, %	8·1	8·9	12·8	—	—	—	—	—	—
m.p. of oil, °C. ..	—	—	—	—	—	—	30°	32°	below 12°
Titre, °C.	—	—	—	—	—	—	50°	52°	47°
Unsap. matter, %	—	—	—	—	—	—	1·23	1·39	0·83
Composition of fatty acids, %									
Linolenic	61	60	41	—	—	—	—	—	—
Linolic	14	12	39	23	32	5	9	3	27
Oleic	17	19	7	53	46	63	39	43	29
Saturated	8	9	13	24	22	32	52	54	44

E. M. P.

Identification of Cow Manure in Dairy Products. **R. E. Duggan** (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 331–336)—Under the microscope manure particles of plant origin are not easily distinguished from fragments of feeding stuffs, and a positive method of separating digested from undigested fragments is required. Two samples of cow manure were first studied, one air-dried and the other in its original moist condition. They consisted of plant particles which had survived digestion bound together with a dark brown gummy mass. By mixing these samples with milk it was found that the visible filth collected on sediment pads or filter cloths represented *ca.* 12% of the original wet manure. After a number of stains had been tested Fast Green FCF (FD & C Green No. 3) was selected. Plant material from which the cell nutrients had not been removed by digestion took the stain well, but manure particles retained their original brown colour. Filter 500 ml of milk through a sediment pad. Transfer with tweezers *ca.* 100 vegetable fragments (max. length 2 mm) to a casserole containing 3–4 ml of water, avoiding inclusion of hair, insect parts and structureless matter. With the aid of a stream of hot water (90° C.), transfer the particles into a Gooch crucible fitted with a circle of hard filter-paper (Sharkskin), and wash thoroughly with hot water, then with hot 95% alcohol and again with hot water. With tweezers replace the fragments in the casserole containing a drop of water. Add, drop by drop, 10 drops of 1% aq. Fast Green FCF soln., immerse floating fragments, stain for 15 min. and promptly wash the particles free from excess of stain by filtering through a Gooch crucible, using a fine stream of cold water. Replace the fragments in the casserole with the aid of a very fine stream of water, restricting the vol. of water to 3–5 ml and boil for 15 sec., keeping the particles away from the sides of the vessel. Filter immediately through a Gooch crucible, replace the particles in the casserole and repeat the staining and boiling procedure. Finally collect the fragments in a Buchner funnel on a 7-cm filter paper cross-sectioned in 6-mm. squares. Place the air-dried paper in a Petri dish with a few ml of mineral oil and separate the manure particles from all others, using a low power microscope (10×). Manure fragments of plant origin after staining have a characteristic dirty, "worn" appearance and a brownish translucent colour. They may or may not be slightly tinged with green. The presence of irregular, amorphous specks on the surface of the fragments is additional evidence of manure origin. Undigested plant fragments have a typical vivid green colour, the surfaces are relatively smooth and clean, and the edges are usually sharp and not frayed or worn. The effect of enzymatic and bacterial actions involved in the manufacture of cheese was determined by addition of alfalfa hay to milk which was then made into cheese by the usual Cheddar process and aged for 10 weeks. Three common methods, using chemical agents (sodium citrate, sodium oxalate and phosphoric acid), were used to isolate the hay from the cheese. Evaporated and condensed milk were prepared from milk contaminated with manure. The above-mentioned treatments had no effect upon the differential staining of feeds and manures. Although it is possible to use staining as the sole criterion, for the best results it is necessary to acquire a thorough knowledge of the appearance of various authentic feeding stuffs and manures after being subjected to the staining procedure, and to apply this knowledge in segregating the manure particles from undigested plant material.

A. O. J.

Oil from the Seeds of *Bombax Malabaricum*. **C. V. Rao, M. N. Rao and A. Venkateswarlu** (*J. Indian Chem. Soc.*, 1943, **20**, 405–406)—Kapok oil is derived from distinct genera of plants, *Eriodendron anfractuosum* and *Bombax malabaricum*. Some earlier work gives constants of the oil without reference to the species (*cf. Bull. Imp. Inst.*, 1926, **24**, 18; ANALYST, 1926, **51**, 465). Characteristics for *Bombax malabaricum* oil were: m.p. 34° C., solidif. p. 30° C., sp.gr. 0·9362 at 35° C., n_D^{40} 1·4611, sap. val. 196·3, iodine val. 68·11, acidity (as oleic acid) 32·58%, unsap. matter 1·761%, Hehner val. 94·8%. Mixed fatty acid from the oil had sap. val. 274·5 and iodine val. 71·78. This oil contained 94·8% of insol. mixed fatty acids composed of 57% of solid and 43% of liquid acids. Their percentage composition, by wt. was: myristic 1·2, palmitic 23·6, arachidic 2·8, oleic 64·9, linolic 7·5, stearic acid no trace. No insol. bromide was obtained on bromination of the fatty acids. After extraction of oil, the seed cake contained 34·4% of crude protein, but was considered unsatisfactory as cattle food. Results previously obtained by Sprinkmeyer for *Bombax* oil (Lewkowitsch, Vol. II) by Georgi for *Eriodendron anfractuosum* (*Malay Agr. J.*, 1922, **10**, 284) and by Meldenbacher for kapok oil from Japan (*Oil and Soap*, 1937, **14**, 118) are also tabulated. Sprinkmeyer's sample had iodine val. 93·78; the difference for the present sample may be due to climatic conditions. E. B. D.

Non-Tertiary Bases in Commercial Nicotine. **K. B. Edwards** (*J. Soc. Chem. Ind.*, 1944, **63**, 186–188)—The nitrous acid method for the estimation of secondary alkaloids in commercial nicotine products is invalidated by presence of so-called polymerised and resinified alkaloids formed during extraction and storage of the alkaloids and their salts. Resinous bases are removed by treatment with light petroleum and potassium carbonate, but it is doubtful if all altered alkaloids are so removed. The presence of these substances is indicated by the slowness of steam distillation and formation of a bulky fluffy silicotungstate ppt. slightly sol. under washing. Bowen and Barthel's estimation of nornicotine in tobacco (*Ind. Eng. Chem., Anal. Edn.*, 1943, **15**, 740) is not applicable to commercial nicotine products, as it does not remove alteration products of the alkaloids. The presence of nornicotine or anabasine in appreciable proportion in commercial nicotine products does not appear to be established. E. M. P.

Determination and Identification of 2-Aminoanthraquinone in D and C Blue No. 9. **O. L. Evenson** (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 317–319)—The intermediate 2-aminoanthraquinone is used in the preparation of D & C Blue No. 9 or Carbanthrene Blue (3,3′-dichloroindanthrene), which occurs in the list of colours that may be certified by the U.S. Food and Drug Administration for use in drugs and cosmetics. The intermediate is converted into 2-amino-1,3-dibromoanthraquinone, which is identified by its m.p. and that of its acetyl derivative and estimated colorimetrically. Dissolve 0·2 g of D & C Blue No. 9 in *ca.* 3 ml of sulphuric acid, using a glass rod to break up lumps. Carefully add 75 ml of alcohol in small portions, stirring after each addition. Heat on the steam-bath with occasional stirring for 30 min., cool, dilute to 100 ml and filter. To 10 ml or less of the filtrate and to a series of standard solns. of 2-aminoanthraquinone containing 0·05–0·15 mg in the same vol. of alcohol acidified with 0·1 ml of sulphuric acid add 0·1 ml of bromine water, 0·1 ml of sat. hydrazine sulphate soln. and 10 ml of water. Mix after each addition and compare the colour of the sample soln. with that of the standards. To obtain the intermediate for identification, dilute the acid alcoholic filtrate (*supra*) with twice its vol. of water, extract with ether, wash the extract with a few ml of water and remove the solvent by evaporation. Dissolve the residue in 95% alcohol (500 ml per 500 mg) and acidify the soln. with sulphuric acid (*ca.* 2 ml per 500 ml). Cool to 50° C., add bromine (2 g per 500 ml), shake in a stoppered flask at intervals for 15 min., filter and wash the derivative with a few ml of 50% alcohol. The orange-brown 2-amino-1,3-dibromoanthraquinone, recrystallised from alcohol, melts at 247° C. To prepare the acetyl derivative, dissolve the dibromo derivative in acetic anhydride and heat under reflux for 10 hr. Dilute with water, collect the product by filtration, wash with water and recrystallise from 95% alcohol. The yellowish-green 2-(N-diacetyl)-1,3-dibromoanthraquinone melts at 211° C. A. O. J.

Improved Dithizone Procedure for Determination of Zinc in Foods. **O. R. Alexander and L. V. Taylor** (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 325–331)—The method involves wet oxidation of the sample, elimination of lead, copper, cadmium, bismuth, antimony, tin, mercury and silver as sulphides after addition of copper to induce complete pptn., separation of nickel and cobalt together by extraction with chloroform after addition of dimethylgloxime and α-nitroso-β-naphthol, extraction of zinc dithizonate with carbon tetrachloride, transference of the zinc to hydrochloric acid and re-extraction of zinc dithizonate for colour measurement. Heat the sample, not exceeding 25 g (25–100μg of zinc) and evaporate to low bulk if liquid, with conc. nitric acid until the first vigorous action subsides, add 5 ml of sulphuric acid and continue heating with addition of small amounts of nitric acid as required until fumes of sulphur trioxide are evolved and the soln. remains clear and almost colourless. Add 0·5 ml of perchloric acid, heat until it has almost all been removed, cool and dilute to *ca.* 40 ml. Add 2 drops of methyl red indicator and 1 ml of copper sulphate soln. (8 g of $CuSO_4.5H_2O$ per litre), neutralise with conc. ammonia (which should be redistilled if appreciably contaminated) and add enough hydrochloric acid to make the soln. *ca.* 0·15 *N* (*ca.* 0·5 ml excess in 50 ml). Pass in hydrogen sulphide, collect the pptd. sulphides on fine textured paper (Whatman No. 42) and wash with 3 or 4 small portions of water. Remove hydrogen sulphide from the filtrate by boiling, adding bromine water to remove the last traces, and dilute to 100 ml. Treat 20 ml of the filtrate with 5 ml of ammonium citrate buffer soln., 2 ml of dimethylglyoxime soln. and 10 ml of α-nitroso-β-naphthol soln. (*infra*) in a separating funnel and shake for 2 min. Discard the chloroform layer and wash the aq. layer with 10 ml of chloroform. To the aq. layer (*p*H, 8·0–8·2) add 2 ml of dithizone soln. (*infra*) and 10 ml of carbon tetrachloride and shake for 2 min. After it has thoroughly settled withdraw the aq. layer by means of a pipette attached to suction apparatus, add *ca.* 25 ml of water and withdraw this without shaking. Add 25 ml of 0·04 *N* hydrochloric acid, shake for 1 min., draw off and discard the carbon tetrachloride layer after dislodging the drop that usually floats on the surface, and add 5 ml of ammonium citrate soln. and 10 ml of carbon tetrachloride.

The *p*H at this stage is 8·8–9·0. Determine the amount of dithizone soln. to be added as follows. To 4 ml of standard zinc soln. (*infra*) made up to 25 ml with 0·04 *N* hydrochloric acid in a separating funnel add 5 ml of ammonium citrate soln. and 10 ml of carbon tetrachloride. Add the dithizone soln. in small increments, shaking briefly after each addition, until a faint yellow colour in the aq. layer indicates slight excess of the reagent. Multiply the vol. of dithizone soln. by 1·5 and add this vol. to all samples. Shake for 2 min., place 5 ml of the carbon tetrachloride layer in the spectrophotometer cell, add 10 ml of carbon tetrachloride and determine the transmission at 530*mμ*. To prepare standard curves, dilute solns. containing 0, 5, 10, 15 and 20*μ*g of zinc to 25 ml with 0·04 *N* hydrochloric acid in separating funnels, add 5 ml of ammonium citrate soln. and extract the zinc as already described. Plot the transmittance on a logarithmic scale against concn. The intercept of the curve may vary slightly from day to day but the slope should be practically constant. Zinc added to fruit cocktail in amounts up to 20 p.p.m. together with 200 p.p.m. of copper and 20 p.p.m. of the other interfering elements was recovered with satisfactory accuracy. To prepare ammonium citrate buffer soln., dissolve 225 g in water, make alkaline to phenol red with ammonia and add 75 ml in excess. Dilute to 2 litres and immediately before use add excess of dithizone soln., extract with carbon tetrachloride until the extract is a clear bright green, remove excess of dithizone by repeated extraction with chloroform and finally extract once more with carbon tetrachloride. Removal of dithizone must be complete to avoid loss of zinc in the separation of nickel and cobalt. To prepare dimethyl glyoxime soln., dissolve 2 g in 10 ml of ammonia and 200–300 ml of water, filter and dilute to 1 litre. To prepare α-nitroso-β-naphthol soln., dissolve 0·25 g in chloroform and dilute to 500 ml. To prepare dithizone soln., dissolve 0·125 g of diphenylthiocarbazone in 5 ml of ammonia, add 250 ml of water, extract repeatedly with carbon tetrachloride until the extract is bright green, discard the extract and filter the aq. soln. which is best prepared as needed. To prepare stock standard zinc soln., dissolve 0·5 g of zinc in dil. hydrochloric acid and make up to 1 litre. For use, dilute 10 ml to 1 litre with 0·04 *N* hydrochloric acid (1 ml ≡ 5*μ*g of zinc). A. O. J.

Biochemical

Determination of Glutamic Acid in Proteins. H. S. Olcott (*J. Biol. Chem.*, 1944, **153**, 71–82)—Glutamic acid can readily be converted into pyrrolidone-carboxylic acid by autoclaving at *p*H 3–4; by determining the decrease in amino nitrogen, corrected for cystine, the amount of glutamic acid can be estimated. With the exception of cystine no other amino acid is affected by this procedure. Dissolve 100-mg samples of the protein in 2 ml of 6 *N* hydrochloric acid by heating on the steam-bath. Attach a small condenser to each of the tubes and heat at 120–125° C. for 48 hr. Cool, transfer the solution to a flask, and dilute to 10 ml. Filter and dilute a 2-ml aliquot portion of the filtrate to 10 ml for the control, and titrate another 2-ml portion to *p*H 3·3 with *N* sodium hydroxide. Adjust a third 2-ml aliquot portion to *p*H 3·3 by adding the requisite amount of alkali thus determined, and dilute with water to 7 to 8 ml. Autoclave the soln. for 4 hr. at *ca.* 19–20 lb. per sq. in. (125–126° C.), cool and dilute to 10 ml. Determine the amino nitrogen in 2-ml aliquot portions of the control, and of the autoclaved sample by the standard Van Slyke manometric method, using a 3-min. reaction period. When a 100-mg sample of protein is used the percentage of glutamic acid equals

$$\frac{57{,}070\,(N_1 - N_2) - 0{\cdot}78\,C\,(100 - M)}{(100 - M)}$$

where N_1 and N_2 are the amounts of amino nitrogen (mg/ml) in the original and autoclaved samples respectively, C is the cystine content (%) of the protein and M the moisture content (%). The constant 57,070 is obtained by multiplying together the dilution factor (50), the factor (147/14) required to convert amino nitrogen to glutamic acid, the correction factor (100/92) for incomplete conversion to pyrrolidone-carboxylic acid and 100 to convert to percentage. The correction factor for cystine is obtained by multiplying the amount (90%) surviving the initial hydrolysis by the amount (65%) lost on autoclaving, and converting the value so obtained into its glutamic acid equivalent. The recoveries of glutamic acid added to casein or gelatin were within 5% of the amounts expected. F. A. R.

Determination of Ammonia Evolved from α-Amino Acids by Ninhydrin. D. A. MacFadyen (*J. Biol. Chem.*, 1944, **153**, 507–513)—*Procedure A—Measurement of Ammonia formed by Action of Ninhydrin*—Treat 1 ml of amino acid soln. (containing 1–1·4 mg of amino-nitrogen), adjusted to *p*H 2·5 with 50–100 mg of citrate buffer mixture, with ninhydrin (50 mg), as described by Van Slyke *et al.* (*J. Biol. Chem.*, 1941, **141**, 627; Abst., ANALYST, 1942, **67**, 104), using a reaction time of 8 min. Remove the carbon dioxide and traces of air from the reaction vessel by suction for 2 min., and then admit hydrogen sulphide to the vessel until atmospheric pressure is restored. Shake vigorously for 4 to 5 min. to precipitate hydrindantin; remove the excess of hydrogen sulphide by applying suction for 4 min. or longer. Transfer the soln. to a 15-ml graduated flask with the aid of 3 washings of citrate buffer soln. (50 mg of citrate mixture of *p*H 2·5 per ml of water), dilute to the mark and filter from the hydrindantin ppt. Estimate the ammonia on 10 ml of filtrate by the aeration method of Van Slyke and Cullen (*J. Biol. Chem.*, 1914, **19**, 211; 1916, **24**, 117). Absorb the aerated ammonia in 25 ml of 4% boric acid soln. and titrate with *N*/70 sulphuric acid or absorb in 12 ml of 0·1 *N* sulphuric acid and measure manometrically by the method of Van Slyke *et al.* (*J. Biol. Chem.*, 1941, **141**, 681). *Procedure B—Measurement of Ammonia and Carbon Dioxide formed by the Action of Ninhydrin*—Follow procedure A, using a smaller sample containing only 0·4 to 0·6 mg of carboxyl nitrogen and, after reaction with ninhydrin, measure the carbon dioxide as described by Van Slyke *et al.* (*J. Biol. Chem.*, 1941, **141**, 627). Evacuate the reaction vessel, fill with hydrogen sulphide, and continue as in procedure A. By either procedure most amino acids yield constant amounts of ammonia, but glycine and alanine yield only 0·86 and 0·90 mol. and tryptophan only 0·34 mol. of ammonia. β-Alanine, α-glucosamine and urea yield none. F. A. R.

Photometric Adaptation of the Somogyi Method for the Determination of Glucose. N. Nelson (*J. Biol. Chem.*, 1944, **153**, 375–380)—The chief objections to the various modifications of the Somogyi method are that the results are not always reproducible and the colour is not very

stable. The following method is said to overcome these difficulties, and has been successfully used for tissue glucose, glycogen, urine reduction equivalents, maltose and glycuronic acid. It was not possible to carry out diastase determinations by the method. The reagents are prepared as follows. *Copper reagent A*—Dissolve 25 g of anhydrous sodium carbonate, 25 g of Rochelle salt, 25 g of sodium bicarbonate and 200 g of anhydrous sodium sulphate in about 800 ml of water and dilute to 1 litre. Filter, if necessary, and store at not less than 20° C. *Copper reagent B*—Dissolve 15 g of $CuSO_4 5H_2O$ in 100 ml of water containing 1–2 drops of conc. sulphuric acid. *Arsenomolybdate colour reagent*—Dissolve 25 g of ammonium molybdate in 450 ml of water, add 21 ml of conc. sulphuric acid, mix, add 3 g of $Na_2HAsO_4 7H_2O$ dissolved in 25 ml of water, mix and incubate at 55° C. for 25 min. with adequate stirring. Store the reagent in a glass-stoppered brown bottle. *Procedure*—Mix one vol. of blood with 15 vols. of water and add 2 vols. of 0·3 *N* barium hydroxide. After the mixture has turned brown, add 2 vols. of 5% $ZnSO_4 6H_2O$ soln. (which should be adjusted so that 5 ml require 4·7 to 4·8 ml of the barium soln. to produce a definite pink with phenolphthalein). Mix, leave for a few min., filter and centrifuge. Pipette 1 ml of the filtrate into a narrow test-tube graduated at 25 ml, and add 1 ml of a mixture of 25 parts of reagent A to one part of reagent B. Also add similar quantities of the reagent mixture to 1 ml of appropriate standard solns. and to 1 ml of water to serve as a blank. Mix, heat all the tubes for 20 min. in a boiling water-bath, cool, and add 1 ml of the arsenomolybdate reagent to each tube. Dilute to the mark and measure the percentage transmission photoelectrically at 500 or 520$m\mu$. Adjust the photometer so as to read 100% transmission through the blank. The colour is quite stable, and the error is generally less than 1·5%. The colorimetric method is alternative to the iodimetric method and, in most instances, gives identical results. F. A. R.

Photometric Method for the Determination of Cholates in Bile and Blood. J. L. Irvin, C. G. Johnston and J. Kopala (*J. Biol. Chem.*, **1944**, **153**, 439–457)—Reinhold and Wilson (*J. Biol. Chem.*, **1932**, **96**, 637) describe a method of estimating cholic acid which was accurate when used for pure solns. or bile, and which was specific for conjugated and unconjugated cholic acid. This method has now been adapted for use with the photoelectric colorimeter, and certain necessary modifications have been introduced. Put 1 ml of solns. of sodium cholate containing 0·02 to 0·3 mg of cholic acid per ml, into a series of test-tubes, and add 6 ml of 16 *N* wulphuric acid and 1 ml of 1% furfural soln. to each. Prepare reagent blanks, using 1 ml of water instead of the cholate soln. Heat all the tubes for exactly 13 min. at 65° C. and then cool rapidly to room temp. Add 5 ml of glacial acetic acid to each tube, and measure the colours at 620 or 660$m\mu$ with the transmittance of the blank set at unity. From the results plot a calibration curve. Reinhold and Wilson used alcohol to deproteinise bile for cholate determinations, and this method proved to be satisfactory, but Dubilet's method (*J. Biol. Chem.*, **1936**, **114**, 289), involving the use of zinc sulphate and sodium hydroxide soln., proved to be superior, as it gave almost colourless solns. and filtrate blanks were negligible. Colour development is carried out directly on 1-ml portions of either of these filtrates, but with alcohol filtrates a correction has to be made, using a blank containing the dry residue from an equiv. portion of alcoholic bile filtrate, 2 ml of distilled water and 6 ml of 16 *N* sulphuric acid, heated for 35 min. at 65° C., and then, after cooling to room temperature, diluted with 5 ml of glacial acetic acid. The estimation of cholate in blood was carried out by two modifications of Josephson's method (*Biochem. J.*, **1935**, **29**, 1519). The first, used for routine estimations, is as follows. Into a 250-ml volumetric flask containing 50 ml of absolute alcohol, add 5 ml of a saturated soln. of barium hydroxide containing 1 g of barium acetate per 100 ml. Then add with shaking exactly 10 ml of heparinised whole blood or plasma, and immerse the flask in boiling water for 5 min. Add abs. alcohol almost to the mark and leave at room temp. for 12 hr. Finally, dilute to the mark and filter. Add a drop of conc. sulphuric acid to the filtrate to remove excess barium and make alkaline to litmus again by addition of 2 to 3 drops of conc. sodium hydroxide soln. Leave for several hr., filter, and evaporate exactly 200 ml of the filtrate to about 10 ml under reduced pressure at below 35° C. Filter and wash with abs. alcohol and distil to dryness under reduced pressure at below 35° C. Dissolve the residue in 3 ml of 0·1 *N* sodium hydroxide, add 10 ml of peroxide-free ether, shake and allow to separate. Repeat the extraction three times and extract the combined ethereal solns. with 1 ml of water made alkaline with a few drops of dil. sodium hydroxide soln. Add this washing to the aqueous soln. and remove dissolved ether by warming. Cool, neutralise to litmus, and adjust to the 5-ml mark. One ml of this soln. is used for colour development, as described above. The second modification of Josephson's method is more accurate, giving greater specificity. The procedure is carried through as described above, except that the final soln. is not neutralised or diluted to 5 ml. Instead, the soln. is transferred to a Pyrex test-tube constricted near the neck, and ethyl alcohol and solid sodium hydroxide are added to give concns. of 40 and 12% respectively. Seal the tube and heat in an autoclave at 115° to 120° C. for 10 hr. to hydrolyse the conjugated bile salts. Remove the contents of the tube, transfer to a flask, and evaporate to small vol. under reduced pressure. Transfer the residue to a separating funnel, and acidify to *p*H 1 with sulphuric acid, cooling the funnel in a bath of ice water. Extract with five 15-ml portions of peroxide-free ether, discard the aqueous phase and wash the combined ethereal extracts once with a 5-ml portion of 0·1 *N* sulphuric acid. Discard the washing. Extract the ether with four 5-ml portions of ether-saturated 6·7 *N* hydrochloric acid, and then adjust the combined acid extracts to *p*H 1 with 20% sodium hydroxide, and extract with five 15-ml portions of peroxide-free ether. Wash the combined ethereal extracts once with 5 ml of 0·1 *N* sulphuric acid and discard the aqueous phases. Make the extract slightly alkaline and evaporate under reduced pressure. Dissolve the ether-free residue in water, make alkaline to litmus with sodium hydroxide soln. and dilute to 5 ml. Estimate cholate in 1-ml samples of this soln., as described above. With pure cholate solns. recoveries of 99·1 ± 0·7% can be obtained, and with bile 96 ± 2%. The average errors of single determinations were ± 1·5% for pure solns., and ± 2·5% for bile. The shorter extraction procedure for blood gave recoveries of 92 ± 5% of the theoretical for concns. of 15 to 20 mg per 100 ml, and 85 ± 6% for concns. of 5 to 10 mg per 100 ml. F. A. R.

Determination of Urinary Amino Nitrogen by the Copper Method. A. A. Albanese and V. Irby (*J. Biol. Chem.*, 1944, **153**, 583–588)—By modifying the method of Pope and Stevens (*Biochem. J.*, 1939, **33**, 1070), free amino acids can be estimated in urine; the presence of ammonia and urea does not interfere. Collect the urine in bottles containing 50 ml of 15% hydrochloric acid (by vol.) and 1 ml of 10% alcoholic thymol, and dilute to 2 litres. To 15 ml, containing at least 1 mg of amino-nitrogen, add 4 drops of thymolphthalein indicator (0·25 g in 100 ml of 50% alcohol) and *N* sodium hydroxide soln. until the colour is faint green or blue. Add 30 ml of a copper phosphate suspension obtained by mixing 1 vol. of 2·73% $CuCl_2 2H_2O$ soln., 2 vols. of 6·45% Na_3PO_4 soln., and 2 vols of borate buffer (dissolve 57·21 g of sodium borate in 1·5 litres of water, add 100 ml of *N* hydrochloric acid, and dilute to 2 litres), dilute to the mark, mix, and, after standing for 5 min., filter into 125-ml flasks. Measure the copper content of two 10-ml aliquot portions of this filtrate iodimetrically as follows. Acidify each portion with 0·5 ml of glacial acetic acid, add 1 ml of potassium iodide soln. (1 g per ml), and titrate with standard 0·01 *N* sodium thiosulphate (prepared from a 0·1 *N* soln., made by dissolving 49·6 g of thiosulphate in 200 ml of water, adding 20 ml of amyl alcohol and diluting to 2 litres) from a micro-burette, adding 6 drops of starch indicator as the end-point is approached (1 ml of 0·01 *N* thiosulphate ≡ 0·28 mg of amino nitrogen). The recovery of amino nitrogen when various amino acids were added to urine was practically theoretical.

F. A. R.

Estimation of Nicotinamide Methochloride in Urine. R. A. Coulson, P. Ellinger and M. Holden (*Biochem. J.*, 1944, **38**, 150–154)—It has been shown that the fluorescent substance "F_2," said to be excreted after ingestion of nicotinamide and related compounds, is only formed after treatment with alkali and transference into *iso*-butanol, and that the substance actually excreted is the non-fluorescent nicotinamide methochloride. A method of estimating this substance has been developed which involves its separation from urine by adsorption and elution, and transformation into fluorescent substances by treatment with alkali. Dilute not more than 5 ml of urine to 10 ml, filter through a small column of Decalso (1 g), and wash with 50 ml of water. Elute the column with 14 ml of 25% potassium chloride soln., and divide the eluate into two equal portions. Add to each 2 ml of non-fluorescent *iso*butanol, and to one of the tubes 1 ml of 15% sodium hydroxide soln. Shake for 5 min. and, after separation, transfer the *iso*-butanol layers into identical non-fluorescent test tubes each containing 100 mg of anhydrous sodium sulphate. Compare the fluorescence intensity of the tube containing the alkali-treated solution with that of standards, using the other tube as a blank. Prepare the standards by passing 10-ml portions of solutions containing 1 to 12μg per ml of crystalline nicotinamide methochloride through 1-g Decalso columns, wash and elute with 14 ml of 25% potassium chloride soln., and treat the eluates as described above. Compare the fluorescence intensities visually in the filtered 366$m\mu$ line of a mercury vapour lamp. Accurate estimations are possible for concns. greater than 0·6μg per ml. Between 0·6 and 1·0μg per ml the error is about 10%, and above 1·0μg per ml it is about 5%.

F. A. R.

Quantitative Micro-method for the Separation of Inorganic Arsenite from Arsenate in Blood and Urine. T. B. B. Crawford and I. D. E. Storey (*Biochem. J.*, 1944, **38**, 195–198)—It has been found that, although arsenite can be extracted from acid soln. with sodium ethyl xanthate dissolved in carbon tetrachloride, arsenates do not react with the xanthate agent to form a carbon tetrachloride-soluble product. Arsenite can be separated quantitatively from arsenate in blood and urine by extraction with this reagent. Dissolve 1 g of sodium hydroxide pellets in 10 ml of pure dry ethanol by warming, shake vigorously for about 15 min., centrifuge, and decant the supernatant liquid. Determine the alkali content by titration and adjust to a concn. of 7%. To 9 vols. of the soln., add slowly, with cooling, one vol. of redistilled carbon disulphide and, after mixing and filtering, store in the ice-box. To prepare the xanthate reagent, dilute 50 ml of the stock soln. (which keeps for a fortnight) to 1 litre with dry redistilled carbon tetrachloride. Transfer not more than 80 ml of the arsenic-containing soln. to a 150-ml separating funnel, and acidify with 10 ml of conc. sulphuric acid diluted with 2–3 vols. of water. Shake vigorously for 2 min. with one 40-ml and two 20-ml portions of the xanthate reagent, rinsing the stem of the funnel with 5 ml of carbon tetrachloride after each extraction. Wash the combined extracts with 10 ml of 2 *N* sulphuric acid and evaporate after addition of water, adding the washing to the aqueous phase. Determine the total arsenic (Levvy, *Biochem. J.*, 1943, **37**, 598) on the evaporated extract (arsenite fraction) and on the aqueous phase (arsenate fraction). Carry blank solns. through the same procedure and apply corrections for the arsenic present in the reagents. Recoveries of arsenite added to blood haemolysates ranged from 81 to 87%, and of added arsenate from 87 to 104%. The corresponding values for urine dialysates were 86 to 104%, and 98 to 111%, respectively.

F. A. R.

New Coupling Component and Simplified Method for the Estimation of Sulphanilamide Drugs. F. L. Rose and H. G. L. Bevan (*Biochem. J.*, 1944, **38**, 116)—The use of a new coupling component, N-β-sulphatoethyl-*m*-toluidine ($HO_3SOC_2H_4NH.C_6H_4.CH_3$) is suggested in place of N-(1-naphthyl)ethylenediamine dihydrochloride, proposed by Bratton and Marshall (*J. Biol. Chem.*, 1939, **128**, 537). The advantage of the new compound is that it gives water-soluble colours with sparingly soluble drugs; in addition, it gives no appreciable colour with nitrous acid, so that treatment with sulphamate to remove excess of the latter is unnecessary. Mix 0·2 ml of blood with 2·8 ml of 0·05% saponin soln. and 1 ml of 15% trichloroacetic acid, centrifuge immediately for 10 min. and transfer the supernatant liquid to a second tube, washing out the first tube with 0·8 ml of 5% trichloroacetic acid. Add 1 ml of 0·1% sodium nitrite soln. and, after 2 min., add 2 ml of 1% N-β-sulphatoethyl-*m*-toluidine soln. (stored in an amber bottle and made freshly each month). Compare the red dye in a visual colorimeter or photoelectric absorptiometer with standards prepared from 0·2 ml of standard solns. containing 5, 10 and 20 mg/100 ml of the test drug. For the estimation of total drug, treat the blood sample as before and transfer to the second tube, note the level of the liquid, and add 1 ml of *N* hydrochloric acid. Heat in a boiling water-bath for 45 min.,

cool, add 0·4 ml of 2·5 *N* sodium hydroxide, dilute to the mark and develop the colour as before. For blood samples of 0·02 to 0·03 ml use one-quarter of the above quantities and adjust the final vol. to 2 ml. The method can generally be applied directly to cerebrospinal fluid and diluted urine. F. A. R.

Urinary 17-Ketosteroids in Metabolism. 1. Standardised Chemical Estimation. R. L. Cahen and W. T. Salter (*J. Biol. Chem.*, 1944, **152**, 489–499)—The methods of Pincus (*Endocrinol.*, 1943, **32**, 176; *J. Clin. Endocrinol.*, 1943, **3**, 195) and Zimmerman (*Z. Physiol. Chem.*, 1935, **233**, 257; 1936, **245**, 47) were compared, and the conditions of the Zimmerman procedure were standardised so that a given concn. of any of the chief 17-ketosteroids would always develop the same degree of colour. In addition, the method was modified so that extraneous pigments and undesirable chromogens were largely eliminated. The improved method comprises four stages. (1) *Hydrolysis of conjugated urinary steroids*—This was carried out as described by Holtorff and Koch (*J. Biol. Chem.*, 1940, **135**, 377; Abst., ANALYST, 1941, **66**, 78). Heat under reflux 250 ml of a 24-hr. sample of the urine with 25 ml of 12 *N* hydrochloric acid for 10 min. and then cool immediately. (2) *Extraction and separation of androgenic 17-ketosteroids*—Extract the hydrolysed urine with three 80-ml portions of ether, remove the acid fraction from the extract by shaking with 80 ml of saturated sodium bicarbonate soln. and then the phenolic fraction by shaking with two 80-ml portions of 15% potassium hydroxide soln. Finally wash the ethereal extract with 80 ml of water, and evaporate to dryness, first on the steam-bath *in vacuo* and then in a desiccator. Transfer the residues quantitatively with 96% alcohol to graduated flasks and then to 20-ml storage bottles; store in the refrigerator until required. (3) *Colorimetric assay*—The method of Pincus and Pearlman (*Endocrinol.*, 1941, **29**, 413) was used, except that the mixture was incubated for 60 instead of 45 min. and the reaction mixture was kept in the dark at 20° C. Prior to the incubation with acetic acid in the dark the soln. is heated with antimony trichloride soln. for 20 min. on the boiling water-bath. Three modifications of the Zimmerman method, were used: first, the procedure of Callow *et al.* (*Biochem. J.*, 1938, **32**, 1312) as modified by Friedgood and Whidden (*Endocrinology*, 1939, **25**, 919; 1941, **28**, 237) in which 2 *N* aqueous potassium hydroxide soln. is used to develop the colour; secondly the method of Holtorff and Koch (*loc. cit.*), in which 5 *N* potassium hydroxide soln. was used; thirdly, measurement of the colour after extraction into 0·5 vol. of chloroform. Cis(β-) and trans (α-) urinary 17-ketosteroids were separated by the method of Baumann and Metzger (*Endocrinology*, 1940, **27**, 664) and each fraction, together with the total 17-ketosteroids, was measured colorimetrically by the Pincus method, by the original Zimmerman method, and by the Zimmerman method using a Klett-Summerson colorimeter with a Corning filter 52. The results obtained for the three methods agreed fairly well with one another, although the original Zimmerman method gave slightly higher results than the modified method or the Pincus method. The recoveries of added androsterone and dehydroandrosterone ranged from 92 to 98% of the theoretical. On the whole, the Pincus reagent was preferred, because the absorption maximum of the green compound formed differs greatly from that of the contaminating red and brown pigments; thus, the method avoids the necessity for a colour correction. F. A. R.

Human-milk Fat. I. Component Fatty Acids. T. P. Hilditch and M. L. Meara (*Biochem. J.*, 1944, **38**, 29–34)—Human milk fat, examined by methods previously described, was found to contain the following fatty acids, expressed as weight and molar percentages respectively:

Acid	Weight, %	Molar, %
Decanoic	0·5– 2·7	0·8– 4·2
Lauric	5·1– 7·0	6·7– 9·0
Myristic	7·6–13·9	8·7–15·8
Palmitic	22·4–24·6	22·8–25·2
Stearic	7·3– 9·6	6·7– 8·8
As Arachidic	0·9– 1·8	0·8– 1·5
As Decenoic	trace	trace
As Dodecenoic ..	0·1	0·2
As Tetradecenoic ..	0·4– 1·3	0·5– 1·5
As Hexadecenoic ..	2·8– 3·7	2·9– 3·8
Oleic	30·2–36·6	27·6–33·9
Octadecadienoic ..	5·5– 8·2	5·1– 7·7
Unsaturated C_{20-22} ..	2·9– 5·7	2·4– 4·7

The acids of human milk fat therefore differ from those of cow milk fat in containing none lower in the series than decanoic acid, and in containing more diethenoid C_{18} acids and unsaturated C_{20-22} acids. Human milk fat resembles a typical margarine fat blend rather than butter fat. F. A. R.

Iodimetric and Colorimetric Methods for the Estimation of Calcium in Serum based on the use of an improved Permanganate Solution. J. A. De Loureiro and G. J. Janz (*Biochem. J.*, 1944, **38**, 16–19)—In the estimation of calcium by the method of Clark and Collip (*J. Biol. Chem.*, 1921, **49**, 487), the final permanganate titration suffers from certain disadvantages, namely, the inconvenience of keeping solns. at 80° C., variations due to differences in speed of titration, and uncertainty of the end-point. To overcome these disadvantages, a modified permanganate reagent has been devised which reacts with oxalic acid at room temperature. The reagent also reacts stoichiometrically with potassium iodide, so that it is possible to titrate the excess permanganate iodimetrically. Alternatively, the excess of permanganate can be measured colorimetrically. To prepare the reagent (approx. 0·1 *N*), dissolve 3·5 g of potassium permanganate in 1 litre of a soln. containing 50 g of $MnSO_4.4H_2O$ and 350 ml of conc. sulphuric acid. The reagent has a characteristic raspberry-red colour and is fairly stable when stored at 0° C.; at room temp. it slowly deposits manganese dioxide, but the supernatant fluid remains perfectly clear, and filtration is therefore unnecessary. To prepare more dilute solns. dilute the 0·1 *N* soln. with the manganese sulphate soln. and not with water. To standardise the soln., add 1 ml of 0·01 *N* sodium oxalate (dissolve 0·335 g in 1 litre of 35% sulphuric acid) to 2 ml of the permanganate soln. and after 30 min. at room temp. dilute with about 50 ml of water and add 3–5 ml of 5% potassium iodide soln.; titrate with 0·01 *N* sodium thiosulphate. Subtract from the titre the vol. of thiosulphate required to titrate a mixture of 2 ml of the permanganate soln., 50 ml of water, and 3–5 ml of potassium iodide soln. One ml of 0·01 *N* thiosulphate ≡ 0·2 mg of calcium. For the titration of calcium oxalate precipitates from serum, dissolve the ppt. in a small drop of 50% nitric acid, add 2 ml of 0·01 *N*

potassium permanganate, and after 30 min. dilute with water and titrate as in the standardisation. For the colorimetric method, 0·002 *N* potassium permanganate is used, and its colour density (L) is measured in an Evelyn photoelectric colorimeter with a green filter transmitting at 520$m\mu$. Dissolve the calcium oxalate ppt. in a drop of 50% nitric acid, add exactly 10 ml of 0·002 *N* potassium permanganate, and, after 30 min. read the colour density (l). The amount of calcium (mg per 100 ml) $= K(L-l)$, where K is a constant depending on the construction of the apparatus. Determine the value of K by partially reducing a standard permanganate soln. by means of a standard reductant, preferably sodium oxalate, though iodide-thiosulphate and ferrous ammonium sulphate give approximately the same results. To 5 ml of 0·002 *N* sodium oxalate in 35% sulphuric acid add 5 ml of 0·004 *N* potassium permanganate and measure the colour density (l^1) in an Evelyn photoelectric colorimeter together with that of 0·002 *N* potassium permanganate (L^1). The difference between these two readings represents the loss of colour due to the action of 10 ml of a 0·001 *N* soln. of standard reductant, so that $K = \frac{10^{-3}}{L^1-l^1}$. The iodimetric method is more accurate than the colorimetric, but the latter is more convenient when a large number of estimations are to be carried out. In the estimation of calcium in serum, the probable error of the latter method is ±1·6%, and that of the former ±0·8%. F. A. R.

Separation of Carotene from Vitamin A for the Determination of Vitamin A in Blood Plasma. P. D. Boyer, P. H. Phillips and J. K. Smith (*J. Biol. Chem.*, 1944, **152**, 445–452)—Since carotene interferes with the estimation of vitamin A by the Carr–Price reaction, it must be removed before satisfactory values for the vitamin A content of cattle blood can be obtained. Phase separation between light petroleum and ethyl alcohol is not satisfactory, but a method based on the differential solubilities of carotene and vitamin A in 50–60% ethyl alcohol gave good results. Add 6 ml of 0·05% sodium chloride soln., with shaking, to 8·0 ml of an abs. ethyl alcohol soln. of the sample in a 50-ml test-tube; leave for 30 min. in a refrigerator, filter and wash the test-tube and filter with 10 ml of a mixture of 4 parts of abs. ethyl alcohol and 3 parts of the sodium chloride soln. Carotene is removed by this procedure, whilst vitamin A and part of the xanthophyll remain in the filtrate; 97% or more of the carotene was removed, and recoveries of 97–99% of the vitamin A were obtained from mixtures of carotene and vitamin A in which the concn. of the former was ten times that of the latter. Before applying the procedure to fish liver oils or concentrates prepared by distillation these must first be saponified. The procedure used for blood analysis is as follows. To 10·0 ml of plasma in a 50-ml test-tube, add 10 ml of 95% ethyl alcohol and 24·0 ml of light petroleum. Stopper the tube, seal with mineral oil and shake gently for 10 min., leave in the refrigerator for 1 hr. to separate and then, according to the amount of carotene present, either use 10 ml of the petroleum extract or dilute a portion (1–4 ml) with light petroleum and estimate the carotene content. Transfer a 20·0 portion of the extract, adding if necessary some of the undiluted soln. used for the carotene estimation, to a 75-ml Pyrex test tube and remove the petroleum *in vacuo*, finally warming to 70° C. Cool the tube, release the vacuum, and dissolve the residue immediately in 8·0 ml of abs. ethyl alcohol. (If saponification is necessary, dissolve the residue in 10 ml of alcoholic 20% potassium hydroxide soln. instead of abs. alcohol, heat for 15–20 min. in a boiling water-bath, add an equal vol. of water and extract with four 5-ml portions of ether. Wash the extracts twice with water and again after addition of 5 ml of light petroleum. Transfer the soln. to a 75-ml Pyrex test-tube, evaporate to dryness, and dissolve the residue in 8·0 ml of abs. ethyl alcohol.) Precipitate the carotene and remove by filtration as described above, dilute the filtrate with water so that the alcohol conc. is *ca.* 40%, and then extract by shaking thoroughly for 10 min. with 13·0 ml of light petroleum. Leave the mixture in the refrigerator and transfer 10 ml of the petroleum layer to an Evelyn colorimeter tube. Measure the colour with a No. 440 filter and estimate the xanthophyll concn. from a standard curve. Remove the light petroleum *in vacuo* as before, dissolve the residue in 1·0 ml of chloroform and estimate the vitamin A by the Carr–Price reaction. Recoveries of added vitamin A alcohol were 95–97% of the theoretical, but the results obtained with milk were not satisfactory, only an 85% recovery being obtained. F. A. R.

Colorimetric Determination of Nicotinic Acid. A. E. Teeri and S. R. Shimer (*J. Biol. Chem.*, 1944, **153**, 307–311)—Difficulties are frequently encountered in determining nicotinic acid by reaction with cyanogen bromide and a primary or secondary aromatic amine, owing to the necessity of waiting for the colour to develop, to the instability and low intensity of the colour, and to turbidity or colour in the blanks. The use of *m*-phenylenediamine and 20% hydrochloric acid overcomes most of these difficulties. To 10 ml of a soln., containing not more than 60μg of nicotinic acid, add 5 ml of buffer soln. *p*H 6·6, and 5 ml of 4% cyanogen bromide soln. Leave for 20 min. and add 1 ml of 5% *m*-phenylenediamine dihydrochloride soln. followed immediately by 1 ml of 20% hydrochloric acid. Measure the colour within 15 min. in a photoelectric colorimeter with filter 400$m\mu$. Calculate the results in the usual way. To overcome the difficulties due to coloured or turbid solns., it is necessary to measure two blanks. First, determine the sample blank by measuring the colour of a mixture of 10 ml of the extract, 5 ml of buffer soln., 6 ml of water, and 1 ml of 20% hydrochloric acid, and then measure in the same way the reagent blank consisting of 16 ml of water, 5 ml of cyanogen bromide soln., and 1 ml of the *m*-phenylenediamine soln. The latter blank is constant and need be determined only once for a series of assays. Set the instrument at zero against distilled water and then subtract the sum of the readings of the two blanks from that of the unknown. When this procedure was used, the recovery of added nicotinic acid was 97 to 104% of the theoretical. F. A. R.

Bacteriological

Direct Count of Micro-organisms in Citrus Products [and Powdered Pectin]. J. W. Stevens and T. C. Manchester (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 302–307)—Since citrus products are sterilised during manufacture, direct microscopical counts must replace plate methods to include the dead organisms that indicate low

quality fruit or insanitary manufacturing conditions. To the sample (1 ml or its weighed equivalent) in a 10- or 25-ml graduated cylinder add from 1 to 11 ml of water (according to the dilution required) and an equal vol. of a freshly prepared 0·5% soln. of gelatin known to be practically free from micro-organisms. Stopper the cylinder and mix the contents by inverting at least 25 times. Transfer two 0·1-ml portions of the soln. immedately to appropriate squares of a slide with etched 1-cm squares and spread the samples evenly with a platinum needle. Dry the film at room temp., heat gently by passing three times through a small flame, fix the film by flooding with formalin and allowing it to stand for 1·5 min. Pour off the excess of formalin, allow the film to become almost dry, wash gently with 35% alcohol and then with water and dry at room temp. Flood the film with methylene blue soln. (prepared by adding excess of the dye to 1 part of 95% alcohol and 1·5 part of water and filtering the soln. after it has stood overnight) and apply gentle heat occasionally during 2 min. Remove excess of the stain and wash the film by dipping the slide in 2 or more portions of water and dry at room temp. Examine the films with an oil immersion objective (*ca.* 95×) and an 8 or 10× eye-piece fitted with a Breed and Brew ocular micrometer disc. Standardise the microscope as described in the Breed method (*Science*, 1943, **98**, 204). The number of fields to be counted depends upon the number of organisms per field and the precision required. If the first few fields show less than 50 organisms of any one class (yeast, mould spores, mould mycelia or bacteria) it will be necessary to count this class in 50 fields (25 on each film) for a probable error of 10%. When the number per field is greater than 50, the counting of 25 fields will suffice to maintain the same probable error. A count of more than 100 fields divided between the two films is seldom necessary, regardless of how few organisms are present. The fields should be so distributed that uniform coverage of each film is attained. Count each type of organism separately for each field and bacteria only within the inner circle of the micrometer disc where definition is best. Determine the mean count per field for each class on the first film and the corresponding figure on the second film and average the two mean values for each class. No. of organisms per ml = (fields per sq.cm. × 100) × average count per field × dilution.

To test powdered pectin, weigh 2 g rapidly in a sterile beaker, transfer quickly to a sterile 250-ml wide-mouthed glass-stoppered bottle, tilting and tapping the bottle so that the pectin collects on one side of the bottom. Tilt the bottle in the opposite direction and add 7 ml of sterile 60° Brix simple syrup from a pipette so that it does not come into contact with the pectin. Mix the syrup and pectin with a sterile glass rod until a smooth suspension is formed, quickly add 92 ml of sterile water and shake until dispersion of the pectin is complete. Alternatively, disperse a mixture of 2 g of pectin and *ca.* 12 g of 4–6 mesh sterile sand in 99 ml of sterile water by stirring with a sterile glass rod. For the direct total count mix the 2% pectin sol with 2 parts of 0·25% gelatin soln. and 1 part of water and prepare stained films as already described. This procedure does not show yeast and bacteria spores because of the difficulty of staining these bodies. Neisser's spore-staining procedure, as described by Tanner ("*Bacteriology and Mycology of Food*," 1st Ed., p. 85; John Wiley & Sons, Inc., New York, 1919), was found satisfactory. Stain the films with aniline water and fuchsin, partly decolorise with hydrochloric · acid, and finally counterstain with methylene blue as previously described. The spores are stained red and the vegetative cells blue, so that total counts and spore counts may be made on the same film. Results are given for a number of citrus juice products and commercial pectins but, as yet, the data are insufficient to indicate a criterion from the sanitary standpoint. The method appears to be adaptable to fruit products other than citrus. A. O. J.

Method for the Assay of Penicillin. N. G. Heatley (*Biochem. J.*, 1944, **38**, 61–65)—Make up the following medium with tap water: Lemco meat extract, 1%; bacteriological peptone, 1%; sodium chloride, 0·5%, agar 2%, and add 50 ml of *M*/1·5 phosphate buffer *p*H 6·8 per litre and phenol red to a final concn. of 0·0025%. Sterilise at 15 lbs. per sq. in. for 20 min. and pour 20-ml quantities into 4 in. Petri dishes. Cool and seed the plates with a 16–24 hr. broth culture of *Staphylococcus aureus* (N.C.T.C. 6571). Tilt and shake to cover the surface of the agar with the culture, draw off the surplus, and mark the edge where the culture is drawn off. Dry in a 37° C. incubator for 1–2 hr. with the lid lifted about ½ in. above the bottom of the dish, and then place on the surface of the agar a number of small glass or vitreous porcelain cylinders, 9·6 mm high and 5·1 and 7·2 mm. internal and external diameter respectively. One end of these cylinders is bevelled internally and the sharp edge is ground practically plane. The cylinders are dry-sterilised in a Petri dish and momentarily flamed before being put on to the agar. Adjust the soln. to be tested so that the *p*H is between 5 and 8, and dilute to a concn. between 0·5 and 2·0 Oxford units/ml with 0·02 *M* phosphate buffer of *p*H 7. Pour the solns., which need not be sterilised, into the cylinders, incubate the plates at 37° C. for 16–24 hr. and measure the diameters of the zones of inhibition either by means of a transparent scale or with calipers. To obtain the best results, a standard soln. of penicillin containing 2, 1 and 0·5 units/ml should be tested with each batch of unknowns. The results are calculated from the curve obtained by plotting the diameters given by the standard solns. against their potencies. It is important that the cylinders should be arranged on the plates in a particular way, as the cylinders near the point at which the plate has been drained tend to give low results. In a quadruplicate assay, it is best to arrange the samples in the same order on all four plates, but on each successive plate the series is begun 90° farther round relative to the side from which surplus culture was drained. If 9% is taken as the coefficient of variation, then for $P = 0{\cdot}01$ the limits of accuracy for a single assay in quadruplicate are ±23·2%, for an assay in triplicate ±26·9% and for an assay derived from only two measurements ±36·7%. The corresponding figures for $P = 0{\cdot}05$ are ±19·5, 22·7 and 27·7% respectively. F. A. R.

Improved Short Time Turbidimetric Assay for Penicillin. J. R. McMahan (*J. Biol. Chem.*, 1944, **153**, 249–258)—Put a number of small glass test tubes, 4 in. by ⅝ in., into suitable racks, cover with inverted stainless steel trays ½ in. deep to keep out dust contamination, and dry sterilise at 170° C. for 1½ hr. Put amounts ranging from 0 to 0·12 ml of a standard solution of penicillin containing 5 Oxford units per ml into one set of tubes, and similar vols. of the solution to be tested into another

set of tubes, using a micro-pipette. Prepare a basal medium containing 16 g per litre of Difco-Bacto nutrient broth and 4 g per litre of yeast extract and sterilise at 15 lb. for 15 to 20 min. Add 65 ml per litre of a 14–18 hr. culture of *Staphylococcus aureus* Strain H, grown on a medium containing 8 g of Difco-Bacto dehydrated powder per litre, put 7 ml of the inoculated basal medium into each tube and incubate at 37° C. for 3 hr. Steam for 10 min. and measure the turbidities with a Lumetron No. 402 E colorimeter, using a 530$m\mu$ filter. Calculate the results from the calibration curve obtained with the standard solutions. The precision of the test is said to be somewhat better than that of the Oxford plate method (*cf.* preceding abstract), and it is considerably more rapid.

F. A. R.

Water

Concentrations of certain Trace Metals in Drinking Waters. R. A. Kehoe, J. Cholak and E. J. Largent (*J. Amer. Water Works Assoc.*, 1944, **36**, 637–644)—Drinking waters from some eastern and central states of the U.S.A. were analysed to determine the degree of conformity with recent limits for inorganic elements (*cf.* ANALYST, 1944, **69**, 186). Spectrographic methods were used to determine manganese, lead, tin, iron, copper, and silver, polarographic methods for arsenic and zinc, and Hubbard's method (*Ind. Eng. Chem. Anal. Edn.*, 1940, **12**, 768) for mercury. A distillation method was used for isolation of fluorine. None of the waters contained concns. of lead, copper, zinc, or arsenic as high as the respective limits. Several exceeded the combined limit for iron and manganese (0·3 p.p.m.), owing, probably, to local corrosion in iron pipes. Concns. of fluorine > 1·0 p.p.m. were found only in areas where dental fluorosis was known to be endemic. No increase in the concn. of iron or aluminium results from the use of salts of these elements in water treatment. Dangerous concns. of lead, copper, or zinc were not found in any instance, although samples were obtained from systems using copper, brass, lead, and galvanised pipes. Tin, mercury, and arsenic were found in only a few samples, and silver was either absent or present in a concn. < 0·0005 p.p.m., the limit of the analytical method employed.

D. D.

Determination of Carbon Dioxide in Water. D. S. McKinney and A. M. Amorosi (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 315–316)—An adaptation of Partridge and Schroeder's evolution method (A.S.T.M., Part III, 1942) is described. The sample is titrated between the end-points at *p*H 8·5 (9 when phosphates are present) and *p*H 5, acidified, boiled to expel carbon dioxide, cooled and re-titrated between the same two end-points. This double titration eliminates the effect of interfering substances, the difference between the two readings giving the amount of carbon dioxide present. Samples should be analysed promptly and transferred from one vessel to another by siphoning. A precision of ±1 p.p.m. is obtainable. *Reagents*—*Mixed indicator*: 0·1 g of methyl red and 0·1 g of *o*-cresolphthalein (or phenolphthalein) in 200 ml of 50% alcohol. *Buffer solns.*: for *p*H 8·5 (or 9·0) and *p*H 5. *Hydrochloric acid*: 0·02 *N*. *Sodium hydroxide*: approx. 0·02 *N*, carbonate-free, stored in waxed bottles fitted with soda-lime traps. *Method*—Add 0·4 ml of mixed indicator to 100 ml of *p*H 8·5 and *p*H 5 buffer solns. in 250-ml stoppered flasks. Add the same amount of indicator to 100 ml (V_3) of the sample to be tested and titrate to *p*H 8·5, using acid or alkali as required and matching the buffered soln. If hydroxide is added record the vol. (V_{1Z} ml). Add 0·02 *N* acid to *p*H 5 (V_1 ml). Add 20% excess of acid (not less than 5 drops), boil strongly for 2 min., cool rapidly to room temp., add alkali to *p*H 8·5 (V_{2Z} ml), and titrate with acid to *p*H 5 (V_2 ml). Make the following correction for carbon dioxide in the sodium hydroxide soln. Titrate 80 ml of carbon-dioxide-free water to *p*H 8·5 with sodium hydroxide soln., then add 0·02 *N* acid to *p*H 5 (A ml). Add immediately 20 ml of sodium hydroxide soln. and again note the vol. of acid required to change the *p*H from 8·5 to 5 (B ml). If X ml is the amount of acid required to remove carbon dioxide in 1 ml of the sodium hydroxide soln. X = (B−A)/20. The amount of carbon dioxide in the sample is given by the equation:

$$CO_2(\text{in p.p.m.}) = 1000.K.N[(V_1-V_1\,X)-(V_2-V_{2Z}X)]/V_3$$

where K = 45·56 for the *p*H 8·5 to 5 or 43·95 for the *p*H 9 to 5 titration, *N* = normality of the acid. Large amounts of sulphite or sulphide destroy the indicators though small amounts of sulphite do not interfere. A correction, for which the original paper should be consulted, can be made for small amounts of sulphide.

C. F. P.

The Determination of Soluble Phosphate and Silica in Water with the Spekker Absorptiometer. T. S. Harrison and H. Storr (*J. Soc. Chem. Ind.*, 1944, **63**, 154–157)—Controlled reductions of phosphomolybdate and silicomolybdate by quinol to molybdenum blue are used for the determination of phosphate and silicate, each in absence of the other, in boiler water. Modifications of acid concn. can be made to give conditions in which either radical may be determined in presence of the other. (i) *Procedure for phosphate in absence of silica*—Filter a suitable portion of the sample and, if necessary, neutralise the filtrate by the calculated addition of *N* sulphuric acid. To a convenient fraction add the following reagents in order, mixing thoroughly after each addition: 2 ml of ammonium molybdate soln. (5% in *N* sulphuric acid), 2 ml of quinol soln. (5% in water containing 1 ml of *N* sulphuric acid) and 3 ml of sodium sulphite soln. (freshly prepared, 20% in water). Make up the blue soln. to 50 ml and fill a 4-cm cell for the absorptiometer measurement, using Ilford red filters (No. 608). Convert the reading to PO_4''', using a graph obtained with standard solns. of AnalaR sodium phosphate (1 part PO_4''' per 100,000) treated exactly as the sample. (ii) *Procedure for silica in absence of phosphate*—Proceed as in (i) but read "silica" for phosphate and ensure that (*a*) at least 2 min. is allowed for the development of the silicomolybdate prior to its reduction, and (*b*) each coloured liquid is set aside for at least 15 min. before making the absorptiometer measurement. Calibrate by using a standard silica soln. (1 part SiO_2 per 100,000) prepared by fusing pure silica with sodium carbonate in a platinum crucible, dissolving in water, adjusting to neutrality with *N*-sulphuric acid and making up to volume. (iii) *Procedure for phosphate in presence of silica*—As in (i), but add 7 ml of *N* sulphuric acid prior to the molybdate to prevent interference from silica, add 7 ml of *N* caustic soda immediately after the sulphite to intensify the colour. Calibrate, using a graph prepared from standard solns. treated exactly

as the sample. (iv) *Procedure for silica in presence of phosphate*—As in (ii), but add 10 ml of 6 *N* sulphuric acid after the molybdate to prevent interference from phosphate, omit the sulphite, take readings after a 20 min. interval and interpret from a similarly prepared calibration graph. B.S.C.

Agricultural

Modification of the Picric Acid Method for Determination of Hydrocyanic Acid in White Clover Plants. J. T. Sullivan (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 320–325)—White clover plants may be grouped into 4 classes—I, plants containing both glucoside and enzyme; II, plants containing the glucoside but not the enzyme; III, plants containing the enzyme but not the glucoside; IV, plants containing neither glucoside nor enzyme (Corkill, *New Zealand J. Sci. Tech.*, 1940, **22B**, 65). The glucoside has been found to be a mixture of linamarin and lotoaustralin (Melville and Doak, *Id.*, 1940, **22B**, 67) and shown to be hydrolysed by linamarase prepared either from linseed or from the clover plant (Coop, *Id.*, 1940, **22B**, 71). Expts. made with classes I and II to establish the conditions under which all the hydrocyanic acid in the glucoside can be determined led to the following method. Grind 10 g of fresh clover leaves with water in a Waring Blendor or with sand in a mortar. To the liquid and leaf fragments in a flask add 1 ml of linamarase soln. (Coop, *loc. cit.*) and a few ml of toluene, stopper the flask tightly and incubate at room temp. for at least 1 day. Remove the stopper and immediately steam distil 80–90 ml, receiving the distillate in a beaker containing 5 ml of 2% potassium hydroxide soln. and keeping the tip of the condenser below the surface of the liquid for the first half of the distillation. Heat an aliquot portion of the distillate with alkaline picrate and compare the colour change with that of a blank expt. by means of the photometer as previously described (*J. Assoc. Off. Agr. Chem.*, 1939, **22**, 781; ANALYST, 1940, **65**, 122). Takadiastase, which was tried as an alternative to linamarase, sometimes gave a lower yield of hydrocyanic acid. A. O. J.

Effect of Fluorine on Dental Health [of Sheep]. G. F. Boddie (*Lancet*, 1944, **247**, 46)—The effect of chronic fluorine poisoning in sheep is described. It is believed that 1 p.p.m. in drinking water is necessary to prevent dental caries, whereas when more than 4 p.p.m. are present, the teeth will show mottling and pitting (*cf.* Ainsworth, ANALYST, 1934, **59**, 380). In the immediate vicinity of an aluminium factory herbage was found to contain between 44 and 61 p.p.m. of fluorine, whilst running water from a stream contained 0·1 p.p.m. Flocks in the neighbourhood were unhealthy, the sheep became debilitated and lambs were born weakly. Three types of dental lesion were shown; in addition to pitting the teeth were so soft that irregular wear occurred and often sharp edges developed. With such teeth the animal was unable to chew its food properly. The third stage was the development of severe inflammatory changes around the teeth. The jaw bones of these animals had a fluorine content of 0·30–1·25% compared with *ca.* 0·24% in normal sheep. J. A.

Gas Analysis

Gasometric Determination of Nitric Oxide. D. J. Le Roy and E. W. R. Steacie (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 341)—Grind together a pellet of potassium hydroxide and sodium sulphite crystals, which will produce a thick paste. Form the paste into a bead on a loop of platinum wire and dry if necessary, but not too much, as otherwise absorption does not take place. Introduce the bead into the gas mixture. Nitric oxide is completely absorbed as sodium hyponitrososulphate in 5–10 min. W. R. S.

Inorganic

Predictable Concentration of Standard Solutions owing to Evaporation. H. A. Liebhafsky (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 349)—A calculation has been made of the concentration of a volumetric soln. in a closed container when the soln. used is displaced by dry air which becomes saturated at the expense of the soln. before the next withdrawal. If 15 litres of soln. are used in this way until 1·5 litres remain, the concentration from this cause will increase the normality by 0·02%. L. A. D.

Determination of Sulphur in Brass and Bronze by the Combustion Method. A. C. Holler and J. P. Yeager (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 349)—The combustion method of determining sulphur (combustion of the metal sample at high temp. in a stream of oxygen, absorption of the sulphur oxides in a suitable soln. and titration of their equivalent of acid) may be used on brass and bronze. The exit end of the combustion tube is packed for half an inch with ignited asbestos fibre in a position where the asbestos is heated to redness. Chromium sesquioxide, ignited in oxygen at 2,400° F. (1320° C.), is the only suitable bedding material for use in the boats. *Method*—Use 0·1 to 1·0 g. of 20 to 60-mesh sample and burn at 2100° F. (1150° C.) in a 2 litre/min. current of oxygen. Titrate with standard NaOH 5 to 10 min. after beginning the combustion and continue the oxygen flow for 5 min. more to confirm that the reaction is complete. Use bromocresol green as indicator to avoid interference by graphite in the sample. L. A. D.

Determination of Iron with o-Phenanthroline. S. L. Baudemer and P. J. Schaible. (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 317–319)—In the photometric determination of iron in ashed egg yolk and white, sodium citrate is recommended as a buffer instead of sodium acetate. Transfer an aliquot portion of soln., suitable for the range of the photometer, to a 25-ml flask, add 1 ml of 1% hydroquinone soln. and 2 ml of *o*-phenanthroline soln. (0·5 g in 200 ml of water) and 25% citrate soln. in the order named; the first two should be freshly prepared or colourless. The amount of citrate required to produce *p*H 3·5 must be determined by titration, but for serial analyses of similar materials an average vol. of buffer may be used. Adjust the volume to 25 ml, set aside for 30 min. at above 20° C., and take the photometric reading with 1-cm absorption cells and a 12·5 mm No. 430 dark-shade blue-green Corning filter. A blank determination should be run. Calculate the iron concn. by reference to a curve made by means of a standard soln. (1 g of electrolytic iron in 50 ml of 10% sulphuric acid, diluted to 1 litre) submitted to the same treatment. W. R. S.

(1) Determination of Carbon by the Low-pressure Combustion Method. W. M. Murray and S. E. Q. Ashley (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 242–248). **(2) Determination of Carbon in Low-carbon Steel. Precision and Accuracy of the Low-pressure Combustion method. R. W. Gurry and H. Trigg** (*Id.*, 248–250)—The method discussed in both papers is basically that of Wooten and Guldner (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 835). The sample, in a refractory-lined platinum alloy crucible, is heated by high frequency induction in oxygen at 150–200 mm pressure in a closed system. The carbon dioxide formed is frozen in a cold trap, the excess of oxygen is pumped away, and the carbon dioxide is allowed to warm up to room temp. and to expand into a known volume in which its pressure is measured.

(1) The design and construction of the apparatus has been modified in several ways and diagrams of two versions are given. A device has been added, consisting of a tube with many branches from which samples can be moved by means of a magnet into the combustion crucible; several samples may thus be burnt without opening the apparatus to the air. The platinum alloy crucible must be strongly reinforced to have a good life; liners of beryllia are considered the best yet used, magnesia also being good. The operation of the apparatus and calculation of results are described in detail. Several conclusions are drawn from experience with the method, including the following. The precision is about ±0·0005% of carbon on samples usually containing more than 0·004%, and the homogeneity of the sample becomes of major importance. The blank averages about 0·0005% of carbon except on the first run of the day, when it may exceed 0·001%. The sample need not be very finely divided—*e.g.*, clippings of sheet silicon steel weighing 30 mg are used, finer division resulting in contamination from the tools employed. Washing the samples with organic solvents tends to cause high results; pre-heating samples *in vacuo* to remove adsorbed gas causes loss of carbon and low results. Determinations may take 2 hr. or less, and the method is preferred to modifications of older procedures which have been tried, *e.g.*, separation of carbon and carbides by solution of the steel in potassium copper chloride soln. or combustion and absorption of the carbon dioxide in barium hydroxide soln.

(2) The precision and accuracy of the results obtained by the Wooten and Guldner method (with slight modifications to the apparatus and procedure) have been determined. The precision, determined from results on 0·5-g samples of 4 steels of carbon content 0·015–0·026%, is about ±0·0005% of carbon and in favourable circumstances may approach the blank value (about 0·0002%). This is at least 3 times as good as the usual combustion method. Again the importance of a homogeneous sample is stressed. The accuracy of the method has been determined by measuring the carbon dioxide evolved from fragments of Iceland spar and is such that the average deviation of the observed result from the calculated result is equivalent to 0·0007% of carbon on a 0·5-g sample. Curves obtained by plotting the number of gram mols. of gas in the apparatus against the trap temperature indicate that the gas measured is virtually pure carbon dioxide. No sulphur dioxide was detected in an expt. with Bureau of Standards Sample 55a, which contains 0·014% C and 0·02% S (see below). Other observations include the following. The blank is normally determined by heating the empty crucible, and the experimental conditions are not identical with those during a determination, when the burning sample raises the crucible temp. The difference between the determined blank and the "true" blank is, however, believed to be negligible. Samples of sheet are cleaned by abrasion and cut up without handling. Samples prepared by machining are washed at least 3 times with pure acetone in a glass tube with a porous Alundum bottom through which the solvent is removed by suction, and kept overnight under 10^{-3} mm pressure. No significant contamination of the sample by this procedure has been found. Results obtained by the low pressure method in various laboratories, including that of Murray and Ashley (above) give a mean value of 0·0114% C for Bureau of Standards Sample 55a and it is suggested that the accuracy of the certificate value of 0·014% needs reconsideration. L. A. D.

Basic Lanthanum Nitrite. G. R. Sherwood (*J. Amer. Chem. Soc.*, 1944, **66**, 1228–1229)—A stable basic nitrite (molar ratio $La_2O_3 : N_2O_3 = 1$) was obtained by treating cold 0·05 *M* lanthanum chloride or nitrate solns. (*p*H 5) with cold sodium nitrite soln. to give 0·5–2 *M* nitrite concn. and subsequently boiling the soln. With the nitrite much below 0·5 *M*, the ppt. formed on boiling is mainly lanthanum hydroxide. Fractional nitrite pptn. has been used for the separation of yttria from other yttria earths (*Id.*, 1914, **36**, 1418).

W. R. S.

Determination of Sulphide Sulphur [in Kraft Paper Mill Liquors]. P. B. Borlew and T. A. Pascoe (*Paper Trade J.*, 1944, **119**, July 6, *T.A.P.P.I. Sect.*, 6–10)—This work is part of a study of methods for the routine analysis of kraft mill liquors. It was established that the methods of Dow, Ripanti and Nolan (ANALYST, 1943, **68**, 63) give satisfactory results so long as the detail of the technique is adhered to. The following method gives good results for the determination of sulphide sulphur. Pass carbon dioxide from a Kipp generator through a conical flask provided with absorption bulbs and containing sat. sodium bicarbonate soln. (to trap acid spray) and then through a long vertical tube into a large test-tube, which is the reaction-vessel. The top of this test-tube is widened into a foam trap, through which the gas (after bubbling through the reaction-mixture) passes into a conical flask. In the neck of the flask is a Meyer absorber (a long straight tube in which 8 bulbs are blown), and the flask and absorber are tilted at an angle of *ca.* 30° to the horizontal; they contain 50 ml of 0·1 *N* iodine. The absorber leads into a small conical flask containing a known vol. of standard sodium thiosulphate soln., to trap any iodine blown over. Place a known vol. of sample in the reaction-tube with a few drops of glycerin (to reduce the danger of oxidation of the sulphur compounds) and 1–2 g of solid calcium chloride; the total vol. should be *ca.* 20 ml. Pass the gas through for 1 hr., and then wash the contents of the vessels containing the iodine and thiosulphate solns. into a beaker, and determine the excess of iodine by titration with standard sodium thiosulphate soln. If the absorbing liquid contains sulphur particles of such a size that they will occlude iodine, collect these particles, dissolve the sulphur in carbon disulphide, and determine the occluded iodine by a separate titration. Blank tests on solns. containing known amounts of sodium sulphide showed that satisfactory results are obtained in presence or in absence of sodium sulphite. J. G.

Microchemical

Micro-titration Method for Determination of Small Amounts of Citric Acid. G. W. Pucher (*J. Biol. Chem.*, 1944, **153**, 133–137)—Citric acid is oxidised by potassium permanganate in presence of potassium bromide to pentabromoacetone, which can be extracted with light petroleum. When treated with sodium sulphide pentabromoacetone yields a yellow colour, the intensity of which is proportional to the amount of citric acid originally present. To suitable aliquot portions of the citric acid solution, add 3 ml of 18 *N* sulphuric acid, boil the mixture for 5 min. or until the vol. is reduced to *ca.* 35 ml, cool to 20–25° C., and add 2 ml of *M* potassium bromide soln. and 5 ml of 1·5 *N* potassium permanganate. Leave for 10 min., cool to below 10° C., and decolorise by dropwise addition of 3% hydrogen peroxide. Transfer the mixture to a 125-ml separating funnel with a short stem, cleaned beforehand with chromic acid, and add 25 ml of light petroleum used for rinsing out the beaker, shake for 30 sec., run off the aqueous layer, and re-extract with 20 ml of light petroleum. Wash the combined petroleum extracts with four 3 to 4-ml quantities of water, shake with a 3- and then with a 1-ml portion of 3% sodium sulphide soln., and then with 2-ml portions of water until the aqueous solns. are no longer coloured. To the combined aqueous extracts, add 0·5 ml of 6 *N* sulphuric acid, boil for 1 to 2 min. to expel hydrogen sulphide, and cool to below 15° C. Add 0·5 ml of 1·5 *N* potassium permanganate, and decolorise the excess with halogen-free hydrogen peroxide. Add 1 ml of conc. nitric acid, 1 ml of 30% ferric ammonium sulphate soln., 1 ml of 20% sodium sulphate soln., and *exactly* 2 ml of 0·01302 *N* silver nitrate. After 5 min. titrate the soln. with 0·01302 *N* ammonium thiocyanate added from a micro-burette. The end-point is indicated by a salmon-pink colour. The amount of citric acid (mg) = 1·166 [0·500 (ml $AgNO_3$ − ml NH_4NCS) − 0·020]. The recoveries of added citric acid was practically theoretical. F. A. R.

Micro-separation of Copper from Nickel by Means of Ethylxanthate. P. Wenger, Z. Besso and R. Duckert (*Helv. Chim. Acta*, 1944, **27**, 291–293)—To the soln. (3 ml; 2 mg of each metal) add 6 drops of strong ammonia and, all at once, 1·5 ml of fresh 2% potassium ethylxanthate soln. Stir until the ppt. is coarsely flocculent, and, if it is not pure yellow, add another drop of ammonia. Set aside for 15 min.; filter in a filter-stick and wash with 1% ammonia soln. The nickel in the filtrate may be determined direct with glyoxime, the ppt. being collected in a micro Gooch crucible and washed with 20% alcohol. Digest the copper xanthate in the pptn. vessel with nitric acid, evaporate to dryness, dissolve the residue in water (1–2 ml) and add sodium acetate (*p*H 5–6) and 1·2 ml of an alcoholic 1% soln. of salicylaldoxime; stir and set aside for 20 min. Collect the ppt. in a dry tared filter-tube, wash alternately with water and alcohol, dry at 105° C., and weigh (Cu factor, 0·1893). W. R. S.

Physical Methods, Apparatus, etc.

Spectrofluorescence with Special Reference to Sugars. S. Judd Lewis (*J. Soc. Chem. Ind.*, 1944, **63**, 157–160)—The author defines "spectrofluorescence" as the fluorescence produced by a fluorescent substance when it is illuminated by a spectrum. The technique adopted for exciting and measuring this radiation is as follows. The main dispersing system is identical with the medium-size Hilger quartz spectrograph of large aperture, but in order to have the plate holder position horizontal, so as to deal with powders and solns., the instrument is turned on its back. A parallel sided cell of quartz is mounted in the plane of the plate and divided into two sections by a wall of tinfoil along the optic axis. One section holds the substance to be examined, the other holds a standard. Light from a powerful tungsten arc (40-50 volts, 8·5–9 amp.) passes through a wide slit, 0·1 mm, on the instrument and is dispersed into a spectrum which is focussed on the upper surface of the quartz plate forming the floor of the cell, *i.e.*, on the under surface of the layer of fluorescent powder or soln. A two-aperture wedge in front of the slit enables the spectrum to be directed on to either section of the cell. From below, the resulting fluorescence is photographed at unit magnification by a camera with a glass lens, this camera and the dispersing system being built into a single light-tight housing. The glass camera lens excludes reflected exciting radiation of wavelength <3500A, but transmits the fluorescent radiation. Intensity measurements are made by varying the time of exposure on the standard to obtain a match at each wavelength with the sample under test. For sugars, exposures of the order of 20 min. are necessary. Commercial mannitol, recrystallised four times from water, was found to be the most satisfactory standard. Fluorescence curves for glucose, fructose, sucrose and lactose were all determined in terms of that of mannitol and show characteristic differences one from another. In addition to this differentiation it is possible to discriminate between a pure substance and one containing a small proportion of another compound, *e.g.*, sucrose alone and when mixed with 1% of raffinose. B. S. C.

Spectrophotometric Determination of Leuco Crystal Violet after Oxidation with Benzoyl Peroxide. W. Seaman, A. R. Norton, J. T. Woods and J. J. Hugonet (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 336–339)—One method for the production of crystal violet involves the initial prepn. of leuco crystal violet, p, p′, p″-methenyltris-(N, N-dimethylaniline), followed by oxidation to the finished product. Proper control of the process necessitates a knowledge of the content of the leuco form. No specific method of determining this compound appeared to be available; it was therefore decided to investigate the conditions under which oxidation to the coloured form under controlled conditions could be used as a basis for a photometric determination. *Procedure*—Dissolve a sample containing 0·15 to 0·23 g of leuco crystal violet in glacial acetic acid and dilute to 100 ml. Dilute 10 ml of this to 100 ml, transfer 10 ml of this dilution to a test-tube, and add 5 ml of standard benzoyl peroxide soln. (0·25 g of C.P. benzoyl peroxide in glacial acetic acid, diluted to 100 ml). Mix the contents of the tube, immerse in a briskly boiling water-bath for exactly 4·5 min. to develop the colour, transfer immediately to an ice-bath and shake for about 1 min. to reduce to room temp. as rapidly as possible. Too long cooling may lead to freezing of the acetic acid. Dilute 5 ml of the coloured soln. to 100 ml with glacial acetic acid. Transfer a suitable portion to a glass cell and measure the transmission of the soln. at $\lambda = 580m\mu$, using a suitable spectrophotometer. The amount

of leuco compound is calculated from the formula

$$\text{Leuco content (\%)} = \frac{-22{\cdot}87 \log_{10} T}{\text{length of cell (cm)} \times \text{wt. of sample (g)}}$$

T, the transmission, is expressed as a decimal. This measurement should be carried out within 1 hr. of preparing the soln. The standard benzoyl peroxide soln. may be kept for a few days in the dark, but must be discarded when it develops a yellow colour. Benzoyl peroxide must not be ground or exposed to heat, as it may explode. Crystal violet, itself, is included in the value so obtained for leuco crystal violet, but it can be determined separately without oxidation and a correction made. The precision of the method is represented by a standard deviation for a single value of $\pm 0{\cdot}25\%$ of the total leuco crystal violet.

B. S. C.

Effect of Wavelength on the Contrast of Photographic Plates in the Ultra-Violet. E. H. Amstein (*J. Soc. Chem. Ind.*, 1944, **63**, 172–177)—This paper extends previous work on the variation of plate contrast (γ) with wavelength (Coates and Amstein, *J. Soc. Chem. Ind.*, 1942, **61**, 65; Abst., ANALYST, 1942, **67**, 310) to 13 types of commercially available photographic plates, all of which might be suitable for spectrographic analysis. The determination was carried out using a rotating stepped sector, and it was found that the presence of spectrum background apparently depressed the value of γ. Care was therefore taken, when determining γ at the various wavelengths, to choose spectrum lines free from background arising from a continuum or from the proximity of another very dense line. The dependence of γ on intensity of irradiation was found to be negligible. Results are as follows.

Plate	Wavelength range over which γ is constant A	Value of γ	Ilford developer No. and processing details
Ilford Process	2450–3100	1·5	I.D.13. 18° 3 min.
,, Thin films, half tone	2450–3200	1·15	I.D.13. 18° 4 ,,
,, Ordinary	2500–3100	1·1	I.D.13. 1:1. 18° 6 ,,
,, ,,	2500–3100	0·85	I.D.2. 1:1. 20° 6 ,,
,, Special Rapid	2550–3250	1·15	I.D.2. 1:2. 20° 5 ,,
,, Press	2550–3100	1·05	I.D.2. 1:2. 20° 4 ,,
,, Zenith	2550–3100	1·15	I.D.2. 1:2. 20° 4 ,,
,, Q.1	2350–3100	1·4	I.D.13. 18° 4 ,,
Kodak Slow spectrographic	2450–3050	1·8	I.D.13. 18° 5 ,,
,, B.5	2450–3150	1·45	I.D.13. 18° 5 ,,
,, B.10	2400–3150	1·5	I.D.13. 18° 5 ,,
,, B.20	2450–3150	1·5	I.D.13. 18° 4 ,,
,, O.120	2500–3250	1·15	I.D.2. 1:2. 20° 7 ,,
,, P.25	2450–3150	1·4	I.D.13. 18° 4 ,,

Although there are slight variations of γ within the range quoted, no appreciable error should arise in assuming it constant between 2500 and 3100A.

B. S. C.

Quantitative Determination of Crystalline Materials by X-ray diffraction. S. T. Gross and D. E. Martin (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 95–98)—In a multi-component mixture of crystalline materials the relative proportions of the constituents can be determined from an X-ray diffraction pattern by using an internal standard. In the examples quoted, a known proportion of sodium chloride, added to the unknown mixture, serves as the internal standard. A reference pattern of the pure sodium chloride must also be photographed, and the intensity ratio of the corresponding diffraction lines of sodium chloride in the two patterns may then be obtained as a function of the "interplanar spacing." This intensity ratio is then applied as a correction factor to the intensities of the appropriate diffraction lines of the constituents of unknown proportions in the mixture. A simple calculation then determines these unknown proportions. The method is stated to give results within 5% of the amount present and is illustrated by the determination of the quartz and tridymite constituents of a rhyolite. The method must be used with caution in presence of possible solid solns. and of materials with particle size in the colloidal range.

B. S. C.

Photoelectric Photometry. R. H. Hamilton (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 123–126)—In using photoelectric photometers of the abridged spectrophotometer type one method of measuring the absorption of light by a solute in the more or less narrow wavelength band selected involves the following operations. (*a*) Setting the galvanometer at zero when no light is reaching the photocell; (*b*) with solvent or blank in the absorption cell, adjusting the circuit so that the galvanometer reads 100; (*c*) reading the galvanometer when the solvent or blank is replaced by the light absorbing soln. Mathematical and experimental proofs show that errors in the two settings and in the one reading produce high relative errors when the soln. being investigated has high or low absorption. For maximum accuracy, conditions should be so chosen that the galvanometer readings resulting from operation (*c*) fall on the centre portion of the scale.

B. S. C.

Apparatus for Rapid Polarographic Analysis. J. J. Lingane (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 329–330)—A new design of polarographic cell has the following features. About 3 to 7 ml of soln. are required. A 2-way tap is fitted which allows pure nitrogen to be passed through the soln. until dissolved oxygen is removed (3 min.), and over the soln. during the recording of a polarogram. The rate of mercury flow through the dropping electrode may be determined automatically from the reading of a synchronous electric clock. This is started and stopped by the action of fixed tungsten contact points in a glass tube from which the mercury is fed to the capillary. The second electrode is a close spiral, 2 cm long, of 22 gauge silver wire wound on the capillary itself, starting 3 to 4 mm from the tip. A thin coating of silver chloride is deposited

electrolytically on the wire. In any given chloride-containing soln. this electrode is 0·046 volt more negative than a saturated calomel electrode in the same soln. It is suitable for use in solns. which do not dissolve silver chloride, *e.g.*, of alkali and alkaline earth halides, hydrochloric acid, acid, neutral or basic tartrates containing chloride ion, sodium hydroxide and tetra-alkyl ammonium halide or hydroxide. It may not be used when stable complex ions of silver can form, *e.g.*, in ammonia or cyanide soln. In general, if 10 ml of the soln. gives a ppt., not necessarily of silver chloride, when a drop of silver nitrate soln. is added, the electrode may safely be used. L. A. D.

Dry Indicator Method for Testing the Water Resistance of Asphalted Papers. E. G. Mullen (*Paper Trade J.*, 1944, **119**, July 12, *T.A.P.P.I. Sect.*, 11–12)—The test described is a modification of the *T.A.P.P.I.* standard dry indicator test (*T.A.P.P.I.* Standard, T433m-41; *cf.* Codwise, ANALYST, 1943, **68**, 292) suitable for papers containing an internal layer of asphalt, since for these the colour change is normally so gradual as to involve a high personal factor (Grant, ANALYST, 1935, **60**, 60). Condition the samples (3 × 3 sq. in.) by the standard method, and seal their edges (to prevent penetration of water) by dipping them in a mixture of molten beeswax and rosin; it may be necessary to do this twice with crêped papers, or with papers containing a fabric re-inforcing layer which may conduct water by capillary action. Sprinkle the indicator powder on the paper surface in a thin even layer, and seal a 2-in. watch glass over it with the molten beeswax-rosin mixture, using a pipette. Float the sample on the surface of the water, avoiding inclusion of any air bubbles; crêped or other uneven surfaces should first be wetted with a soft brush. Record the time required to produce an easily perceptible colour-change over the whole area of the paper; make 4 tests on each side. Results are presented for 1 triplex and 8 duplex papers of this type. Different papers gave curves of a similar type relating the penetration time and the temp. of the water used, and a definite relationship between these two variables is indicated. Since the rate of penetration at 100° F. is *ca.* 5 times that at 73° F., it is possible, by using the former temp., to accelerate the test considerably; this modification is recommended for routine and control work. Moreover, sharper end-points are obtained in the accelerated tests. J. G.

Effect of Humidity on the Physical Properties of Paper. F. T. Carson (*U.S. Nat. Bur. of Standards, Circular* 445, 31.1.44, 1–12)—Most physical tests on paper must be made at a specified temp. and relative humidity (R.H.) if they are to be compared with similar tests on other papers. It is, however, undesirable to use graphs based on experimental data to convert a particular value obtained at a non-controlled (though known) temp. and R.H. to the value for the specified conditions, because papers which are apparently similar may not behave alike in this respect (hysteresis effects may result when a paper is transferred from one set of conditions to another), and because variations in the natural humidity of the room may occur while the paper is attaining equilibrium in it. Graphs showing the % change in various properties of paper with change in % R.H. have been compiled from data from 11 (book, writing, bond, ledger, index Bristol, kraft and rope manila) papers over the range 15–83% R.H. at 15·5° or 21° C. A typical sigmoid curve usually relates moisture content and R.H., and the change in the former is approx. proportional to the change in basic ream wt., since the % dimensional change of the paper is small. The bursting-strength attains a max. at 30–50% R.H., and then falls off rapidly; the tensile strength behaves similarly, except that the rate of increase with R.H. is greater. The graph relating the % increase in tearing strength and the % increase in R.H. is approx. linear throughout the range measured. In general, the folding strength increases with increase in R.H., but its variation is the least predictable of those of the physical properties examined, although individual papers behave consistently. The increase in stretch is approx. proportional to the increase in R.H. up to *ca.* 65% R.H., after which it is greater. J. G.

Reviews

VALENCY, CLASSICAL AND MODERN. By W. G. PALMER. Pp. x + 242. Cambridge University Press, 1944. Price 10s. 6d. net.

This valuable and interesting book should command the attention of anyone who can claim to be a serious student of chemistry. Although the subject of valency dates back to the middle years of the nineteenth century, and much progress was made in chemical theory on the classical conceptions of valency which were wholly independent of any ideas regarding atomic structure, the modern development of the subject on electronic lines may be dated from Bohr's theory of atomic spectra (1913) and the work of Kossel, Lewis, and Langmuir since 1916, which resulted in a clear recognition of the fundamental distinction between electrovalent and covalent linkages.

After a clear, but possibly all too brief, historical sketch of the development of the origins of valency from the foundations of the atomic theory by Dalton and Avogadro, the author has traced the evolution of the subject as the result of the work of Berzelius, Gerhardt, Frankland, Kekulé, Cannizzaro and others up to the elaboration of the periodic law by Mendeléeff. This is followed by a most interesting application of the classical conceptions of valency to a number of problems in inorganic and organic chemistry, and particular care has been exercised in pointing out some of the limitations which are inseparable from that mode of treatment. This is extremely important, because there is a widespread misconception in the minds of many students that the theory of valency is in some manner conditioned with the acceptance of the Rutherford–Bohr theory of atomic structure. The author has succeeded

admirably in showing how much the conclusions reached by the classical methods of pure chemistry have been extended by the aid of the array of new experimental methods, such as those of X-ray analysis of crystals and electron diffraction for gases. The treatment of the subjects of bond lengths and of the shapes of some of the simpler types of molecules is full of interest.

Viewing the work as a whole it may be said that the first half of the book will make a strong appeal to the majority of chemists, including those whose interests are centred in analytical chemistry. Thus in a discussion of the quadrivalency of cerium, attention is directed to the high oxidation potential of the ceric ion and the use of ceric sulphate as a volumetric oxidising agent. Another example is to be found in dealing with the electropositive character of iodine in iodine monochloride, in which reference is made to Andrews' method of direct titration with iodates. The latter half of the book, which is largely concerned with the electronic spin theory of valency, is much more physical in outlook, and is definitely difficult. Nevertheless, the topics which are dealt with, such as the application of the spin theory to the heavier elements, co-ordination compounds and aromatic character are highly important. Resonance, as understood by Pauling and others, is treated in a most interesting manner.

The book is attractively produced and contains a very complete and well-arranged index.

A. J. BERRY

COLORIMETRIC DETERMINATION OF TRACES OF METALS. By E. B. SANDELL, Ph.D. Pp. xvi + 487. New York: Interscience Publishers, Inc., 1944. Price $7.00.

Almost every analyst, whatever his special field of activity, has occasion to determine traces of metals present in commodities either as essential elements or as impurities; consequently a fair-sized book entirely concerned with the question is certain to arouse interest. This important work is divided into a General Part consisting of four chapters occupying 109 pages and a Special Part, including detailed accounts of the methods used for the colorimetric determination of 45 metals, together with the rare earths considered as a group.

Throughout, the treatment is spacious. The first chapter comprises a discussion of the general methods of trace analysis with which, however, one might presume most chemists are already familiar, although few may realise that scandium is more abundant than either mercury or bismuth. Then follows a disquisition on the principles employed in the isolation of substances present in traces and a third chapter expounding the physical basis of colour measurement. The final section of this General Part consists of a detailed discussion of those colorimetric reagents which are applicable to the determination of several metals and is replete with graphical formulae, tables, discourses on keto and enol tautomeric forms, absorption curves and mathematical expressions. Nearly half this section is devoted to dithizone which, perhaps not altogether wisely, has been forced into service for the colorimetric determination of many metals.

Coming to the Special Part, which is the real substance of the book, a separate chapter is assigned to the colorimetric determination of each metal, excepting that columbium (niobium) and tantalum are discussed together and a short section is allotted to the rare earths exclusive of cerium. Each monograph opens with an account of the methods for preliminary separation, then follow details for conducting the actual determinations by well-selected procedures which are amplified by notes on applications to certain inorganic materials, such as alloys and silicate rocks or other appropriate substances and, finally, the technique to be employed in the examination of biological materials. Actually, the treatment is more comprehensive than the above description suggests and digressions occur wherever the complexity of the subject demands, while there is abundant information concerning deficiences inherent in the methods and errors likely to be encountered owing to interference.

In turning over these pages the reviewer is acutely conscious how much easier it is to criticise than to produce and, therefore, it is hoped that the remarks which follow will not be interpreted as intended to minimise the worth of this book. One notices many instances of repetition, a feature largely occasioned by the existence of the somewhat redundant General Part. Again, the American predilection for reproducing curves showing the relation between concentration and light extinction is evident throughout and, according to the author, they are included "with the purpose of indicating the sensitivity . . . the reproducibility, and, sometimes, adherence to Beer's law." In most instances small tables would have provided all the data required, and the space could have been more profitably used for additional diagrams of special apparatus such as, for example, Melaven's electrolytic cell for the removal

of alloying metals in the determination of aluminium (*Ind. Eng. Chem., Anal. Ed.*, 1930, **2**, 180) or the still recommended by Law (ANALYST, 1942, **67**, 283) for the isolation of tin as stannic bromide. While the applications to inorganic materials are well described, there is an evident weakness in the corresponding accounts referable to the determination of metals in biological materials, which may be attributable to the author's particular interest in the study of silicate rocks.

One cannot but feel disappointed at the paucity of British work included for description. Out of a total of approximately 790 references to scientific papers, about 390 are taken from journals of the United States, some 220 allude to German literature, while British sources account for little more than 60, about two-thirds of these being from THE ANALYST. Surely this is out of proportion to the work accomplished in this country. Thus, under Arsenic the Gutzeit method is not described, and it is natural for the author to refer his readers to the *Official and Tentative Methods of Analysis* of the A.O.A.C.; but would not the Society's Report on the Determination of Arsenic (ANALYST, 1930, **55**, 102) make a helpful complementary study? In the monograph on Lead the sulphide method is not mentioned, dithizone being favoured for the actual colorimetric determination following the preliminary separation by means of the same reagent, and no allusion is made to the work of Monier-Williams entitled *Lead in Food* (Reports on Public Health and Medical Subjects, No. 88, 1938), although there can be little doubt that this represents the best that has been written on the theme. Dithizone is also recommended for the determination of mercury, and one looks in vain for an account of the method due to Strafford and Wyatt (ANALYST, 1936, **61**, 528), using *p*-dimethylamino-benzal-rhodanine, although, strangely enough, the reference to this classic paper is given on page 104 in the chapter on general colorimetric reagents. In considering the question of the determination of traces of nickel present in steel the useful procedure recommended by B. Jones (ANALYST, 1929, **54**, 582) is not noticed, although he was the first to apply the reaction between dimethylglyoxime and quadrivalent nickel to this particular purpose.

But these remarks invite the charge of insularity and must certainly not be interpreted in a spirit of condemnation; on the contrary, this is a truly excellent book and one that every analyst will be glad to possess. We British owe much indeed to our American friends, and not the least to American scholarship. Concluding, one notices that this handsome volume is produced in full compliance with the Government's regulations for conserving paper and other essential materials: O what plenitude prevails in that mighty Federation of United States!

N. L. ALLPORT

INSECT PESTS OF FOOD. By H. E. HINTON, A. S. CORBET and W. H. T. TAMS. H.M. Stationery Office, 1944. Price 5s.

Although modestly described as a "pamphlet," this booklet extends to 148 pages and consists of two elaborate papers prepared for the Ministry of Food under the direction of the Keeper of the Department of Entomology of the Natural History Branch of the British Museum. Two of the authors (Dr. Hinton and Dr. Corbet) are at present serving as officers in the Ministry of Food, and these papers, as explained in a foreword by Mr. W. McAuley Gracie, Director of Infestation Control, have been written primarily for the use of inspectors of the Ministry.

The first paper, by Dr. Hinton, is concerned with the identification of the larvae of the many species of lepidoptera associated with stored food—chiefly by minute observation of the form and arrangement of their setae (chaetotaxy), in illustration of which the author has prepared as many as 128 finely executed drawings, although it may perhaps be regretted that no pictures are given of the larvae themselves either as seen by the naked eye or under magnification, such, for example, as those by Mr. Britten contained in Mr. Hayhurst's more popular book on *"Insect Pests in Stored Products"* previously reviewed in these pages (ANALYST, 1942, **67**, 377).

The second paper, by Dr. Corbet and Mr. Tams, deals with the identification of the lepidoptera in the mature insect form, and this is prefaced by nearly forty good photographic pictures. Many drawings are given of wing venation, but the greater part of the paper is devoted to the morphology of the male and female genitalia of the many species discussed and to their microscopical investigation as an aid to identification, the text being illustrated by some 250 drawings.

To prevent possible disappointment, it should perhaps be added that both papers are confined to the problems of identification, there being no description or discussion of measures for the control of infestation.

BERNARD DYER

THE CHEMICAL ANALYSIS OF WATERS, BOILER- AND FEED-WATERS, SEWAGE, AND EFFLUENTS. By DENIS DICKINSON, M.Sc., F.R.I.C. Pp. xii + 140. London and Glasgow: Blackie & Son, Ltd., 1944. Price 6s. net.

This excellent book is an amplification of the author's practical notebook, and the needs of the industrial chemist have been his primary consideration. It is essentially a practical guide, but the principles of methods described have been mentioned where necessary, and there are many references to original papers—given, fortunately, at the foot of the pages concerned.

The usual methods of water and sewage analysis are concisely and accurately dealt with in separate chapters, followed by a section on residual chlorine, fluorine and silica, and others on boiler-feed water, grease in sewage effluents and the expression of results. This last chapter is not as clear as it might be. There is a good description of a simple apparatus for electrometric titration and for determining hydrogen ion concentration using antimony electrodes fluorescent indicators for determining acidity and alkalinity of coloured trade effluents are mentioned, and the use of methyl orange screened with xylene cyanole F.F. or methylene blue is advised. The author states that it has been possible to include only a fraction of the known methods, but it would have been an advantage to describe some of the newer ones, such as the determinations of magnesium by precipitation as the oxine complex; of sodium as magnesium uranyl sodium acetate; of aluminium, colorimetrically, with aurin tricarboxylic acid. The process described for the determination of lead in coloured waters is tedious and the modification of Manley (ANALYST, 1940, **65**, 403) entailing wet oxidation, alcoholic precipitation of lead sulphate and solution in ammonium acetate is simpler and accurate. Sintered glass crucibles are rightly dismissed with contumely for the determination of suspended matter in sewage, but they have their uses, *e.g.*, the filtration of calcium oxalate prior to volumetric determination or the filtration of the magnesium oxine and sodium uranyl precipitates. No mention is made of the determination of traces of phenol or other tar acids in water; these cause tastes in chlorinated water supplies and are detrimental to fish life. The method of Fox and Gauge or that of Houghton and Pelly (ANALYST, 1937, **62**, 117) is satisfactory.

These, however, are minor criticisms and suggestions and may form subject matter for subsequent editions. The author covers the ground of "the very wide subject of water analysis" in a competent manner, and his book is worthy of a place on the bookshelf of every water analyst, in the august company of "Thresh" and A.P.H.A. Standard Methods—and that is high praise.

The book is well printed and produced and is very reasonable in price.

W. GORDON CAREY

FORMATION OF A GROUP DEALING WITH PHYSICAL METHODS OF ANALYSIS

SEVENTY members have signified their wish that a Group dealing with physical methods of analysis should be formed within the Society, and the Council has decided to proceed with the formation of such a Group. The Hon. Secretary will be pleased to receive the names of any further members of the Society who may wish to become members of the Group.

SOCIETY FOR VISITING SCIENTISTS

5, Old Burlington Street, London, W.1. Telephone: Regent 1069

WE are asked to make known the formation of this Society and the following particulars:

OFFICERS: *President:* Professor F. G. Donnan, C.B.E., F.R.S.
Secretary: Mr. J. G. Crowther.

OBJECTS: To serve as a focus for scientists from overseas visiting Britain, and to provide an information centre and hospitality.

ACCOMMODATION: Reception rooms, a refectory, and two dormitories.

MEMBERSHIP: No fee will be charged for the duration of the war.

ADVICE TO AUTHORS

THE Council has approved the following notice by the Publication Committee, which is here given in condensed form.

The Society publishes papers concerned with all aspects of analytical chemistry, inorganic and organic, as, for example, food and drugs analysis, analysis of water (including its bacteriological examination), gas analysis, metallurgical assays, biological standardisation and micro-analysis. Papers on these and allied subjects may be submitted for presentation and publication; they may:

(1) Record the results of original investigations into known methods or improvements therein;
(2) Record proposals for new methods and the investigations on which the proposals are based;
(3) Record analytical results obtained by known methods where these results, by virtue of special circumstances, such as their range or the novelty of the materials examined, make a useful addition to analytical information;
(4) Record the application of new apparatus and new devices in analytical technique and the interpretation of results.

Communications.—Papers (which should be sent to the Editor) will normally be submitted to at least one referee, on whose advice the Publication Committee will be guided as to the acceptance or rejection of any communication. Papers or Notes accepted by the Publication Committee may not be published elsewhere except by permission of the Committee.

Abstracts.—The MS. should be accompanied by a brief abstract of about 100 to 150 words indicating the scope and results of the investigation.

Notes on the writing of papers for THE ANALYST

Manuscript.—Papers and Notes should preferably be typewritten.

The title should be descriptive and should set out clearly the scope of the paper.

Conciseness of expression should be aimed at; clarity is facilitated by the adoption of a logical order of presentation, with the insertion of suitable paragraph or sectional headings.

Generally, the best order of presentation is as indicated below:

(*a*) General, including historical, introduction.
(*b*) Statement of object of investigation.
(*c*) Description of methods used. Working details of methods are usually most concisely and clearly given in the imperative mood, and should be given in this form, at least while economy of paper is pressing, *e.g.*, "Dissolve 1 g in 10 ml of water and add . . .". Well-known procedures must not be described in detail.
(*d*) Presentation of results.
(*e*) Discussion of results.
(*f*) Conclusions.

To be followed by a short summary (100 to 250 words) of the whole paper: items (*e*) and (*f*) can often be combined.

Illustrations, diagrams, etc.—The cost of setting up tabular matter is high and columns should therefore be as few as possible. Column headings should be brief or replaced by a number or letter to be used in combination with an explanatory footnote to the table.

Sketches or diagrams should be on white Bristol board, not larger than foolscap size, in Indian ink. Lettering should be in light pencil.

Tables or graphs may be used, but normally not both for the same set of results. Graphs should have the co-ordinate lines clearly drawn in ink.

References.—References should be numbered serially in the text and collected in that order under "REFERENCES" at the end of the paper. They should be given in the following form:

1. Dunn, J. T., and Bloxam, H. C. L., *J. Soc. Chem. Ind.*, 1933, **52**, 189T.
2. Allen, A. H., "*Commercial Organic Analysis*," Churchill, London, 1882.

Notes on the Presentation of Papers before Meetings of the Society are appended to the "ADVICE," copies of which may be obtained on application to the Secretary, 7/8, Idol Lane, London, E.C.3.

Volume 69 October, 1944
No. 823, Pages 291—328

Contents

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

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