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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held at 6 p.m. on Wednesday, April 3rd, at The Chemical Society's Rooms, Burlington House, London, W.1. The chair was taken by Mr. Eric Voelcker, Vice-President. The following papers were presented and discussed: "The Determination of Boron," by E. C. Owen, B.A., M.Sc., Ph.D.; "Separation of the Cobalt Complex of β-Nitroso-α-Naphthol from other Coloured Metallic Complexes," by E. Boyland, D.Sc. The following pieces of apparatus were then described and exhibited by J. T. Stock, M.Sc., F.R.I.C., and M. A. Fill: (1) "For the Control of Delivery from Burettes"; (2) "A Vacuum-operated Circulating Pump"; (3) "A Thermostatically-controlled Low Temperature Bath"; (4) "For the Continuous Production of Doubly-distilled Water."

NEW MEMBERS

Mark Barent, B.Sc., Ph.D. (Lond.), A.R.I.C.; John Tracey Greaves, B.A. (Oxon.); Herbert Kent Hartley, B.Sc. (Manc.), F.R.I.C.; Alice Lacourt, D.Sc.; Walter Wilfred Stevenson, A.Met. (Sheff.), F.R.I.C., A.M.I.C.E.; Ronald Herbert Thorp, B.Sc. (Lond.); George Ernest Tunnicliffe, B.Sc. (Lond.); Ronald Dixon Windross.

DEATHS

WE regret to have to record the deaths of

William John Atkinson Butterfield. Herbert Firth.

PHYSICAL METHODS GROUP

THE First Annual General Meeting of the Group was held on November 28th, 1945, in the Chemical Society's rooms, Burlington House, London, W.1, at 6 p.m. The Chairman, Mr. R. C. Chirnside, presided. He explained that, in accordance with the provisional rules of the Group, the present Officers and Members of Committee (see ANALYST, **1945, 70, 67**) would remain in office for the coming year. The Hon. Secretary's Report (*cf.* ANALYST, **1946, 71,** 160) and the Financial Statement for the past year were presented and approved. The Chairman invited members to contribute papers during the coming year. After conclusion of the Group business the following papers were read and discussed: "The Barker Index, a means of Identifying Crystals from their Shape," by R. C. Spiller, and "Examples of the Utility of the Barker Index," by M. W. Porter.

MICROCHEMISTRY GROUP

THE Annual General Meeting of the Group was held on January 26th, at Imperial College, South Kensington, London, S.W.7. The Chairman, Professor H. V. A. Briscoe, presided. The Hon. Secretary's Report (*cf.* ANALYST, **1946, 71,** 159) and the Financial Statement were presented and approved. The Officers and Members of Committee remain as last year. After conclusion of the Group business the following papers were read and discussed: "Chemical Microscopy in Metallurgical Analysis," by Miss I. H. Hadfield, and "A Review of Methods for the Micro-analysis of Gases," by Dr. W. A. Kirkby.

BIOLOGICAL METHODS GROUP

THE First Annual General Meeting of the Group was held on February 25th, at 6 p.m., in the Chemical Society's Rooms, Burlington House, London, W.1. The Chairman, Mr. A. L. Bacharach, was in the chair. The appointment of the Officers and Members of Committee elected at the Inaugural Meeting on October 17th, 1945 (see ANALYST, **1945, 70,** 393) was confirmed. Messrs. D. M. Freeland and J. H. Hamence were elected as Honorary Auditors for the coming year. An Ordinary Meeting of the Group followed at 6.30 p.m. for the reading of papers by Messrs. N. T. Gridgeman and E. C. Feiller entitled respectively "The Transformation of Metamers with special reference to Vitamin D Assays" and "Some Remarks on the Statistical Background of Bio-Assays."

NORTH OF ENGLAND SECTION

THE Twenty-first Annual General Meeting of the Section was held at Manchester on Saturday, January 26th, 1946. The Vice-Chairman, Mr. C. H. Manley, presided over an attendance of forty. The Hon. Secretary presented the Report and Financial Statement, which were adopted. Appointments were made as follows. *Chairman*, H. M. Mason. *Vice-Chairman*, C. H. Manley. *Hon. Secretary and Treasurer*, Arnold Lees. *Other Members of Committee*, H. F. Bamford, W. Gordon Carey, C. J. House, A. N. Leather, R. Mallinder, J. G. Sherratt. *Hon. Auditors*, U. A. Coates and J. R. Walmsley.

The following papers were read and discussed:—"A Photoelectric Method of Assaying Vitamin A in Margarine," by J. L. Bowen, N. T. Gridgeman, B.Sc., and G. F. Longman, B.Sc., F.R.I.C.; "The Determination of Carotene and Vitamine A in Butter and Margarine," by T. W. Goodwin, M.Sc., A.R.I.C., and Professor R. A. Morton, Ph.D., D.Sc., F.R.I.C.; "An Application of Photoelectric Spectrophotometry to the Analysis of Mixtures," by Professor R. A. Morton and Dr. A. L. Stubbs.

An Ordinary Meeting of the Section was held in Manchester on Saturday, April 27th. The Vice-Chairman, Mr. C. H. Manley, presided over an attendance of thirty-one. The following papers were read and discussed:—"The Rapid Determination of Sodium in 50% Potassium Hydroxide Liquor, 50% Potassium Carbonate Liquor and Solid Potassium Carbonate,"* by J. Haslam, M.Sc., F.R.I.C., and J. Beeley; "The Determination of the Pyridine Content of Technical Pyridine," by A. Hamer, B.Sc., A.R.I.C., R. Pomfret, A.R.I.C., and W. V. Stubbings, B.Sc., A.R.I.C.; "Rapid Methods of Analysis of Boiler Water in Power Station Practice," by C. J. House, B.Sc., A.R.C.S., F.R.I.C.

Obituary

JAMES FOWLER TOCHER

ON 8th November, 1945, Dr. James Fowler Tocher passed away at the mellow age of 81. A native of Fyvie and reared in the cauld grey north he was, like his native granite, a man of strong character, purposeful of mind and a true and faithful friend.

He served his apprenticeship as a pharmaceutical chemist and started on his own account in Peterhead, where he carried on the practice for 26 years until 1912. In 1909 he was President of the Pharmaceutical Society. He also served on the Board of Examiners of the Society and on various Committees such as those connected with dangerous drugs and therapeutic substances. He studied at Aberdeen University and graduated B.Sc., and later took his Doctorate in Science. He then established himself in Aberdeen as a Public Analyst, and at his death held a number of public appointments including that for the County of Aberdeen, which he held for 33 years.

Tocher, as he was familiarly known to his friends, was a man of wide interests; he was Lecturer in Statistics in Aberdeen University and consulting chemist to the Highland and Agricultural Society, and served on numerous public bodies, including the governing bodies of the Rowett Institute, the Macaulay Institute for Soil Research and the North of Scotland Agricultural College. He was also on several occasions external examiner for the Diplomas of N.D.A. and N.D.D., being nominated by the Highland and Agricultural Society.

Amongst his other interests was the science of anthropometry, in which he made a special study of heads and statistically determined their variations and classified them into their respective categories. In his native Buchan he was an authority on the cultural and historical associations of the district and for more than half a century guided the activities of the Buchan Club as its Honorary Secretary. He edited and wrote articles for the "Majority" and "Jubilee" Volumes of the Book of Buchan. In point of fact he was the Buchan Club.

One cannot fail to be impressed by the high regard in which he was held by his contemporaries; he always had an admiring circle of friends. In 1937 his fellow scientists and public and professional friends presented him with his portrait in oils, and in the same year the University of Aberdeen conferred on him the honorary degree of LL.D.

His catholicity of interest and versatility were matched by his golden commonsense and his fantasy of wit and humour; he was a grand spinner of Aberdeen tales. He held a high place in the Councils of his profession as a Public Analyst, and one wonders how far he would have reached had he restricted his energies to that branch of science. He published a number of articles on analytical chemistry, and his *"Variations in the Composition of Milk"* will remain a memorial to his scientific and scholarly attainments.

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* See this Vol., p. 223.

His friends will remember him as the hillman who roamed and loved his Benachie and they will cherish the memory of his uniqueness now that he has gone from them. Let me quote his own words written in 1933.

"May all the fondest dreams of youth
To every worker here come true,
May hard and honest search for Truth
Bestow on each what is his due."

ANDREW DARGIE

Notes on the Selective Oxidation of Vinegar

BY F. A. LYNE AND T. MCLACHLAN

(*Read at the Meeting of the Society on February 6th, 1946*)

VARIOUS modifications have been proposed for the determination of the oxidation values of vinegars, following the work of Wustenfeld,[1] who suggested direct titration with permanganate. Bailey[2] gave reasons why this method failed to give a sharp end-point, and Edwards and Nanji[3] introduced the principle of distillation and an improved method of determination. Illing and Whittle[4] suggested a modified distillation technique, thereby considerably reducing the amount of sample required. Whitmarsh[5] gave an account of the volatile reducing substances present in various types of vinegar, and O'Neill and Henry[6] have investigated the influence of time, temperature and concentration of reactants on the oxidation value.

The object of the present investigation was to find a method of oxidation which would differentiate more sharply between the various types of vinegar than those previously published, and incorporate, if possible, some of the advantages of the iodine value.

Lines of Investigation—Vinegar consists of a number of components, many of which occur in traces but contribute in a marked degree to the flavour and aroma. The varying proportions in which these occur account for the characteristics of different types of vinegar. Of the oxidisable substances present the most important are ethyl alcohol, acetyl methyl carbinol, fusel oil, ethyl acetate, diacetyl and acetaldehyde. Whitmarsh (*loc. cit.*) has shown that whereas both malt and spirit vinegars contain alcohol, acetyl methyl carbinol is present in malt vinegar in much greater proportion than in spirit vinegar, and that these two compounds are mainly responsible for the oxidation and iodine values. If, therefore, a method could be found which would oxidise acetyl methyl carbinol much more readily than alcohol, a greater differentiation between the various types of vinegar would result.

A number of acid oxidising agents such as potassium dichromate, bromide - bromate solution, etc., were tried at various temperatures and dilutions, and with different reaction times, but none of these was found to give greater selectivity than the acid permanganate method of Edwards and Nanji.[3] An alkaline permanganate oxidation method appeared, however, to hold promise of more satisfactory results, and the conditions of oxidation were studied in order to obtain maximum differentiation.

Effect of Concentration—The alkaline oxidation values of samples of malt and spirit vinegars were determined, using the same quantities of distillate and the same conditions

TABLE I

EFFECT OF CONCENTRATION OF REACTANTS

Other conditions of oxidation being those given in method adopted

Volume of water added in mls.	Alkaline oxidation value	
	Malt vinegar	Spirit vinegar
0	104·2	76·8
20	96·0	50·4
50	86·4	27·2
100	81·6	12·8
150	78·4	8·8
200	76·8	8·8

as in the method finally adopted (see later) but varying the quantities of distilled water added. The results (Table I) show that no greater separation of values is obtained by addition of more than 100 ml. of water, and this quantity was adopted.

Effect of Temperature—Determinations of alkaline oxidation values at various temperatures from room temperature up to 80° C. were made, but these appeared to offer no advantage over room temperature, and this was adopted for convenience.

Effect of Time of Reaction—The alkaline oxidation values of samples of malt and spirit vinegar were similarly determined, using various reaction times, but keeping the other conditions constant. The results (Table II) show that no advantage is obtained by prolonging the reaction time beyond 30 minutes.

TABLE II

EFFECT OF TIME OF REACTION

Other conditions of oxidation being those given in method adopted

Time of reaction in minutes	Alkaline oxidation value	
	Malt vinegar	Spirit vinegar
5	91·6	2·10
10	99·5	3·43
20	103	5·28
40	105	7·45

Effect of Light—Pairs of parallel determinations were made by exposing one bottle to direct sunlight and another to ordinary daylight such as from a north light. Compared with determinations done in the dark, those exposed to direct sunlight showed significant differences, but those exposed to normal daylight did not. As the reaction appears to be photosensitive it is recommended that the oxidation be performed in the dark.

Effect of Caramel—As commercial samples of vinegar invariably contain caramel, which might possibly contain volatile reducing substances, solutions of several commercial caramels of the strength normally employed in vinegar were distilled. There was no evidence that caramel had any effect on the oxidation value.

METHOD ADOPTED—The following conditions of oxidation were adopted as a result of the foregoing experiments:

Pipette 2 ml. of the distillate, obtained by the modified method of Illing and Whittle, into a stoppered bottle of about 250 ml. capacity and add 100 ml. of water, 10 ml. of 10% sodium hydroxide solution and 10 ml. of $N/10$ permanganate solution, in that order. Place the mixture in the dark for 30 minutes, and then acidify by addition of 10 ml. of 25% sulphuric acid; add 0·5 g. of potassium iodide and titrate the liberated iodine with $N/50$ thiosulphate solution. Carry out a blank determination on the reagents at the same time.

Then, if the alkaline oxidation value is defined as "the number of parts by weight of oxygen required to oxidise 100,000 parts of vinegar under the conditions laid down,"

$$\text{Alkaline Oxidation Value (A.O.V.)} = 8\,(A - B),$$

where A ml. is the blank titration and B ml. the vinegar titration.

There is a striking difference in appearance between different types of vinegar during the alkaline oxidation. Within a minute of adding the permanganate a deep green colour develops with malt and wine vinegars, whereas with spirit or artificial vinegars the liquid remains pink or purple. This change is so striking that it can be used as a rapid sorting test.

Whitmarsh recommends dilution of the vinegar to 4% acetic acid strength before distillation, but this has been found unnecessary except, perhaps, with spirit vinegars of 12% strength, which are seldom encountered in the retail trade.

Results Obtained—Figures obtained from 10 typical samples of malt vinegar (37 were examined), 10 wine vinegars, 8 spirit vinegars, and 8 artificial vinegars are shown in Table III. The results have further been calculated to 4% acidity as acetic acid to provide a standard basis of comparison. Maxima, minima, and mean figures for all samples examined are shown in Table IV. It is seen that malt and wine vinegars gave acid oxidation values ranging from 1585 down to 323, spirit vinegars gave values from 385 down to 162 and artificial vinegars values from 100 to 5·9. On the other hand the corresponding values for the alkaline oxidation method with malt and wine vinegars ranged from 158 down to 50, with spirit vinegars from 13·5 to 5·5, and with artificial vinegars from 6·7 down to 0·5. There is thus a sharp line of demarcation between malt and wine vinegars on the one hand and spirit and artificial vinegars on the other by means of the alkaline oxidation method, whereas there is a definite overlap between these two groups by the acid oxidation method. On the other hand the acid oxidation method appears to be rather more efficient than the alkaline oxidation method for distinguishing between spirit vinegars and artificial vinegars. It is impossible to

differentiate between malt and wine vinegars by the alkaline or the acid method of oxidation or by determination of iodine or ester values, and recourse must be had to determinations of tartaric acid, flavour, aroma, or other factors for this purpose.

Reasons for Increased Efficiency—As pointed out above, the selectivity of the method is dependent upon the amounts and the relative oxidation values of components present in

TABLE III

TABLE OF RESULTS

Type of vinegar	No.	Acidity	Calculated on original sample				Calculated to 4% acetic acid			
			A.O.V.	Oxid. value	Iodine value	Ester value	A.O.V.	Oxid. value	Iodine value	Ester value
Malt—	1	4·25	168	1400	1136	133	158	1318	1070	125
	2	4·62	176	758	1428	49	153	666	1235	42
	3	5·22	176	1316	952	70	135	1010	730	54
	4	4·00	133	1036	940	131	133	1036	940	131
	5	4·00	116	1456	388	86	116	1456	388	86
	6	4·45	123	1768	752	32	111	1585	662	29
	7	4·62	82	1064	268	40	72	925	232	35
	8	5·01	83	820	453	39	66	655	362	31
	9	6·47	103	527	869	57	64	323	351	35
	10	7·04	88	1224	492	38	50	696	280	22
Wine—	1	7·20	178	2406	568	217	99	1340	315	121
	2	4·68	141	1606	380	102	121	1370	324	87
	3	4·74	142	1648	512	181	120	1390	432	152
	4	4·80	143	1596	460	90	119	1330	383	75
	5	6·18	130	1672	665	87	84	1082	430	51
	6	5·95	122	1555	640	58	82	1045	430	39
	7	6·10	118	1380	550	100	77	905	360	65
	8	6·03	103	1480	608	73	68	982	403	48
	9	5·52	131	1560	835	84	95	1130	605	61
	10	5·40	126	1380	765	74	94	1021	516	51
Spirit—	1	4·74	16·0	456	8·0	0	13·5	385	6·8	0
	2	4·14	12·8	200	20·0	—	12·4	193	19·3	—
	3	4·38	12·8	372	6·0	0	11·7	330	5·5	0
	4	4·65	12·0	188	16·0	—	11·3	162	13·8	—
	5	4·00	8·0	164	·14·0	15·2	8·0	164	14·0	15·2
	6	3·96	7·6	224	12·0	13·6	7·7	226	12·2	13·8
	7	12·00	22·4	646	40·0	8·8	7·4	215	13·3	2·9
	8	12·17	16·8	688	12·0	15·6	5·5	266	3·9	4·9
Artificial—	1	3·60	5·6	12	14·0	6·4	6·7	13·3	15·5	7·1
	2	4·20	6·4	12	10·0	13·6	6·1	11·4	9·5	12·9
	3	8·05	8·0	40	26·0	2·2	4·0	20·0	13·0	1·1
	4	3·50	2·4	88	12·0	4·0	2·7	100·0	13·7	4·6
	5	4·32	2·4	64	8·0	8·8	2·2	59·0	7·4	8·1
	6	5·40	2·4	8	12·0	5·6	2·0	5·9	8·9	4·2
	7	4·98	1·6	48	0	8·0	1·3	38·5	0	6·4
	8	6·20	0·8	96	0	0	0·5	62·0	0	0

NOTES: *Malt vinegar*
Sample No. 7 was distilled malt vinegar.

Wine vinegars
No. 1. Described as "Wine Vinegar."
Nos. 2, 3 and 4. Described as "Red Wine Vinegar."
Nos. 9 and 10. Described as "Tarragon Vinegar."
— implies insufficient sample to carry out the determination.

different types of vinegars, *i.e.*, the best method will tend to accentuate the differences and suppress the common factors.

In order to investigate this point, dilute solutions of ethyl alcohol, acetyl methyl carbinol, fusel oil, ethyl acetate, diacetyl and acetaldehyde were distilled in exactly the same way as vinegar, and the acid oxidation, alkaline oxidation, and iodine values were determined on the distillates. The results so obtained are given in Table V and, in order to bring out the extent to which chemical action occurs with these individual substances the acid oxidation values and the iodine values have been recalculated on the basis of parts by weight of oxygen absorbed per 100,000 of solution by dividing by 12·5. Taking the oxidation by the alkaline oxidation method as 100, the relative oxidation values by the acid method and the iodine

method have then been calculated. As might be anticipated, the attack of dilute iodine on ethyl acetate, fusel oil, ethyl alcohol and acetaldehyde is very slight, whilst acetyl methyl carbinol is attacked most vigorously. Dilute acid permanganate solution attacks all six compounds more or less equally, whereas there is a definite ascending rate of oxygen absorbed from alkaline permanganate solution by these substances. These ratios will

TABLE IV

SUMMARY OF RESULTS IN TABLE III

All results calculated to 4% acidity as acetic acid

		Malt vinegar	Wine vinegar	Spirit vinegar	Artificial vinegar
Acid oxidation value	Max.	1585	1390	385	100
	Min.	323	905	162	5·9
	Aver.	974	1160	243	39
Recalculated to parts by wt. oxygen per 100,000 vinegar	Max.	127	111	30	8
	Min.	26	72	13	0·5
	Aver.	78	93	19	3·1
Alkaline oxidation value	Max.	158	121	13·5	6·7
	Min.	50	68	5·5	0·5
	Aver.	108	96	9·7	3·2
Iodine value	Max.	1235	605	19·3	15·5
	Min.	232	315	4·0	0
	Aver.	622	420	11·1	8·5
Ester value	Max.	131	152	15·2	12·9
	Min.	19	39	0	0
	Aver.	55	75	6·1	5·5

doubtless be of value in determining eventually the proportions in which various minor constituents are present in different types of vinegar, but it would appear that the large difference in the figures for the acid oxidation values of spirit and artificial vinegars is caused by the presence of small amounts of alcohol, whereas the difference between the alkaline oxidation values and the iodine values of malt and wine vinegars as compared with spirit

TABLE V

		Calcd. as equiv. of oxygen absorbed per 100,000			Relative absorption of oxygen by the three methods		
Substance	Concn. %	Acid O.V.	Iodine val.	Alk. O.V.	Acid O.V.	Iodine val.	Alk. : Acid : Iodine
Ethyl acetate	0·1	132	8	13	11	0·6	100 : 85 : 4·6
	0·4	418	12	24	33	0·9	100 : 137 : 3·7
Fusel oil	0·1	174	8	24	14	0·6	100 : 58 : 2·5
	0·4	376	28	50	30	2·2	100 : 60 : 4·4
Ethyl alcohol	0·1	176	20	29	14	1·6	100 : 48 : 5·5
	0·4	516	40	50	41	3·2	100 : 82 : 6·4
Acetaldehyde	0·1	204	64	74	16	5·1	100 : 22 : 7·0
	0·4	574	160	169	46	12·8	100 : 28 : 8·9
Diacetyl	0·1	178	360	93	14	29	100 : 15 : 31
	0·4	528	996	238	42	80	100 : 18 : 34
Acetyl methyl carbinol	0·1	150	464	60	12	37	100 : 20 : 62
	0·4	532	1332	150	43	107	100 : 29 : 71

vinegars must be due largely to diacetyl and acetyl methyl carbinol. There is a considerable difference in the iodine values for acetaldehyde found by Whitmarsh and by the present authors, and this point is being further investigated.

Conclusion—In order to differentiate between types of vinegar an alkaline oxidation method is given, which is more efficient than those previously described. The alkaline method of oxidation can also be used as a rapid sorting test to differentiate malt and wine vinegars from spirit and artificial vinegars.

We should like to thank British Vinegars Ltd., Hill, Evans & Co. Ltd., the Midland Vinegar Co. Ltd., the Derby Malt Vinegar Co. Ltd., the Yorkshire Vinegar Co. Ltd., the Victoria Vinegar Co. Ltd., Holbrooks Ltd., and Fardon's Vinegar Co. Ltd., for supplying us with samples of vinegar, and Gillman & Spencer Ltd., for samples of caramel.

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4, HANWAY PLACE
LONDON, W.1

The Volumetric Determination of Nitroguanidine

BY T. L. COTTRELL, C. A. MACINNES AND E. M. PATTERSON

IT has been known for some time that a solution of nitroguanidine in sulphuric acid contains nitrate ion or a substance capable of yielding nitrate ion under suitable conditions.[1] A method has been devised by Bowman and Scott[2] for the determination of nitrates by solution in sulphuric acid and titration with a solution of ferrous sulphate. The ferrous sulphate reduces the nitrate, and the end-point is indicated by persistence of the colour of the "brown-ring" compound, $FeSO_4.NO$ on addition of a small excess of reagent. Cope and Barab[3] have shown that under the conditions of the Lunge nitrometer a quantitative yield of nitric oxide is obtained from nitramines, the compounds considered by these workers being nitro-urea, nitroguanidine, and trinitrophenylmethylnitramine ("Tetryl"). This made it seem probable that nitroguanidine might be capable of quantitative determination by the ferrous sulphate method of Bowman and Scott. Tetryl has recently been determined by this method.[4]

Preliminary work showed that, while the reaction was approximately quantitative, the true end-point was very difficult to observe. Towards the end of the reaction, when most of the nitroguanidine was decomposed, the brown colour of the ferrous sulphate - nitric oxide complex tended to persist in the solution and to fade away very slowly. Premature appearance of the brown colour made it seem that the end-point had been reached, but after a period varying from several seconds to many minutes the colour gradually faded; this cycle was repeated on successive small additions of the reagent. Attainment of a true end-point by this procedure was obviously difficult, and work was at first directed to finding a means of increasing the rate of fading of the premature "brown-ring" colour. At room temperatures, however, this fading was unmanageably slow, and although rise in temperature to 40° C. or above was found to produce a marked acceleration of the fading, the use of a comparatively high temperature was considered objectionable for the reasons given by Bowman and Scott (*loc. cit.*). The prolonged standing at moderate temperature was found to result in gradual precipitation of finely divided ferric sulphate from the solution. The resulting opalescence obscured the end-point and it was possible to over-run it.

The speed of fading of the premature end-point was also found to be affected by the amount of sulphuric acid present in the reaction flask. Increase in volume from 80 ml. to 200 ml. was tried, and it was found that the larger volume gave considerably accelerated fading. It was necessary to subtract a "blank" from the volume of ferrous sulphate used, to allow for the excess needed to give a visible brown coloration to the solution, and as this "blank" was proportional to the volume of the solution the larger volumes of sulphuric acid which were used to accelerate the end-point were inevitably associated with an undesirably large "blank". The use of added oleum was also tried, thereby maintaining the H_2SO_4 content of the solution at a high value towards the end-point, but the troublesome opalescence caused by precipitated ferric sulphate became more obvious, while no acceleration of the fading was obtained.

It was found in the course of these experiments that the decomposition of nitroguanidine in sulphuric acid according to the equation

$$NH_2.C(NH)NHNO_2 + H_2O \rightarrow N_2O + CO_2 + 2NH_3$$

described by Davis[5] took place at a measurable rate even at room temperature.

This showed that titration had to be carried out immediately after solution if low results were to be avoided.

TITRATION TO VISUAL END-POINT—*Reagent*—Dissolve 160 g. of ferrous sulphate crystals in 400 ml. of water and stir into a cooled mixture of 250 ml. of water and 250 ml. of concentrated sulphuric acid. Cool to room temperature and dilute to 1 litre.

Standardisation—Weigh accurately about 0·6 g. of pure potassium nitrate, dried for 1 hour at 140° C., into a 400-ml. conical flask, dissolve in 100-ml. of concentrated sulphuric acid and titrate with the ferrous sulphate solution until a permanent pink colour appears, cooling the flask during the titration to maintain the temperature below 40° C.

101·1 g. of $KNO_3 \equiv$ 104·1 g. of nitroguanidine.

Procedure—Dissolve a weighed quantity of nitroguanidine in 200 ml. of concentrated sulphuric acid and titrate as above. Since a larger volume of acid is used as solvent, a larger volume of excess reagent is required to give the pink coloration at the end-point. Comparison of results obtained with different volumes of acid makes it possible to deduce a correction to allow for this.

Results—In successive determinations on the same sample the following results were obtained for % nitroguanidine: 100·7; 101·8; 101·5; 97·3; 101·3; 99·6; 101·2; 99·9. These results show considerable lack of precision.

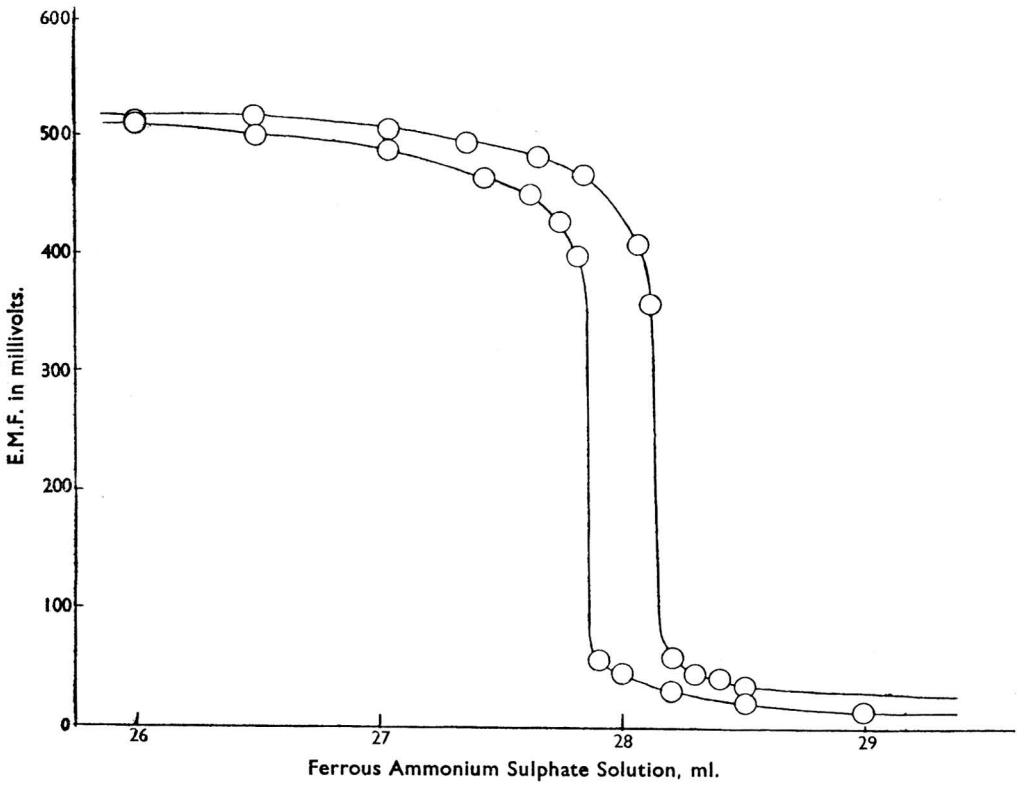

Fig. 1.

Owing to difficulty in observing the end-point, it was decided to carry out the titration potentiometrically. Treadwell and Vontobel[6] have described the potentiometric titration of nitrate with ferrous sulphate, using a Pb/PbO_2 electrode system, and found it necessary to titrate in an atmosphere of nitrogen. Though this precaution has been found essential on the micro-scale,[7] the work here reported shows that with the volumes and concentrations used an inert atmosphere is not necessary. In the present instance a platinum-tungsten electrode system was used (*cf.* Willard and Fenwick[8]).

Results with a precision within ±0·2% were obtained potentiometrically; the sharpness of the end-point is shown by the curves in Fig. 1.

TITRATION TO POTENTIOMETRIC END-POINT—*Apparatus*—The titration was carried out in a 300-ml. CO_2 flask, fitted with a rubber bung through which passed a thermometer, the burette tip, a mechanical stirrer, a platinum and a tungsten electrode, which were connected (platinum to positive) to a Cambridge *p*H meter adjusted to read in millivolts.

Reagent—At this stage in the work ferrous sulphate was replaced by ferrous ammonium sulphate, which had the advantage of increased stability to atmospheric oxidation. It was made up as described for ferrous sulphate.

Standardisation—Weigh accurately about 0·6 g. of dry potassium nitrate into the titration flask, dissolve in 100 ml. of concentrated sulphuric acid, and titrate with the reagent until the potentiometer shows a sudden drop in E.M.F. from about 400 to 140 mv. This is taken as the end-point. Maintain the temperature below 45° C., and allow it to fall to a little below 40° C. near the end-point; this is achieved by cooling the flask in ice-water. Allow a total time of 15–20 mins. for the titration, which uses approx. 30-ml. of reagent.

Procedure—Treat nitroguanidine as above. The end-point is taken as the drop in E.M.F. which is not followed by a rise. The drop is from about 300 to 170 mv.

Results—Typical results obtained with a sample of nitroguanidine were as follows: % nitroguanidine, 99·80 99·53; 99·70; 99·82; 99·72; 99·77; 99·70. Determination of impurities in this material showed that it was 99·85% nitroguanidine. The precision and accuracy of these results are excellent.

The reaction has been stated[1] to be a general one for nitramines. Davis and Abrams[9] have shown that the concentration of nitrate in solutions of nitroguanidine is sufficient to effect certain nitrations. This is of course only a particular example of the general reaction discovered by Thiele and Lachmann, which involves the production of nitrate in sulphuric acid by all compounds containing the $N.NO_2$ grouping. For nitroguanidine, it is suggested that the compound is "de-arranged" into nitramide and cyanamide. The former functions as a source of nitric acid by a process of hydrolysis, apparently only so long as free nitric acid is removed from the system. This latter condition is obtained when a substance capable of nitration is present; or, as in the present instance, when the nitric acid is immediately reduced by the ferrous ion. Davis and Abrams's mechanism is feasible with nitro-guanidine and nitrourea, (I) and (II), but is obviously not applicable to such compounds as nitrourethane (III) or "tetryl" (IV), both of which give the Thiele and Lachmann reaction, though not quantitatively under the conditions of the test. These compounds would require to undergo hydrolysis in order to give the intermediates postulated.

(I) $H.N(H).C(NH)NHNO_2 \longrightarrow NH_2NO_2 + HN{:}C{:}NH$

(II) $H.N(H).CO.NHNO_2 \longrightarrow NH_2NO_2 + HN{:}CO$

(III) $EtO.C(=O).NHNO_2$

(IV) $(NO_2)_3.C_6H_2.N(CH_3).NO_2$

Summary—The work described was undertaken in order to find out whether a quantitative method for the determination of nitroguanidine could be founded on the observation that nitroguanidine, when dissolved in sulphuric acid, yields nitrate ion or a substance capable of yielding nitrate ion under suitable conditions. Solutions of nitroguanidine in concentrated sulphuric acid were accordingly titrated with ferrous salt solutions, using both a colour-change end-point and a potentiometric one. The colour-change end-point was found to be unsatisfactory, but the potentiometric method gave results which could be reproduced to within ±0·2%. The mechanism of the reaction is discussed.

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The Determination of Boron by the Use of Quinalizarin

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INTRODUCTION—Recently Owen[1] reported an experiment on the metabolism of boric acid in the milking cow, and Owen, Snow and Thom[2] reported experiments on the effect of borax on the growth and yield of field beans. The method which was established for the determination of boron in these experiments is described in the present paper.

Preliminary investigations showed that, owing to the difficulty of ascertaining exactly the initial and final end-points of the titration of boric acid, the well-known mannitol method was not sufficiently sensitive for estimating very small amounts of boron. The sensitive reaction of boric acid with quinalizarin[3,4] was therefore investigated.

Quinalizarin, 1:2:5:8-tetrahydroxy-anthraquinone, in concentrated sulphuric acid gives a bluish purple solution which becomes bluer in the presence of boric acid. This colour change has been used by several investigators for estimating boron. In the present experiments the reaction was adapted for use with the Spekker Absorptiometer.

MATERIALS—Preliminary attempts to determine boron in urine failed owing to solution of boron from the glass vessels which were used for the concentration of ammoniacal samples under reduced pressure. The amounts of boron dissolved from the glass were much greater than the amounts in the aliquots of urine taken for analysis. All efforts to obtain vessels made of glass not containing boric oxide were unsuccessful, although glass tubing without this disadvantage was readily procured. The difficulty was overcome by employing vessels of fused silica and of pure nickel. Copper or stainless steel could replace nickel, but copper vessels, such as are used for pyrolyses, were found unsuitable, presumably because borax had been used in soldering their joints.

REAGENTS—(1) *Stock Quinalizarin Solution*—0·5 g. of quinalizarin was weighed into a 100-ml. beaker, transferred to a 500-ml. glass-stoppered volumetric flask with 173 ml. of water and made up to volume with 98% sulphuric acid (AnalaR). Next day, after the mixture had cooled to room temperature, it was made up once again to volume with 98% sulphuric acid, mixed thoroughly and filtered through glass-wool into a dry glass-stoppered bottle. This reagent contained 78·1% of H_2SO_4.

(2) *Quinalizarin Reagent*—Acid was poured out of a freshly opened winchester quart bottle of 98% sulphuric acid (AnalaR) until the liquid remaining was level with the top of the vertical sides of the bottle. The bottle was then found to contain about 2·24 litres. The quinalizarin reagent was prepared by pouring 100 ml. of the stock quinalizarin solution into the bottle containing the 2·24 litres of 98% sulphuric acid and mixing thoroughly. This procedure was followed in order to minimise uptake of atmospheric moisture. The reagent thus contained 97·2% of H_2SO_4.

(3) *Standard Solutions of Boric Acid*—By dilution of an aqueous solution of boric acid (AnalaR) containing 1·6679 g. of boric acid per litre, solutions containing 25, 50, 75, 100, 150 and 200 μg. of boric acid, *i.e.*, 4·38, 8·75, 13·13, 17·50, 26·25 and 35·00 μg. of boron per 3 ml., were prepared. These solutions were used in the preparation of standards for the colorimetric estimation of boron.

(4) *Orthophosphoric Acid*—100% orthophosphoric acid was used without further purification. Different batches of the acid gave different blanks.

(5) *Methanol*—This was purified by first allowing it to stand over granulated calcium oxide, with intermittent shaking, for a day or two, and then distilling through a fractionating column.

(6) 0·1 *N Sodium Hydroxide*—AnalaR sodium hydroxide, in sticks, was dissolved in water in a 300-ml. nickel crucible to form a saturated solution, which was diluted with freshly boiled distilled water. When this procedure was followed no boron was detected in the reagent.

(7) *α-Naphtholphthalein*—A 0·2% solution in 50% ethyl alcohol.

(8) *Sulphuric acid*—AnalaR sulphuric acid was used.

PREPARATION OF SAMPLES FOR ANALYSIS—Materials rich in boron, *e.g.*, samples of heat resistant glass, silicates, or soil, were analysed by grinding them to a powder and then subjecting them to fusion with pure sodium carbonate[5] in a platinum vessel. The cool melt was dissolved in water, neutralised with sulphuric acid and then considerably diluted. The diluted solution was used, without distillation, for colorimetric assay.

Plant materials, *e.g.*, leaves, seeds or stems, were lightly ashed in vitreosil basins, first over a bunsen burner with the air-vent closed and then at a dull red heat in an electric muffle furnace. The resulting ash was friable and easily removed from the basin. By means of a pestle the ash was ground to a fine powder while still in the basin and either all of it or a known fraction was subjected to distillation in the apparatus about to be described.

Before milk was ashed, in order to prevent possible loss of boron, calcium hydroxide was added at the rate of 4 g. per litre and followed by concentrated ammonia until the smell of ammonia persisted. The milk was then evaporated to dryness on a sandbath, with occasional stirring to break the encrusted surface. The ashing of the dried material from 50–200 g. of milk was completed in small vitreosil basins. The subsequent procedure was the same as that for other biological materials.

Borax excreted in the dung of cows was determined by distillation of material which had been dried overnight in an electric oven at 100° C. The urine of cows was evaporated to dryness after spontaneous ammoniacal fermentation. This fermentation was found to be necessary to prevent loss of boron by evaporation. Either the residue or its ash was found suitable for distillation with methanol and phosphoric acid.

The ground ash, or if suitable (see above) the ground dried material, was transferred into a 500-ml. dry silica flask. The vitreosil basin was washed with 10 ml. of methanol, which was then poured on to the ash in the silica flask. The wetting of the ash with methanol before it came into contact with orthophosphoric acid prevented the ash from caking. In a measuring cylinder 10 ml. of 100% orthophosphoric acid were dissolved in 30 ml. of methanol and with this solution the remaining contents of the basin were washed into the distillation flask. Finally a further 50 ml. of methanol was used to rinse the measuring cylinder and the basin. Thus the total volume of methanol used was 90 ml.

DISTILLATION APPARATUS—The distillation apparatus (see Fig. 1) was a modification of that of McHargue and Calfee.[6] All connections in the apparatus were made with rubber tubing and rubber bungs.

The 500-ml. distillation flask A was of transparent fused silica. The receiver B was of pure nickel and was made by soldering the lid on to a 300-ml. crucible and providing it with two outlet tubes of copper. Both A and B were immersed in water-baths. When the still was in use methanol vapour from A was condensed by the condenser C and passed into B. In the nickel receiver B were 40 ml. of 0·1 *N* sodium hydroxide, which hydrolysed the methyl borate, and 2 drops of the α-naphtholphthalein. Methanol vapour from B passed back through the by-pass tube into the vertical double-surface condenser above A, and after condensation passed into the tube D and thence back into the reaction mixture in A. The tube conducting the methanol from D to A was fitted at its lower end with a rubber bunsen valve F to prevent the vapour of methanol from leaving A except by way of the condenser C. Thus the apparatus provided a closed system for the continuous extraction of boric acid from the sample and phosphoric acid in A. The continual dropwise return of condensed methanol into A minimised "bumping." The tube between C and B contained a bulb of 100-ml. capacity to prevent any possibility of liquid in B being sucked back into A. After 2 or 3 hours the extraction was discontinued by removing the heat from A. Increase of the heat under B then served to remove methanol from it and to concentrate its contents to any desired volume. The safety tube E had a spring clip which could be released to admit air to A during cooling,

either after the completion of a distillation or when cold water was being added to the water-baths.

The α-naphtholphthalein indicated the reaction of the contents of the receiving vessel during the distillation (the colour could be observed during momentary suck-backs into the expansion bulb, due to irregularity of boiling) and also served to mark the end-point of the subsequent neutralisation with sulphuric acid. If in the course of a distillation the solution in the receiver became colourless, the extraction was interrupted and more 0·1 *N* sodium hydroxide was added to the contents of the receiver. This was, however, seldom necessary.

The final volume of distillates was such that, after being neutralised with sulphuric acid and made up to a suitable volume (say 50 ml.), they contained from 3 to 35 μg. of boron in 3 ml.

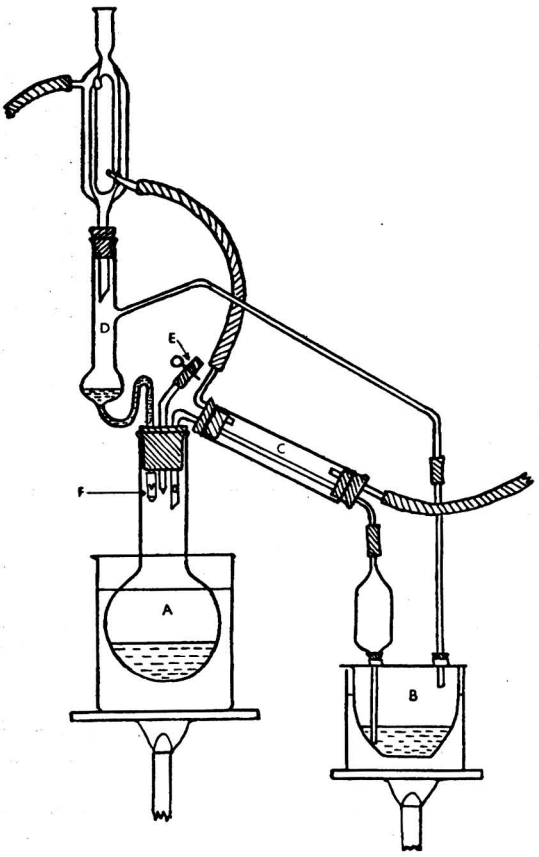

Fig. 1. The Distillation Apparatus.

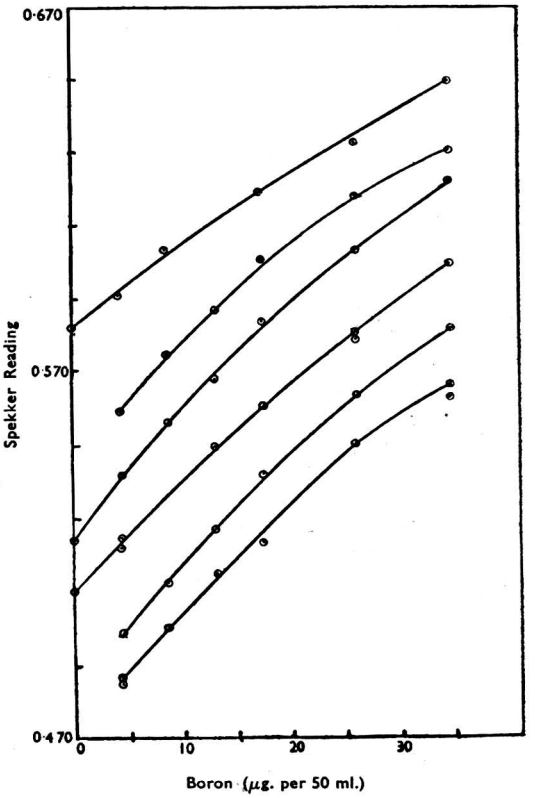

Fig. 2. Calibration curves of the Spekker Absorptiometer for the same standard solutions of $B(OH)_3$, but different batches of quinalizarin reagent.

The distillates were removed from vessel B as follows. B was detached after the apparatus had cooled and its contents were transferred to a 50-ml. flask. The expansion bulb was then removed and rinsed with distilled water into vessel B. The lower portion of the by-pass tube was also removed and rinsed similarly into B. Two tightly-fitting rubber bungs were then placed in the exits of B and the contents were thoroughly shaken and transferred into the 50-ml. flask. This process, including the rinsing of the expansion bulb and by-pass tube, was repeated until B had been rinsed with five successive lots of distilled water. The contents of the flask were then neutralised, first with drops of strong H_2SO_4 and then with dilute alkali, and adjusted to the mark.

Blanks were estimated by subjecting a solution of 10 ml. of phosphoric acid, in 90 ml. of methanol, to distillation. For any one batch of phosphoric acid the blanks were comparable, but different batches gave the following blanks: 111, 291, 105, 70, 115 μg. of boron per 10 ml. of phosphoric acid.

COLORIMETRIC—Three ml. samples of the standard solutions of boric acid and of the distillates were transferred to 50 ml. glass-stoppered flasks. The winchester quart bottle in which the quinalizarin reagent was stored was fitted with a rubber bung through which passed a siphon-tube controlled by a glass tap, and an air-inlet tube connected to a U-tube containing glass wool and 98% sulphuric acid. When the siphon was running, air entering the bottle was freed from moisture by the sulphuric acid in the U-tube. The free end of the siphon passed through a second rubber bung. When the apparatus was not in use a closed tube was slipped over the end of the siphon and on to this latter bung, and a spring-clip was placed on a rubber tube fitted to the inlet of the U-tube. By means of this apparatus moisture from the atmosphere was prevented from reaching the reagent and the reagent itself made contact with glass only. By being momentarily loosened the tap of the siphon was lubricated with some of the quinalizarin reagent.

Three or 4 ml. of the quinalizarin reagent were run into each of the 50-ml. flasks and the stoppers quickly replaced. The reagent was carefully mixed with the 3 ml. of aqueous solution by slowly rotating the flask about its central axis, while holding it inclined at 45°. Care was taken to avoid turbulence in the contents of the flasks, which would cause a sudden evolution of heat and a consequent generation of water vapour from some of the water before it had had time to mix with the sulphuric acid.

The flasks were then set aside to cool. After the flasks had all been filled with the quinalizarin reagent they were allowed to stand at room temperature for at least an hour, or preferably overnight in a desiccator with concentrated sulphuric acid. Some contraction of the contents then took place and the temperatures of the various flasks became equal. Their contents were made up accurately to the mark and were then ready to have their absorptions compared in a Spekker Absorptiometer. To do this the 1 cm. rectangular-sided cell of the Spekker Absorptiometer (capacity about 8 ml.) was filled with the solution to be tested. If the cell had already been used for a previous determination, it was rinsed four or five times with the solution under test, before the final filling. The cell was then covered with a ground glass lid and the absorption of its contents was measured in the Spekker. The remaining two cells of the Spekker contained distilled water. The No. 1 colour filters were used, and the instrument was calibrated for the current batch of quinalizarin reagent.

Fig. 2 shows the relationship between the Spekker readings and the amounts of boron in the 3 ml. samples. It is noticeable that the calibration curves show a slight departure from Beer's Law. The points shown on the graphs at zero concentration were obtained from solutions made by neutralising 40 ml. of 0·1 *N* sodium hydroxide with sulphuric acid and making up to 50 ml. They demonstrate that the boron content of the sodium hydroxide was negligible.

A new set of colour standards was prepared for every new batch of quinalizarin reagent. The calibration curves shown in Fig. 2 were obtained with the same set of standard solutions of boric acid but with different batches of quinalizarin reagent. Different batches of quinalizarin reagent gave different calibrations, as illustrated by the data in Fig. 2. The variation of position of the curves in Fig. 2 is only partly attributable to variation in strength of sulphuric acid, but shows the inadvisibility of using either permanent or artificial standards[9] for routine estimations of boron. A more probable cause is the varying solubility of the quinalizarin in the acid. The graphs may be compared with those of Hove, Elvehjem and Hart[7] and of Olsen and De Turk.[8]

RESULTS

THE EFFECT OF WATER—The effect of the concentration of water in the quinalizarin reagent on its chromogenic power was investigated. Increasing amounts of water were added to 25-ml. flasks in which the colour was produced by 1·5 ml. of solution containing 12·5 μg. of $B(OH)_3$. The results of this experiment are recorded in Table I, which shows that the displacement of the Spekker reading by as much as 2% by weight of added water was not as large as was expected.

RECOVERY OF BORON—79·8 mg. of boric acid were mixed with 2 g. of anhydrous sodium carbonate in a platinum crucible and fused in an electric muffle. The melt was transferred to a 2-litre flask, neutralised with sulphuric acid and made to volume. Three-ml. samples of the solution were taken for colorimetry. Duplicate blank determinations made on the sodium carbonate showed no boron in it. The Spekker readings were compared with those of the standards containing 100 and 150 μg. of boric acid prepared at the same time. In

each estimation of the boric acid solution the Spekker readings were identical. The recovery of the boric acid was 99·1%. The amount of boron found in the 3 ml. samples taken in this experiment was 20·9 μg.

TABLE I

EFFECT OF EXTRA WATER ON CHROMOGENIC POWER OF 1·5 ML. OF SOLUTION CONTAINING 12·5 μG. OF $B(OH)_3$

Water added ml.	$B(OH)_3$ ml.	Quinalizarin reagent to volume ml.	H_2SO_4 in final test soln. % w/w	Spekker reading
0·0	1·5	25	94·0	0·339
0·1	1·5	25	93·8	0·332
0·2	1·5	25	93·6	0·329
0·4	1·5	25	93·2	0·311
0·7	1·5	25	92·7	0·302
1·0	1·5	25	92·1	0·274

Table II shows the percentage of boric acid recoverable by 3 hours' distillation with 10 ml. of orthophosphoric acid and 90 ml. of methanol. The weights of boron shown in this table were measured by taking aliquots from a methanol solution containing 500 mg. of boric

TABLE II

RECOVERY OF BORON BY DISTILLATION FROM A METHANOL SOLUTION CONTAINING 500 MG. OF $B(OH)_3$ PER LITRE

Micrograms of Boron		
Taken	Found (corrected for blank)	Percentage recovered
263	248	94·3
263	237	90·0
438	397	90·7
438	400	91·3
875	740	84·6
875	770	88·0

Note—All figures are corrected for the blank on reagents. On distilling 90 ml. of methanol with 10 ml. of H_3PO_4, in duplicate, 88 μg. and 78 μg. of boron respectively were obtained. The average of these, *i.e.*, 83 μg., was therefore subtracted from the amount of boron found, in order to obtain the figures in the middle column of this Table.

acid per litre. The Table illustrates the difficulty, also encountered by numerous other investigators, of recovering boric acid quantitatively by distillation with methanol.

The recovery by distillation of boron from urine, faeces and milk of cows was tested by addition of a solution of borax, containing 1·0296 g. per litre. These materials were evaporated to dryness in nickel vessels at an alkaline *p*H both in the presence and in the absence of the

TABLE III

RECOVERY OF BORON BY DISTILLATION

	Before addition of boron		After adding boron				
Material	Weight of substance analysed g.	Boron found p.p.m. (*a*)	Amount of boron added p.p.m. (*b*)	Weight of substance analysed g.	Boron found p.p.m. (*c*)	Extra boron found, p.p.m. (c−a)	% Boron recovered 100(c−a)/b
Urine of cow	50	2·3	116·9	5	86·4	84·1	71·9
	50	2·7	116·9	5	86·4	83·7	71·6
Faeces of cow	27	1·4	21·6	27	19·2	17·8	82·4
	27	0·8	21·6	27	19·2	18·4	85·2
Milk of cow	60·2	none	5·34	67·4	4·1	4·1	77
	60·2	,,	5·34	67·4	4·1	4·1	77

Note—All figures are corrected for blanks on the reagents but not for incomplete recovery on distillation.

added boron. The results in Table III show that the recovery of added boron from biological material (72–85%) was somewhat less than the recovery from the pure samples of boric acid (85–94%, Table II).

In Table IV the results of analysis of some feeding-stuffs are shown. The replicate samples analysed were taken from the same main sample after it had been ground to a fine powder in a Christie and Norris laboratory mill. The results have not been corrected for incomplete recovery on distillation but experiments showed that over 90% of the borax added to feeding stuffs could be recovered by distillation. All samples were distilled under the same conditions, the volumes of orthophosphoric acid and methanol used being 10 ml. and 90 ml. respectively. Thus the same reagent blank was subtracted in every case. The results show that with boron contents ranging from 15 to 47 p.p.m. replicates may differ from one another by 11 to 14%. Where the boron contents are considerably lower, as with wheat bran and cocksfoot and clover mixtures, the replicates are of the same order though the percentage difference between them may be relatively high.

TABLE IV

BORON IN PLANT MATERIALS

Feeding stuffs:	p.p.m.	Pastures:	p.p.m.
Beetpulp	33·3	1. Ryegrass + clover mixture	1·5
	30·3		1·5
	30·3	2. Cocksfoot + clover mixture	2·9
	34·6		4·0
Earthnut meal	41·6		
	46·7		
Vetch leaves	15·3		
	17·8		
Wheat bran	3·2		
	4·7		
	3·5		
Oats (with husks)	nil		
	,,		
	,,		
	,,		
	,,		

Note—None of these figures is corrected for incomplete recovery of $B(OH)_3$ by distillation, but all are corrected for the blank on the reagents, which were in duplicate, 79 μg. B and 73 μg. B, respectively.

As has been recorded by Sinyakova,[10] the boron content of material from dicotyledonous plants (beet, earthnut and vetches) is found to be high compared with that of cereals (wheat, oats, etc.). No boron could be detected in oats.

DISCUSSION AND GENERAL CONCLUSIONS

Many authors, among them Rader and Hill,[11] and Hove, Elvehjem and Hart,[7] have called attention to the fact that the recovery of boron on distillation is not complete. The figures in Table II and III demonstrate this phenomenon. No satisfactory explanation of the incomplete recovery has yet been advanced. Distillation is, however, essential in order to separate boron from substances such as carbon and salts in the ash, which would interfere with the colorimetry, and from germanium which, like boron, gives the quinalizarin reaction.[3] Germanium also resembles boron in giving the mannitol reaction.[3]

In routine distillations of boron it was found that the mixture being distilled attained a temperature of 73° C. Germanium chloride boils at 83° C. In view of the nearness of these two temperatures the possibility of interference from germanium was investigated. A sample of sodium germanate was prepared from 2·5 mg. of pure germanium metal (Hilger's spectroscopically standardised substances, Laboratory No. 9237), and sodium peroxide (AnalaR). On distillation with methanol and phosphoric acid in presence of chloride the germanium was retained in the distillation flask. A solution of sodium germanate prepared from the same source was tested with quinalizarin reagent without distillation. The colour produced was of density comparable with that produced by boron if the results were compared on an atom for atom basis. Since, however, the atomic weight of germanium is 72·6 while that of boron is only 10·82 the sensitivity of the reaction with germanium was much less on a weight for weight basis.

The colour development when boric acid reacts with quinalizarin is inhibited by the presence of fluoride. No means of preventing this interference has been found. Interference

by fluorine makes the present method inapplicable to the analysis of superphosphate or rock phosphate. In attempts to apply the method to superphosphate it was found that fluorine passed into the distillate.

Nitrates interfere with the colour reaction by bleaching the quinalizarin but they are readily destroyed by ashing.

The accuracy of the method which has just been described is limited by the magnitude of the blank determination which makes it very difficult to estimate boron in materials containing less than 3 p.p.m. This difficulty could be partly obviated by the construction of a smaller still and using less of the reagents or alternatively by using more highly purified phosphoric acid. A great saving of time in routine estimations would result if the final colorimetric solutions were prepared in standard flasks of such a shape that they could be placed directly in the Spekker for comparison of the colours. Wartime difficulties in obtaining materials made it impracticable to give more serious attention to these possibilities.

SUMMARY—The micro-determination of boric acid by its colour reaction with quinalizarin has been studied. The boron was isolated either from the material itself or from its ash by distillation with a solution of phosphoric acid in methanol. The distillate was collected in a solution of sodium hydroxide. After removal of the methanol, aliquots of the distillate were treated with a solution of quinalizarin in concentrated sulphuric acid. The colour so developed was compared in a Spekker Absorptiometer with the colours of similarly prepared standards.

The boron contents of some biological materials are reported.

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September, 1945

DISCUSSION

Mr. A. MORE said he had had an opportunity to read this paper beforehand. The large range in calibration values, indicated in Fig. 2, suggested investigation of the variants in the test. The well-known effect of the strength of the sulphuric acid had been looked into by the author, and had been effectively guarded against, both by his method of storing the quinalizarin reagent and by the conditions laid down for mixing the strongly acid reagent with the test solutions. The author had also confirmed that standard solutions of boric acid had not lost boric acid by reaction with the glass of the container when left for as long as 2½ years. It would appear, therefore, that the variations must be due to variations in the solubility or stability of the quinalizarin in the sulphuric acid used in making up the reagent. However, the author had specified conditions under which he could determine the amount of boron with accuracy. With reference to the author's method of preparing samples for distillation, there was no question that they should be alkaline at all stages of the drying process. As in the determination of traces of chlorides, however, he (Mr. More) would prefer to have samples containing traces of boric acid strongly alkaline before ashing.

Dr. W. F. ELVIDGE asked what colour filters the author had used in the photometry. He had found that by careful choice of filters, absorbing at the correct wavelengths, the colour due to alizarin could be practically completely cut off.

Mr. J. HASLAM said he understood that the author prepared a fresh calibration curve for each batch of reagent. Had he studied the variation of the curve with time? *E.g.*, would the same curve be obtained after the lapse of a fortnight? Also, had the author examined the silica basins used in the ashing process for the presence of silicyl borate in them?

Mr. J. H. HIGH suggested that variations in the calibration could be eliminated by setting the Spekker with a boron standard solution instead of water.

Mr. R. F. MILTON also asked if the differences in the graphs with different batches of reagent would be apparent if Spekker differences (*i.e.*, with boron readings as blanks) were taken.

Mr. W. FURNESS asked if the author had a particular preference for the use of phosphoric acid in the distillation flask, as it seemed the main cause of a blank of about 80 μg. of boron. If the phosphoric acid could not be purified before use, would not a slight excess of sulphuric or hydrochloric acid have been equally satisfactory? He (the speaker) had been interested in the estimation of small quantities of boron of the order of 1–10 μg. and had been able to recover 75–90% of the boron present by distillation in a stream of methanol vapour from an aqueous solution just acid to methyl red with hydrochloric acid, and in such experiments his blanks had been less than 0·5 μg. of boron. Commenting on the author's results for the

boron content of samples of oats and ash or faeces, he wondered whether boron had been lost by volatilisation during preparation of the ash, and said that his experience had shown that certain carbonaceous materials should be ashed in presence of an alkali, *e.g.*, calcium hydroxide, in order to avoid or materially reduce loss of boron by volatilisation.

Dr. OWEN, replying, thanked Mr. More for his criticism of the paper before it was read at the meeting, and said that he greatly appreciated the suggestions Mr. More had made. He (Dr. Owen) was satisfied that either the solubility or the stability of the quinalizarin was responsible for variations of the positions of the calibration curves, though he regretted that pressure of other work had prevented him from investigating the point raised by Mr. Haslam about the keeping quality of the reagent. Neither had he investigated the suggestions of Mr. R. F. Milton or of Mr. J. H. High. Hove *et al.* had set their photoelectric colorimeter against concentrated sulphuric acid instead of water but he failed to see the wisdom of such a procedure. Probably the setting of the Spekker in the way suggested by Mr. High and Mr. Milton would serve to eliminate the wide variations of position of the calibration curves but it would not obviate the necessity for using the same batch of sulphuric acid both for standardisation and for determination of boron.

Mr. More had referred to the keeping quality of boric acid standards. Each of the standards was stored in a separate litre bottle. Boric acid rather than borax was chosen for the standards in order to avoid contact of the alkaline borax with the glass. When the standard solutions containing 200 μg., 100 μg. and 50 μg. $B(OH)_3$ were diluted 1 : 8, 1 : 4 and 1 : 2 respectively the resulting solutions gave the same reading as the 25 μg. standard. These standards being 2½ years old, this experiment demonstrates the absence of interaction of the boric acid and the glass.

With reference to Mr. More's remark as to alkalinity during all stages of ashing there was no doubt that it was desirable, the best method being fusion with sodium carbonate. When, however, large numbers of samples were to be analysed the choice of vessels for ashing was a limiting factor. Platinum would be ideal but cannot be used on account of expense. Nickel is unsuitable. Thus vitreosil was used for all ashings. When no addition was made before ashing materials the *p*H of the final ash was always tested and found to be alkaline. In ashing such materials as leaves, stems and seeds intimate mixture of them with a dry alkali such as lime, to which Mr. Furness had referred, is difficult and, for routine analysis, very time-consuming. On the other hand addition of an aqueous alkali means that the resulting ash makes intimate contact with the vitreosil, a result not to be desired. Mr. Haslam had asked about the formation of silicyl borate. Dr. Owen had not specifically investigated this point but wished to stress that all ashings had been completed in an electric muffle at a temperature so controlled that there was at the end of determination no sign of interaction of the resulting ash with the silica of the vitreosil dish. He had, nevertheless, found it advisable to analyse cows' faeces by drying it at 100° C. and then subjecting it to distillation without ashing. Of all the materials investigated such faeces would be richest in constituent silica, which may have served to bind the boron in a non-distillable form. Such material could, of course, be analysed by fusion with sodium carbonate in a platinum vessel. Oats, in which he had failed to demonstrate boron resembled other cereals in being rich in constituent silica. Thus, in answer to Mr. Furness, he could not say whether the absence of boron in oats was real or apparent as he had not yet carried out fusions of them with sodium carbonate. If there were traces which he had not been able to detect he could not say whether the supposed failure of detection was due to fixation by silica or to volatilisation. He was grateful for Mr. Furness's suggestion for reducing blanks to negligible proportions. He had used 100% H_3PO_4 for two main reasons: (1) because many investigators advised against presence of water in the still and (2) because H_3PO_4 did not itself form esters volatile under the conditions used in distillation. In answer to Dr. Elvidge, Dr. Owen stated that he used the red filters No. 1 in colorimetry. Of the set of glass filters supplied with the Spekker these gave the best calibration curve. He had made no attempt to obtain a more highly selective filter.

The Gravimetric Determination of Tin in Alloys by the Tannin Method

BY W. R. SCHOELLER AND H. HOLNESS

IN a recent paper[1]—the third on the subject of further analytical applications of tannin—we have advocated the use of the reagent for the gravimetric determination of tin and its separation from a number of metals, having satisfied ourselves that the tin-tannin complex is a much more tractable precipitate than either metastannic acid or stannic sulphide and is free from occlusions after one precipitation at the correct acidity. Our test analyses were conducted on pure solutions of known metal content. We have now elaborated and tested methods for the determination of tin in the following alloys: white metal (lead base), brass, bronze, and zinc-base die-casting alloy. For the first three, we have used the standardised alloys supplied by the Bureau of Analysed Samples, Ltd. (Messrs. Ridsdale & Co.).

Before proceeding to describe the new methods we may usefully explain their common features, *viz.*, the elimination of antimony if present and our perfected procedure for securing acidity control of the solution during tannin precipitation. This improved technique simplifies the assay and makes it unnecessary to test the tannin filtrate for complete precipitation.

A. SEPARATION OF TIN FROM ANTIMONY—In the determination of tin in alloys by the tannin method antimony is the most likely interfering element. It is co-precipitated to a

certain extent from either chloride or oxalate solution, and although some antimony volatilises during ignition of the tannin precipitate the tin results nevertheless always show a substantial positive error. Fortunately such contamination of the ignited stannic oxide (which is white when pure) is detected with certainty by its greyish-blue to dark blue tint.[2]

In order not to complicate the tin assay of alloys containing several metals of the hydrogen sulphide group, we decided to avoid the separation methods based on the precipitation of antimony sulphide from oxalic,[3] hydrofluoric,[4] phosphoric[5] or 4 *N* hydrochloric acid[6] solution. This led us to adopt metallic precipitation of the antimony, and in our early test analyses of the white metal (*C*) we used very fine soft iron wire for that purpose. Some of our results were fairly close, but others were definitely low, and in every determination the ignited stannic oxide was slightly discoloured. It became evident that a small variable amount of tin was being co-precipitated with the antimony, and the latter was proved to re-dissolve to a small extent during the washing on the filter. By a compensation of errors of opposite sign, seemingly accurate results were obtained in some of the tests. These observations confirmed the conclusions of Järvinen's careful study of the separation of tin from antimony by reduced iron.[7] We then investigated the action of nickel, shown by Evans and Higgs[8] not to induce co-precipitation of tin with antimony or copper. The result was very satisfactory, the tin figures becoming concordant and the white colour of the ignited stannic oxide proving its freedom from antimony. Evans and Higgs's investigation appears to us to be an important contribution to the analytical chemistry of tin and to establish the superiority of nickel over other metals used as reducing agents in the volumetric iodine method.

B. Acidity Control during Tannin Precipitation—The vital adjustment of acidity required for quantitative tannin separations is obviously very much simplified if the initial acidity of the solution is accurately known. Such is not the case when the solution to be precipitated has been obtained by an acid attack of the assay material, a large excess of acid being normally used and an indefinite fraction of this eliminated by evaporation. The optimum acidity must then be approached with care, and complete precipitation ascertained by testing the filtrate from the tannin precipitate. Since in rapid and serial routine work it is highly desirable to be able to dispense with such tests, we have been at pains to work out a procedure in which the acidity is under complete control: the tin is first recovered from the solution of the alloy in the form of an ammonia or "basic acetate" precipitate. This need not be pure—in fact the presence of iron is desirable and we add it if necessary. It acts as a sensitive indicator and thus provides an additional safeguard against accidental over-neutralisation, which will cause discoloration of the white tin-tannin complex.

Procedure—Wash the ammonia or basic acetate precipitate a few times with water and return it to the 600-ml. precipitation beaker provided with a mark at the 250-ml. level. Dissolve the precipitate in a boiling-hot solution of 3–5 g. of ammonium oxalate in 50 ml. of *N* hydrochloric acid, which is poured through the filter. Thoroughly wash the filter with hot water, and reserve it. The beaker now contains the whole of the tin in an oxalate solution of known acidity. Dilute the solution with 25 ml. of saturated ammonium chloride solution and hot water to about 200 ml., heat to boiling, and add 1 g. of tannin dissolved in a little hot water. Stir the boiling solution while adding 35–40 ml. of *N* ammonia previously standardised against the *N* acid, and adjust the volume to 250 ml. This results in a final acidity of 0·06 to 0·04 *N*, at which the tin precipitation is complete. The approach towards neutrality is indicated by the more gradual disappearance of the dark colour of the iron-tannin complex upon agitation; permanent discoloration of the precipitate must be avoided and does not take place if the above directions are followed. Allow the precipitate to settle, collect it in the reserved filter containing a little filter pulp, wash with 2% ammonium nitrate solution until free from chloride and ignite to SnO_2 in a tared porcelain crucible. The oxide should be white without any bluish-grey tinge.

C. White Metal (Lead Base "A")—The standard sample was certified to contain Pb 82·6, Sb 12·04 and Sn 4·64%, totalling 99·28%. The sequence of operations is as follows: solution in brominated hydrochloric acid (1+1); precipitation of the antimony with nickel; recovery of the tin from the filtrate by basic acetate precipitation and treatment of the precipitate as under *B*. Lead and nickel, which would interfere in the precipitation of the tin on account of the sparing solubility of their oxalates, are eliminated as soluble acetates.

Procedure—Dissolve 0·5 g. in 50 ml. of diluted hydrochloric acid (1+1) saturated with bromine; boil off excess bromine and dilute to 150 ml. Boil gently for ½ hour with 1 g. of nickel powder, filter hot through a pad of filter pulp sprinkled with nickel and wash with hot

N hydrochloric acid. To the boiling filtrate add 20 ml. of bromine water (or 5 ml. of conc. nitric acid), 10 g. of ammonium acetate and diluted ammonia (1+1) until most of the acid is neutralised; this is indicated by the incipient colour change from green to pale blue of the dissolved nickel salt. Restore the green colour, if necessary, with a minimum of acid. Allow the precipitate to flocculate in the hot solution, collect it on an 11 cm. No. 41 Whatman filter, wash a few times with hot water and proceed as under *B*.

Found: Sn 4·71, 4·78, 4·71, 4·67%.

We believe the true tin content of this alloy to be slightly higher than 4·7%. The certified figure, 4·64%, is the average of ten determinations by a variety of methods. The highest and lowest figures are 4·94% and 4·16% respectively and the probable error of the mean is ±0·06%.

D. BRASS—The certified figures for this alloy ("Manganese brass B") include Cu 58·8, Zn 33·9, Sn 1·75, Fe 0·91 and Sb 0·05% (total 95·4%).

The time-honoured method for the determination of the small quantities of tin in brass consists in nitric acid attack and ignition of the insoluble residue, which is weighed as SnO_2. The method is subject to more or less serious errors which may be either positive (occlusion of the oxides of other metals) or negative (presence of more than about 0·5% of iron, which interferes with the complete recovery of the metastannic acid[9]). For the accurate and rapid determination of tin in brass the method given below is probably unrivalled. If the brass is free from or very low in antimony a determination can be made within 3½ hours.

Procedure—Dissolve 1 g. of sample, together with about 0·05 g. of pure iron (6 inches of iron wire), in. 10 ml. of *aqua regia* in a 600-ml. beaker. Dilute to 200 ml., add 25 ml. of saturated ammonium chloride solution and precipitate the hot solution with excess of ammonia. Collect the precipitate on an 11 cm. No. 41 Whatman filter, wash to remove most of the copper and proceed as under *B*.

In three of our test analyses (all on 1 g. of brass) we added known amounts of AnalaR tin. The amount added was deducted from the total tin found.

Tin added g.	Total SnO_2 found g.	Equivalent to tin g.	Tin in brass %
none	0·0222	0·0175	1·75
,,	0·0222	0·0175	1·75
0·0150	0·0413	0·0325 − 0·0150 = 0·0175	1·75
0·0247	0·0540	0·0425 − 0·0247 = 0·0178	1·78
0·0431	0·0771	0·0607 − 0·0431 = 0·0176	1·76

The results are in excellent agreement and confirm the certified figure, 1·75%.

E. BRONZE "A"—The standard alloy is declared to contain Cu 85·5, Sn 9·96, Pb 1·83, P 0·25, Sb 0·24, and Fe 0·07%. When assayed for tin by the preceding method the results were high (10·15 and 10·20%) and the ignited stannic oxide was slate-grey. The method was therefore modified to include nickel reduction, the procedure being as follows.

Procedure—Dissolve 0·5 g. of alloy and 0·05 g. of iron in *aqua regia* and precipitate with ammonia as under *D*. Collect the precipitate, wash it free from most of the copper and dissolve the washed precipitate in 50 ml. of diluted hydrochloric acid (1+1). Reduce the solution with nickel powder and proceed as under *C*.

Found: Sn 9·71, 9·80, 9·69, 9·69, 9·71%.

We believe the certified figure, 9·96%, to be somewhat high, the *ten* analyses of which it is the average including *five* results exceeding 10%. The highest and lowest results given are 10·10% and 9·70% respectively.

F. ZINC-BASE DIE-CASTING ALLOY—The accurate determination of tin in this type of alloy (containing about 4% of aluminium, with or without copper) is of very great practical importance, since minute amounts of tin (more than 0·001%) are now known to produce most serious effects, such as inter-crystalline corrosion and gradual disintegration of the alloy. In actual practice the tin is detected spectrographically, but no chemical method for its accurate determination appears to be available. Neither the direct volumetric determination nor gravimetric methods, necessarily involving the separation of a trace of tin from large quantities of zinc, aluminium and probably copper, have given trustworthy results. We are satisfied that the simple tannin procedure given below offers a completely satisfactory solution of the problem.

Procedure—Cautiously dissolve 100 g. of the alloy in 700 ml. of diluted hydrochloric acid (1+1) in a 2-litre beaker, adding the acid in small portions at first. When attacked, add

bromine water or a little nitric acid to the hot liquid if copper is present, to complete solution; boil off bromine or nitrous fumes. Dilute to 1000 ml. and add 25 ml. of saturated ammonium chloride solution followed by 1 g. of tannin dissolved in a little hot water. Stir the boiling-hot solution and add 2 *N* ammonia slowly and with stirring until partial precipitation of the aluminium-tannin complex takes place (about 200 ml. are required). Digest to flocculate. Precipitate as much aluminium as can be easily accommodated on an 11 cm. No. 41 filter; it is better to add a slight excess of ammonia and adjust the quantity of precipitate by careful addition of *N* hydrochloric acid than to be over-cautious in the addition of the ammonia. Filter hot and wash with 2% ammonium chloride soln. Dissolve 5 g. of ammonium oxalate in 100 ml. of *N* hydrochloric acid in the 2-litre beaker and pour the hot solution on to the tannin precipitate on the filter, collecting the filtrate and washes (hot 2% ammonium chloride solution) in a 600-ml. beaker. Any specks of dirt or other insoluble particles present in the sample will be retained by the filter. To the hot filtrate (about 300 ml.) add 25 ml. of saturated ammonium chloride solution, and 5 drops of 10% ferric chloride solution. Should any zinc oxalate crystallise out it will redissolve during the subsequent reduction of the acidity. Stir the boiling solution, add 0·5 g. of tannin dissolved in 10 ml. of hot water, and *N* ammonia (75–80 ml.) until the discoloration due to iron fades rather slowly.

Set aside to flocculate on a hot plate and leave overnight at room temperature. Collect the precipitate on a 9 cm. No. 41 filter, wash it with 2% ammonium nitrate solution containing a few drops of nitric acid, ignite in a tared porcelain crucible and weigh as SnO_2.

Our test determinations were conducted on 100-g. portions of alloy spectroscopically free from tin, to which the quantities of tin shown in column 1 of the table were added.

Tin added	SnO_2 found	Equivalent to tin
g.	g.	g.
0·0004	0·0006	0·00047
0·0009	0·0013	0·00102
0·0013	0·0018	0·00141
0·0021	0·0026	0·00205
0·0039	0·0049	0·00386
0·0044	0·0056	0·00441

A sample of alloy containing tin was tested, with the following results (100 g. samples taken):

Tin added	SnO_2 found	Tin in alloy
g.	g.	%
none	0·0040	0·00315
,,	0·0040	0·00315
0·0019	0·0065	0·00512 − 0·0019 = 0·00322

Such effortless complete extraction of minute quantities of tin from 100 g. of alloy proves the above procedure to be a precision method which, moreover, utilises the simplest apparatus and ordinary reagents and renders the use of the costly spectrograph unnecessary.

SUMMARY—Rapid and reliable assay methods are given for the gravimentric determination of tin in white metal (lead base), brass, bronze, and zinc-base die-casting alloy. A procedure is described by means of which the optimum acidity for the quantitative separation and precipitation of tin by tannin is secured with certainty and ease, no test for complete precipitation being required.

We thank the Governors, Principal, and Head of the Science Department of the South West Essex Technical College for permission to carry out this investigation.

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The Determination of Selenium in Copper Mattes

By W. B. POLLARD

THREE methods have been used for the determination of selenium in copper mattes:

1. Attack with nitric acid.[1]
2. Extraction with potassium cyanide solution.[2]
3. Fusion with sodium peroxide.[3]

The first method was studied by a number of analysts using a very carefully prepared sample of O'Okiep copper matte. The matte was attacked in a mixture of nitric and sulphuric acids, residual sulphur was dissolved in fuming nitric acid and the solutions were treated by a method closely resembling that described by Challis[4] for the determination of selenium in copper. The results of the different analysts using this method vary very considerably.

A few determinations were also made on the same sample by the second method and certain difficulties in its use were reported.

At this stage the author was asked to continue the investigation and the same sample of O'Okiep copper matte was placed at his disposal.

It was decided to re-examine the third method although it had been adversely criticised.[1]

A modification (described below) has been devised which enables the peroxide oxidation to take place quietly, and by a simplified method of separation and titration the selenium can be determined in approximately four hours. This modification has been checked on synthetic mixtures of known selenium content.

The results obtained on the same sample of O'Okiep copper matte by all three methods are given below:

ANALYSES OF O'OKIEP COPPER MATTE

Analyst	Method	Finish	Selenium found, %
A	Nitric and sulphuric digestion	Gravimetric	0·031
B	,,	,,	0·030
C	,,	,,	0·027
D	,,	,,	0·039
E	,,	,,	0·037
F	Extraction with KCN soln.	,,	0·036
G	,,	,,	0·039
Author	Na_2O_2 and NaOH fusion	,,	0·041
,,	,,	Volumetric	0·041
,,	,,	,,	0·041
,,	,,	,,	0·040
C	,,	,,	0·040
,,	,,	,,	0·039
,,	,,	,,	0·040

IRON VESSEL FOR PEROXIDE FUSIONS—An iron vessel in the shape of a saucepan was made by cutting out the pieces from 16-gauge mild steel sheet and welding them together. The dimensions were:

Diameter	5 in.
Height	3¼ ,,
Length of handle (steel tube) ..	6½ ,,
Width of spout at base	1½ ,,
Length of spout	2 ,,

The spout was turned downwards at the tip to allow the extract and washings to be poured without risk of spilling them down the side. For convenience in washing, the handle should be placed so that the vessel pours left-handed.

A stirrer of ¼ in. iron rod with a small "T" welded to the end was found convenient for rubbing down any lumps left after extraction.

METHOD

Fuse 75 g. of sodium hydroxide in the iron saucepan over a large flame, cool and allow to set. Add 20 g. of powdered copper matte, re-fuse and mix by rotating the saucepan over the flame. Add 40 g. of sodium peroxide in 5-g. portions at a time (the only sign of reaction is a faint reddish glow at the point where the peroxide falls). Keep the melt rotating till it

becomes viscous; then stir well with an iron stirrer, but do not use this at the beginning or lumps will be produced.

Allow the melt to cool, cover the saucepan with a clock glass, add 200 ml. of water and heat gently until the sodium salts have dissolved and oxygen ceases to be given off. Stir well and pour the contents into a 1500 ml. beaker. Rub down any small lumps of oxide and wash all sediment into the beaker, keeping the total volume below 400 ml.

Dilute 350 ml. of hydrochloric acid (sp.gr. 1·18) with 300 ml. of water and pour this down a glass rod into the covered beaker. Stir the solution meanwhile from time to time to prevent a sudden evolution of gas. Rub any small lumps with the glass rod, so that these will pass into the green solution leaving only a trace of scale and the like undissolved.

Add a little asbestos pulp and then a clear saturated solution of stannous chloride in hydrochloric acid (sp.gr. 1·18) until the copper and iron salts have been reduced and the solution is almost colourless. Add a further 10 ml. of stannous chloride solution, heat to boiling and then allow to simmer for 15 minutes to precipitate the selenium and tellurium in combination with copper as described by Schoeller.[5] Filter the hot solution on a Gooch crucible (3 cm. diam.) and wash the precipitate three times with 2% hydrochloric acid.

Fix the Gooch in a 100-ml. receiving bottle by means of a two-hole rubber stopper and pour 2 ml. of 10% bromine in hydrobromic acid (sp.gr. 1·46)[4] into the Gooch; cover with a watch glass and allow to stand for two minutes. Apply gentle suction and then wash four times with 5-ml. quantities of hydrochloric acid (sp.gr. 1·18).

Add to the filtrate a little asbestos pulp suspended in hydrochloric acid (sp.gr. 1·18) and precipitate the selenium free from tellurium, copper, etc., by Lenher and Kao's method[6] with a saturated solution of sulphur dioxide in hydrochloric acid (sp.gr. 1·18). Warm the solution to between 25° and 30° C. and filter on a Gooch (3 cm. diam.). Wash out the precipitation bottle four times with 5-ml. portions of hydrochloric acid (sp.gr. 1·18) and transfer these to the Gooch, but do not attempt to remove the film of selenium on the inside of the bottle. Reserve the filtrate for the determination of tellurium should this be required.

Wash the selenium with water and test the washings to make sure that all copper has been removed. Fix the Gooch in the 100-ml. bottle in which the selenium was precipitated, add 1 ml. of 10% bromine in hydrobromic acid, cover with a watch glass and allow to stand for two minutes. Apply gentle suction and then wash with water until the washings reach a 50-ml. mark scratched on the outside of the bottle. Add 2 ml. of hydrochloric acid (sp.gr. 1·18) and then 2 ml. of a 12% solution of sodium salicylate. Close the bottle with a rubber stopper, shake well and allow to stand two minutes while the bromine is being removed and determine the selenium by Berg and Teitelbaum's method[7] as follows.

Add 5 ml. of 4% potassium iodide solution and then 4 ml. of carbon tetrachloride. Close with a rubber stopper, shake well and titrate the liberated iodine with sodium thiosulphate solution (12·5 g. per litre); shake well after each addition and add starch indicator as the end point is approached.

To standardise the thiosulphate solution weigh 0·25 g. of pure selenium powder into a beaker and dissolve it in 2 ml. of nitric acid (sp.gr. 1·42). Evaporate off all nitric acid on a covered water bath. Dissolve the residue in water and make up the volume to 250 ml. (1 ml. ≡ 1·0 mg. Se). Transfer 10 ml. of the solution to the 100-ml. bottle, add water to the 50-ml. mark and then add 1 ml. of 10% bromine in hydrobromic acid, 2 ml. of hydrochloric acid (sp.gr. 1·18) and 2 ml. of 12% sodium salicylate and proceed exactly as described above.

TESTS OF THE METHOD—Owing to difficulties in preparation, it was decided not to attempt to produce a matte with a definite known selenium content, as this would have been open to doubt unless checked by a proved method of analysis.

Ferrous sulphate and copper sulphate can be obtained free from selenium, and if these in the form of powder are fused with caustic soda and sodium peroxide and the water extract of the melt is acidified with hydrochloric acid the solution will closely resemble one obtained from a matte. Known amounts of a standard selenate solution can be added at this point and the analytical procedure checked with absolute certainty. Using 10 g. of copper sulphate and 10 g. of ferrous sulphate, the following results were obtained:

Selenium added as selenate	Selenium found
10·0 mg.	9·95 mg.
10·0 ,,	9·95 ,,
5·0 ,,	4·95 ,,
5·0 ,,	5·05 ,,

Tests of the method were also taken back to the fusion stage with weighed amounts of selenium mixed with the powdered copper and iron sulphates. 12 g. of each salt were used, together with the stated amount of selenium.

Selenium added	Selenium found
11·4 mg.	11·45 mg.
3·2 ,,	3·10 ,,

Further tests were then made with a copper matte free from selenium and tellurium. Various weights of selenium were thoroughly mixed with about 20 g. of the matte and then tested by this method.

Selenium taken	Matte taken	Selenium found
5·0 mg.	20·3318 g.	4·90 mg.
12.8 ,,	19·9968 ,,	12·70 ,,
7·2 ,,	20·3052 ,,	7·05 ,,

DISCUSSION OF THE METHOD—When dissolving selenium from the Gooch in bromine and hydrobromic acid under slightly reduced pressure it was thought that a loss of selenium at this stage might occur. By interposing a bubbling tube containing hydrochloric acid saturated with sulphur dioxide between the receiving bottle and the filter pump it was found that selenium was not carried over.

Evans[8] has suggested that oxidation of selenium with bromine might go partially beyond the quadrivalent stage. Standard solutions of selenium prepared by solution in nitric acid and by solution in bromine and hydrobromic acid were found to require the same amount of thiosulphate.

The removal of bromine by sodium salicylate is almost complete, but a very slight colour is given with starch and potassium iodide (which contains no free iodine). This is discharged by 1 drop of thiosulphate, and corresponds to less than 0·05 mg. of selenium.

If a gravimetric finish is required, asbestos is omitted and the final precipitation made at 15° to 22° C. The selenium can then be collected on a porosity 4 glass crucible. It is washed free from copper easily, but the last trace of bromides is very difficult to remove.

In conclusion the author wishes to acknowledge the ready collaboration and assistance he has received from the British Non-Ferrous Metals Research Association and, in particular, from their chief chemist, Mr. B. W. Drinkwater. He is also indebted to the British Insulated Callender's Cables, Ltd., for permission to publish.

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ROYAL SCHOOL OF MINES
SOUTH KENSINGTON, LONDON, S.W.7

February, 1946

The Rapid Determination of Sodium in 50% Potassium Hydroxide Liquor, 50% Potassium Carbonate Liquor and Solid Potassium Carbonate

BY J. HASLAM AND J. BEELEY

(*Read at a Meeting of the North of England Section, on April* 27, 1946)

IN connection with the manufacture of potassium carbonate from potassium hydroxide liquor, it recently became necessary to devise methods for the rapid determination of

(*a*) up to 1·5% of sodium hydroxide in 50% w/w potassium hydroxide liquor;
(*b*) up to 5% of sodium carbonate in 50–55% w/w potassium carbonate liquor;
(*c*) up to 3% of sodium carbonate in solid potassium carbonate.

From our experience in the determination of sodium in various products it seemed most promising to employ the zinc uranyl acetate method of sodium determination in such a way that the interference of potassium in the test was reduced to a minimum. It has long been known that large amounts of potassium interfere seriously in the sodium determination. Further, as the test to be devised should be as simple as possible a technique based on measurement of the volume of the sodium zinc uranyl acetate precipitate after centrifuging appeared to be the most promising.

Determination of Sodium in 50% Potassium Hydroxide Liquor

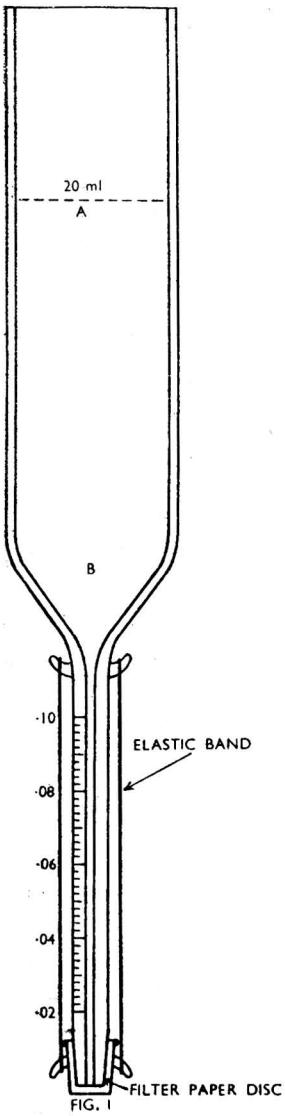

FIG. 1

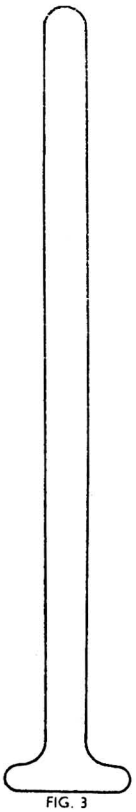
FIG. 3
PLUNGER


FIG. 2
SLIPPER

Preliminary tests indicated that a satisfactory method could be based on the use of a solution equivalent to 0·05 ml. of the original 50% potassium hydroxide liquor, with 20 ml. of zinc uranyl acetate reagent to precipitate the sodium.

Centrifuge tubes were therefore designed for these conditions. Fig. 1 shows the type of tube. The upper calibration mark allowed for the collection of a maximum amount of 0·1 ml. of sodium zinc uranyl acetate precipitate. The range 0 to 0·1 ml. was divided into 50 parts. Each tube was carefully checked at the upper limit of 0·1 ml. by weighing the appropriate volume of mercury on the lines laid down by the Analytical Methods Committee of the Society of Public Analysts and Other Analytical Chemists, in connection with their work on the determination of dirt in milk.[1] The tubes which we used gave the following results on calibration.

No. of tube	True vol. at 0·1 ml. graduation
1	0·1004
2	0·1012
3	0·1001
4	0·1000
5	0·0996

As the volume of precipitate obtained in a given test would vary with the particular method of centrifuging, a standard method was adopted for the centrifuging process. Although we should have preferred to use an electrically-driven centrifuge driven at a constant speed we were restricted to the use of a hand centrifuge.

After a few preliminary experiments the first series of tests were carried out with the same amount of potassium present in each test (≡ 0·0377 g. of KOH) but with different amounts of sodium. The test solutions were prepared by making up 50% potassium hydroxide liquors of known NaOH contents. Five ml. of the liquor were then taken, diluted with water, treated with 5 ml. of glacial acetic acid and diluted to 100 ml. The sodium zinc uranyl acetate precipitation method was applied to 1 ml. of this solution, particular attention being directed to the following points.

1. Method of addition of the potassium solution.
2. Mixing of the potassium solution with the zinc uranyl acetate reagent.
3. Method of centrifuging and collection of the sodium zinc uranyl acetate precipitate.

The manner of application of the test was as follows.

Precipitation was carried out in the centrifuge tubes already mentioned (Fig. 1), each

consisting of two parts: (*a*) the body of the tube with its graduated stem, and (*b*) the small slipper (Fig. 2). In assembling a tube, a filter paper disc (diam. 6 mm.) was placed in the slipper and the slipper and body of the tube were held together by two elastic bands.

Zinc uranyl acetate reagent (1) was run into the assembled tube up to the 20 ml. mark and the graduated portion of the tube was allowed to fill completely by releasing the slipper slightly. The volume of reagent was then readjusted to the 20 ml. mark.

One ml. of the solution under test was now added drop by drop from a 1 ml. pipette. Throughout the addition the reagent solution was stirred by movement of a glass plunger (Fig. 3) upwards and downwards from A to B.

The glass plunger was then rinsed with a little zinc uranyl acetate reagent and removed from the tube and the tube transferred to the centrifuge. After standing for 5 minutes the centrifuge was rotated in a clockwise direction for one minute at approximately 1100 r.p.m. The tubes were then turned half-round in their carriers and the centrifuge was rotated for half a minute in an anti-clockwise direction.

A small amount of precipitate had collected at point B and this was dislodged with the aid of a glass rod and rubber "policeman." The centrifuging was now repeated, *i.e.*, for 1 minute in a clockwise direction and, after the tubes had been turned in their carriers, for half a minute in an anti-clockwise direction.

Readings of the volume of precipitates were taken immediately, the shape of the meniscus being taken into account.

The following results were obtained.

	Volume of precipitate in ml.	
	1	2
1 ml. of diluted pot. acetate solution ≡ 0·0377 g. KOH	0·004	0·004
,, ,, ,, ,, ,, containing 0·004 g. of added NaOH	0·0225	0·022
,, ,, ,, ,, ,, ,, 0·008 g. ,, ,,	0·0385	0·037
,, ,, ,, ,, ,, ,, 0·0016 g. ,, ,,	0·070	0·071

Taking into account the control test on the potassium acetate solution, it was concluded from the above observations that 0·069 ml. of sodium zinc uranyl acetate precipitate, when precipitated in the presence of potassium acetate equivalent to 0·0377 g. of potassium hydroxide, corresponds with 0·0016 g. of sodium hydroxide, *i.e.*, 0·1 ml. of precipitate ≡ 0·00232 g. NaOH (or 0·00133 g. Na). This factor was used in all our subsequent tests.

The effect of variation of the amount of potassium present was shown by applying the test to solutions which contained the same amount of sodium but different amounts of potassium and which were faintly acid with acetic acid. The following results were obtained.

Potassium present in individual test equiv. to g. KOH	Sodium present in individual test equiv. to g. NaOH	Amount of NaOH equiv. to 0·1 ml. of sodium zinc uranyl acetate precipitate g.
nil	0·00151	0·00285
0·0081	0·00151	0·00302
0·0162	0·00151	0·00280
0·0325	0·00151	0·00248
0·0406	0·00151	0·00234

On application of the method outlined above to 50% potassium hydroxide liquors containing different amounts of sodium hydroxide, which were unknown to the operator at the time of test, the following results were obtained.

Sodium Hydroxide in Liquor, %	
Added	Found
0·53	0·45
1·06	1·0
1·58	1·65

In deducing the proportion of sodium hydroxide the factor 0·1 ml. sodium zinc uranyl precipitate ≡ 0·00232 g. NaOH was used.

Application of the Method to the Determination of Sodium in 50–55% Potassium Carbonate Liquor

In order to carry out the final precipitation test on an amount of sample containing the equivalent of 0·0377 g. of potassium hydroxide the preliminary treatment of the liquor is carried out as follows:

Measure 4 ml. of the strong potassium carbonate solution from a graduated pipette into a 100 ml. beaker. Dilute with water and acidify carefully with 15 ml. of 6 *N* acetic acid. Boil to remove carbon dioxide, cool, and add 10 ml. of potassium acetate solution (2). Transfer to a 100-ml. graduated flask and dilute to the mark with water. Carry out the test on 1 ml. of this solution, measured from a graduated pipette or burette as for potassium hydroxide above.

0·1 ml. of precipitate ≡ 0·00306 g. Na_2CO_3

Applied to 50% potassium carbonate liquor containing known amounts of sodium carbonate, the following results were obtained.

Sodium Carbonate in Liquor, %	
Added	Found
1·9	1·8
3·98	4·28
3·98	3·95
3·98	4·09

Application of the Method to the Determination of Sodium in Solid Potassium Carbonate

In order to carry out the final sodium test on an amount of sample containing the equivalent of 0·0377 g. of potassium hydroxide the preliminary treatment of the sample is carried out as follows.

Weigh 4·7 g. of sample, dissolve in a little water in a 100-ml. beaker and acidify carefully with 15 ml. of 6 *N* acetic acid. Boil to remove carbon dioxide, cool, transfer to a 100-ml. flask and dilute to the mark with distilled water. Carry out the test on 1 ml. of this solution measured from a graduated pipette or burette as for potassium hydroxide above.

0·1 ml. of precipitate = 0·00306 g. Na_2CO_3

The result of the application of the test to known mixtures of sodium carbonate with potassium carbonate was as follows:

Sodium Carbonate, %	
Added	Found
1·02	0·94
1·7	1·63
2·04	2·08
2·77	2·93
3·06	3·16

Reagent Solutions Used

(1) *Zinc uranyl acetate reagent*—

Solution A: zinc acetate $2H_2O$, 427 g.; acetic acid 30% v/v, 46 g.; water, 527 g.
,, B: uranyl ,, $2H_2O$, 154 g.; ,, ,, 30% ,, 92 g.; ,, 748 g.

Solutions A and B were prepared separately on the water-bath, care being taken to avoid loss by evaporation. The hot solutions were mixed, 2 ml. of *N*/10 sodium chloride were added and the mixture was allowed to stand for 24 hrs. and then filtered.

(2) *Potassium acetate solution.*

13·8 g. of potassium carbonate (AnalaR) were dissolved in water, 12 ml. of glacial acetic acid were added and the solution was boiled to remove carbon dioxide, cooled and diluted to 100 ml.

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Imperial Chemical Industries, Research Dept.
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November, 1945

Errata

April issue, p. 176, line 21: *for* "potassium silicofluoride" *read* "fluosilicic acid."
p. 177, line 4 from bottom: *delete* "from" now in front of "2 ml. to 50 ml."
p. 178, Graph I, along ordinate axis: *for* 0·009 *N*" *read* "0·0009 *N*."
p. 179, Graph II, along ordinate axis and in heading: *for* "Th/F" *read* "Th/F_4," and in heading *delete* brackets round "Range."

Notes

NOTE ON THE PRESENCE OF HEAT-LABILE SULPHUR IN MILK POWDER MADE FROM MILK PRE-HEATED AT A HIGH TEMPERATURE (190° TO 200° F.)

IN recent publications,[1,2] the efficacy of high temperature pre-heating of the liquid milk as a means of stabilising full cream milk powders against development of tallowy "off" flavours, and of retarding loss of vitamins A and C during storage, has been pointed out, and the improvement in keeping properties has been attributed mainly to production of anti-oxidant active sulphydryl compounds by the action of heat on the protein. This process is now in commercial use. Details of a method, based on the work of Almy[3] and of Townley and Gould,[4] which has been found useful as a qualitative and as a rough quantitative test for labile sulphur in milk powders made from milk pre-heated at a high temperature may therefore be of interest.

A quantity of milk powder containing 50 g. of milk solids is reconstituted to the solids content of fresh milk (12·5%) and placed in a specially shaped vessel (see Fig. 1) made from a "350" ml. Pyrex beaker by

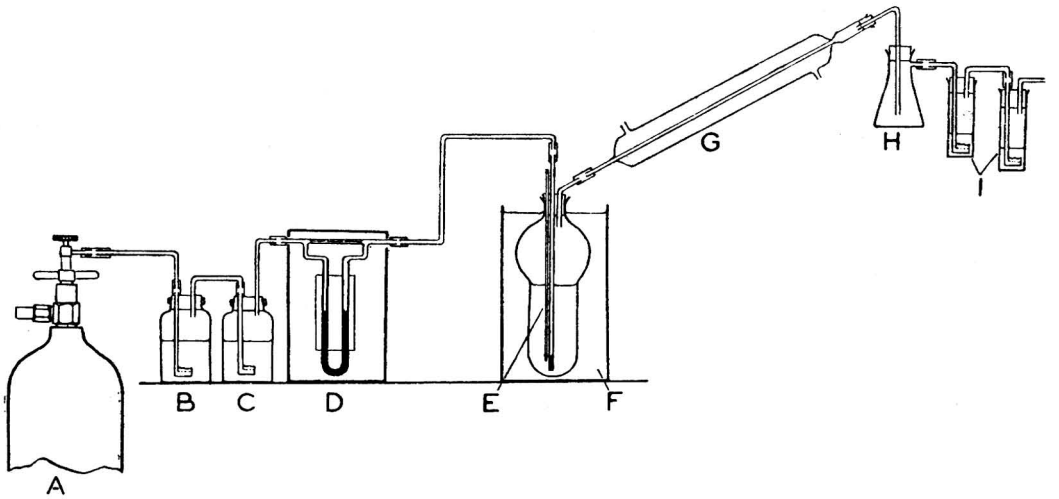

Fig. 1.

A = Cylinder of "oxygen-free" nitrogen.
B = Wash bottle containing alkaline triacetoxybenzene (1:2:4-triacetoxybenzene 20 g., potassium hydroxide 27 g., water 200 ml.).
C = Wash bottle containing water.
D = Flow meter.
E = Specially shaped flask.
F = Thermostatically controlled water bath.
G = Condenser.
H = Trap.
I = Absorption tubes containing alkaline zinc acetate solution.

rounding the bottom and fusing to the mouth a 500-ml. round-bottomed flask from which the bottom has been removed and of which the neck has been shortened. The milk roughly fills the lower, straight sided portion of the vessel, and the upper bulb and a loose plug of glass wool in the neck of the flask serve as a foam trap. Oxygen-free nitrogen is passed through the milk and thence, *via* a reflux condenser and trap, to a sintered glass bubbler immersed in a few ml. of alkaline zinc acetate solution contained in a boiling tube, of which the bottom is flattened. A second absorption tube placed in series with the first has never been found to contain any sulphur. The flow of gas is controlled at 60 litres per hour by means of a simple, glass U tube flowmeter. In experiments to date, commercial "oxygen-free" nitrogen has been further purified by passage through alkaline 1 : 2 : 4-triacetoxybenzene, but this grade of gas has already been purified over copper and it is very doubtful whether the additional precaution is necessary. Various multiple aperture jets were tried and found unsatisfactory owing to gradual choking, which tended to cause non-uniform gassing during the run; a single jet made from 2 mm. tubing was finally adopted. The temperature of the milk is controlled at 60° C. by immersion of the vessel in a thermostat at about 60·5° C. Air is swept out of the apparatus before heating is commenced, and the flow of gas cut down to a trickle during the 20 minutes necessary to bring the milk to the required temperature.

Colorimetric estimation—Place 6 ml. of 1% zinc acetate solution and 6 ml. of 0·8% sodium hydroxide solution in the absorption tube. After absorption, wash the bubbler with 4 or 5 ml. of 1% hydrochloric acid, dilute the liquid to a 17·5 ml. mark on the tube and insert a glass rod for mixing. Add 2 ml. of a freshly prepared 0·05% solution of *p*-aminodimethylaniline hydrochloride in diluted hydrochloric acid (1+1), followed by 0·5 ml. of 0·02 *M* ferric chloride and allow to stand in darkness for 20–24 hours. Dilute the ferric chloride from a stock solution prepared by dissolving 6·75 g. of the hexahydrate in 125 ml. of concentrated hydrochloric acid and making up to 250 ml. Measure the blue colour developed by means

of a photometer and filter at about 650 mμ. The Zeiss Pulfrich instrument with filter No. 7 and ½–3 cm. cell was quite suitable. A zero blank was easily obtained without any precautions other than limitation of the area of exposed rubber to a minimum.

Calibration against sodium sulphide—Standardise an approximately 0·01 *N* sodium sulphide solution by addition of 25 ml. to 50 ml. of 0·01 *N* iodine and titration of the excess iodine with 0·01 *N* thiosulphate. Prepare a series of dilutions in oxygen-free water (de-oxygenated by passing nitrogen) and immediately add 1 ml. portions to tubes containing the alkaline zinc acetate reagent, and develop and measure the colour as described above. A suitable range of concentrations is 0–3 mg. of sulphur per litre of coloured solution.

Production of volatile sulphur from fresh milk—388 ml. of milk, corresponding to 50 g. of milk solids, was used for each determination, three-hour runs being made at 50, 60, 65, 70 and 75° C. The milk was obtained from consecutive morning milkings of the same cow, and all runs duplicated on a different day, the mean of the two sets of data being recorded. The result (Table I) shows that no volatile sulphur could be detected at 50° C., even after heating for 3 hours. At 60° C. a very faint reaction was obtained, and the response increased rapidly as the temperature of heating was raised.

Production of volatile sulphur from milk powder—Several samples of high temperature (190 or 200° F. for 20 seconds) pre-heated spray-dried full cream powder were examined for the effect of time and temperature on the liberation of volatile sulphur. Owing to the season, freshly made powder was not available and the materials used had been held for 2 months in 21 lb. gas-packed cans. The results showed that practically no volatile sulphur was obtained at 20° C., in contradistinction to liquid milk which, after heating at 190° F., gives a strong positive reaction when nitrogen is passed through it at 22° C.[4] At 40, 50, 60 and 70° C. increasing quantities of volatile sulphur were obtained (Table I). Milk reconstituted from powder therefore contains little or no free H_2S, that formed during pre-heating of the liquid milk presumably having been lost during the drying process. The proteins, however, have been so altered by the pre-heating treatment that they liberate volatile sulphur at a much lower temperature than does fresh milk.

TABLE I

PRODUCTION OF VOLATILE SULPHUR FROM FRESH MILK AND FROM MILK RECONSTITUTED FROM A HIGH TEMPERATURE PRE-HEATED FULL CREAM POWDER

	Duration of heating (hours)	Mg. of sulphur produced per kg. of milk solids* at						
		20° C.	40° C.	50° C.	60° C.	65° C.	70° C.	75° C.
Fresh milk	½	—	—	0·00	0·01	0·04	0·08	0·43
	1	—	—	0·00	0·03	0·08	0·18	0·73
	2	—	—	0·00	0·05	0·15	0·48	1·12
	3	—	—	0·00	0·07	0·23	0·76	1·50
Reconstituted milk ..	½	0·01	0·05	0·09	0·20	—	0·35	—
	1	0·01	0·09	0·16	0·32	—	0·56	—
	2	0·01	0·14	0·24	0·51	—	0·89	—
	3	0·01	0·18	0·30	0·65	—	1·22	—

* 1 kg. of milk solids ≡ approx. 7·77 litres of milk.

For the routine examination of milk powders, 60° C. is probably the most suitable temperature and 1 hour an adequate period of heating. Under these conditions fresh milk gives a barely detectable reaction. Low temperature (160°, 165° or 170° F. for 20 seconds) pre-heated powders tested to date have all given a completely negative reaction when examined some little time after manufacture, possibly owing to destruction, by oxidising fat or by traces of contaminating copper, of any minute quantities of volatile sulphur which might have been produced by the pre-heating treatment or during the determination. High temperature pre-heated powders give a strong positive reaction.

This work formed part of a joint programme of the Agricultural Research Council and the Food Investigation Board of the Department of Scientific and Industrial Research.

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November, 1945

NOTE ON TECHNIQUE FOR MICROBIOLOGICAL ASSAY OF VITAMINS BY METHODS INVOLVING MOULD GROWTH

THE difficulties of manipulation for methods of microbiological assay involving the harvesting, drying and weighing of mycelia of numerous replicates may well prevent the general adoption of these methods on account of the time and space involved.

The following technique has been found to save a considerable amount of time, labour and space in incubators, etc., and renders the assay of large numbers of samples easy.

The moulds (*Neurospora sitophila* for pyridoxine or *Phycomyces Blakesleeanus* for aneurine) are grown in the usual way on 10 ml. of medium containing the appropriate standard or sample under assay, in 50-ml.

Erlenmeyer Pyrex flasks. These flasks are placed in order in racks holding 50 (Fig. 1). A number of these racks, made of sheet copper, are convenient for routine work. They and their contents can be steamed or autoclaved according to the method used and the flasks are replaced on them after inoculation. Since the racks are interchangeable they can be placed one on top of the other for incubation in the constant temperature room, and so a considerable economy of shelf space is effected.

When the mycelial growth is ready for harvesting we steam the racks and contents for 10 minutes to kill and toughen the mould. The contents of each flask are then filtered on a small Buchner funnel (diameter 5 cm.) fitted with a hard filter paper such as Whatman No. 54. It is convenient to remove the mycelium from the flask by twisting it round a thin glass rod flattened at one end. The flask and mould are washed with two 5-ml. quantities of distilled water from a tilting pipette fitted to a wash bottle. The mould mycelium is then carefully detached from the filter paper and rolled into a ball which is pressed between two filter papers (Whatman No. 54) to remove excess moisture. Care should be taken that the thin edges of the growth are well rolled in because they become brittle when dry. The balls of mycelium are then dried in batches of ten. This is effected by placing limiting rings of "Alda" (British Oxygen Co. aluminium-silicon alloy welding rod, gauge $\frac{1}{8}$th inch) of about 1·3 cm. internal diameter in the bottom of a flat aluminium dish of diameter 7·5 cm. (Fig. 2). The rings are easily made. The mycelia can be coded by a pencil mark on

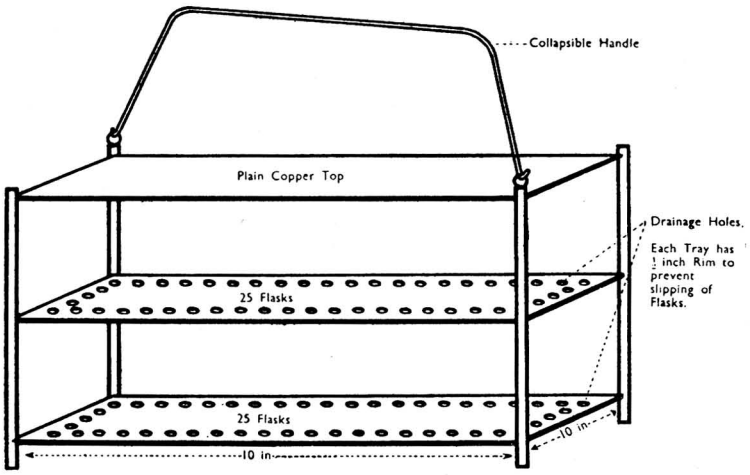

Fig. 1. Rack for Flasks.

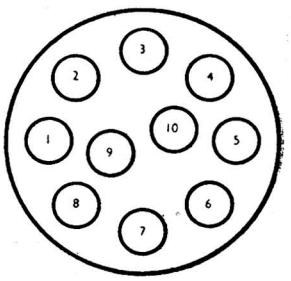

Fig. 2. Drying Dish with Loose Rings.

the Petri dish inside each limiting ring. The Petri dishes and contents are dried to constant weight, for which we find that 3 hours in a vacuum oven at 100° C. is suitable. The dishes and mycelia are then weighed in equilibrium with air and each mycelium is removed with forceps in turn. This gives the weight of each mycelium by difference from the preceding total weight and saves much time. It is convenient to use a good air-damped balance.

A series of ten replicates on three levels of aneurine, using *Phycomyces Blakesleeanus*, gave the following error calculated as maximum deviation from the mean.

Level of assay	Average weight of ten replicates	Maximum deviation from mean	Error
0·1 μg.	29·7 mg.	± 1·3 mg.	4·3%
0·2 ,,	49·6 ,,	± 3·1 ,,	6·2 ,,
0·4 ,,	79·5 ,,	± 5·6 ,,	7·0 ,,

As with all these microbiological assay methods the higher levels give more erratic results, since the relation between standard dose and response is only linear over a given range. There was no progressive variation between individuals in a series, so there appears to be no absorption of moisture during weighing.

The technique seems reasonably accurate (considering the small quantities assayed) over levels normally encountered in the assay of foods and would save much time and labour where large numbers of samples have to be handled, *e.g.*, in nutritional surveys.

I acknowledge with gratitude the generosity of Messrs. J. Lyons & Co. in permitting the publication of this note on a technique which was developed in their laboratories.

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SEPARATION OF THE COBALT COMPLEX OF β-NITROSO α-NAPHTHOL FROM OTHER COLOURED METALLIC COMPLEXES

(*Read at the Meeting of the Society on Wednesday, April* 3, 1946)

SEVERAL α-nitrosophenols form coloured co-ordination compounds with cobalt and can be used for the colorimetric determination of the metal. Coloured complexes are also formed with tin, copper, silver, bismuth, nickel, chromium and iron. It has been found possible to separate cobalt from these metals even when they are present in great excess, and the reactions now described should be of service in determining cobalt in their presence. The main elements likely to interfere with the colorimetric determination of cobalt in biological materials are iron and copper, and since 1 μg. of cobalt can be quantitatively separated from 5000 μg. of iron it should be possible to apply the technique after destruction of organic matter. Preliminary attempts to determine cobalt in biological materials by direct application of the suggested method after wet ashing have given low results, but it may prove possible to overcome this difficulty.

In order to increase intensity and specificity it is an advantage to be able to extract the coloured complex in a small amount of organic solvent. Of the *o*-nitrosophenol derivatives available β-nitroso α-naphthol appeared to be the most promising, as the cobalt complex formed with it is intensely coloured[1] and only slightly soluble in water[2] (1 part in 6×10^6). The red complex formed by cobalt and β-nitroso α-naphthol is produced rapidly in the cold over the *p*H range 4–9; it is readily extracted by organic solvents such as amyl alcohol, benzene, chloroform or carbon tetrachloride and is not destroyed by strong acid or alkali. Under similar conditions copper forms an orange-coloured complex and iron gives both green and red complexes. The two iron compounds can be separated from each other and from the cobalt complex by chromatographic adsorption from a carbon tetrachloride solution on a magnesium oxide or alumina column. The red derivative is converted into the green ferrous form on treatment with alcoholic sodium hydroxide (0·5 *N*) or reducing agents such as sodium hydrosulphite. The green form is only slightly soluble in carbon tetrachloride but is soluble in 0·5 *N* sodium hydroxide in 50% aqueous alcohol. The copper complex is destroyed by aqueous or alcoholic alkali. The chromium complex is red and stable to alkali, but destroyed by acid.

When solutions of the β-nitroso α-naphthol complexes of the metals in carbon tetrachloride are shaken with concentrated hydrochloric acid the cobalt derivative is unchanged, but the coloured complexes of other metals are immediately destroyed. This reaction permits estimation of cobalt in presence of large excess of iron, copper or other metals. The specific destruction of complexes other than that of cobalt also occurs with 90% sulphuric acid or 90% acetic acid.

The complexes of cobalt, copper and iron can also be separated by chromatographic adsorption of the carbon tetrachloride solution on a column of alumina or magnesium oxide and development with alcohol. The cobalt complex passes through first, but if a large excess of iron (*e.g.*, 10 times as much iron as cobalt) is present this separation is difficult. The separation is made more difficult by the presence of the two different iron complexes, one of which has a similar colour to that of the cobalt derivative. The red iron complex has a rather bluer shade than the cobalt complex. Quantitative separation of the complexes formed from 2–5 μg. of each metal is possible with a column 1 cm. in diameter and 5 cm. in length.

The β-nitroso α-naphthol cobalt complex in carbon tetrachloride solution can then be separated from complexes of other metals in three ways.

1. By chromatographic analysis when only two or three metals are present and none in great excess.
2. By destruction of the complexes formed by other metals on shaking with concentrated hydrochloric acid.
3. By treatment with 0·5 *N* sodium hydroxide in 50% alcohol the iron complex is extracted and only cobalt and chromium complexes remain.

A method of estimation of cobalt depending on methods 2 and 3 has been worked out. The amount of β-nitroso α-naphthol used must be sufficient to combine with all metals present in the solution and capable of reacting.

METHOD—The solution, containing between 0·5 and 10 μg. of cobalt, is neutralised with sodium hydroxide and acetic acid, and methyl red as indicator, so that the *p*H lies between 5·0 and 7·0. The volume of this neutralised solution should be under 20 ml. and 5 ml. of a 1% solution of β-nitroso α-naphthol* in a mixture of 40% carbon tetrachloride and 60% of absolute alcohol by volume are added and shaken for 10 minutes to allow the cobalt complex to form and to be dissolved in the carbon tetrachloride. The carbon tetrachloride layer is then allowed to settle and the supernatant aqueous layer removed by suction. The carbon tetrachloride solution is transferred to a 10- or 15-ml. stoppered measuring cylinder or graduated tube, being washed in with conc. hydrochloric acid of AnalaR grade. It is twice extracted with 10 ml. of conc. hydrochloric acid by shaking, settling and removal of the upper layer by suction. This treatment decomposes the complexes of other metals and removes some of the excess reagent. The remaining acid is removed by two washings with water and the remaining excess reagent dissolved out by shaking with 10 ml. of *N* sodium hydroxide, the excess of which is in turn removed by washing with 10 ml. of water. If the carbon tetrachloride solution at this stage is not a clear pink or red it should be washed with 1 volume of 0·5 *N* sodium hydroxide in 50% alcohol to remove any remaining iron complex, which will impart a green colour to the aqueous alcohol layer. Excess alkali is then removed by twice washing with water. As much water as possible is removed by suction. If the colour is to be read in a microcolorimeter, absolute alcohol is added to make the total volume equal to twice that of the remaining carbon tetrachloride solution (*e.g.*, 1·5 ml. of carbon tetrachloride solution with 0·2–0·3 ml. of remaining water are made up to 3 ml.). If readings are taken in a photometer requiring 1 ml. or less of solution the water can be removed by filtration through dry filter paper. The absorption of green light, which is proportional to the amount of cobalt present, is determined and the amount of cobalt is then ascertained by reference to a standard curve obtained by determination of the absorption produced by known amounts of cobalt ranging from 0 to 10 μg. A

* The β-nitroso α-naphthol is twice recrystalled, from glass-distilled water. Filtration of the hot solution is necessary to remove dark coloured impurities.

blank determination is carried out, using water in place of cobalt solution and the final extract is then placed in one cell of the photometer or above the neutral density screen of the colorimeter. If a colorimeter is used the depth of the blank extract is adjusted so as to be equal to that of the unknown solution.

SUMMARY—The β-nitroso α-naphthol complexes of several metals can be dissolved in carbon tetrachloride. If this carbon tetrachloride extract is washed with concentrated hydrochloric acid the complexes formed by metals other than cobalt are destroyed. The excess reagent can be removed by washing with sodium hydroxide solution. Iron forms two complexes with β-nitroso α-naphthol; a red iron complex is converted into a green derivative by reduction. The green compound can be extracted from carbon tetrachloride solution by a solution of sodium hydroxide in 50% aqueous alcohol. These reactions are used in a method for the colorimetric determination of cobalt.

I should like to thank Dr. H. H. Green for his interest in this work.

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December, 1945

DISCUSSION

Mr. L. A. HADDOCK said he used a similar method several years ago for the determination of traces of cobalt. He found it necessary, however, to buffer the solution at *p*H 3 to 4 and to boil and cool before extracting the cobalt complex. Without boiling, low results were obtained. Amyl alcohol was found to be a better solvent than the chlorinated hydrocarbons. Interference from chromium and tin could be circumvented to some extent by working in a tartrate solution.

Mr. R. F. MILTON asked why the author used β-nitroso-α-naphthol in preference to α-nitroso-β-naphthol. In his experience loss of cobalt on ashing was due to entrainment in the silicaceous residue, and could be overcome by ashing in a platinum basin at a low temperature and then removing silica by treatment with hydrogen fluoride and volatilisation as silicon fluoride.

In reply, Dr. BOYLAND said that if excess of the reagent was present the colour development by cobalt did not appear to be increased by heating the reaction mixture. The advantages of carbon tetrachloride over amyl alcohol as a solvent for extraction were: (*a*) its lower solubility in water, so that with repeated washing very little solvent was lost and (*b*) being heavier than water, it enabled washings with aqueous reagents to be carried out rapidly by shaking in tubes, allowing to settle and removing the aqueous layer by suction through a capillary tube. The advantages of β-nitroso-α-naphthol over α-nitroso-β-naphthol are: (*a*) the greater ease of purification of the reagent, (*b*) the higher partition coefficient of the cobalt complex between organic solvents and water, and (*c*) the greater intensity of the colour of the complex and suitability for colorimetric estimation.

THE USE OF CUPFERRON FOR THE ESTIMATION OF LARGE QUANTITIES OF IRON IN FAECES

IT has been fashionable for a number of years to administer large quantities of iron, usually in the form of iron ammonium citrate (of the order of 90 grains per day), to the human subject in certain cases of anaemia. The necessity arose to carry out balance experiments in such cases, *i.e.*, to compare the excretion of iron in the faeces and urine with the intake in the diet and the administration of iron ammonium citrate. Since the absorption of iron, even with such large doses, is small, a number of problems arose.

URINE—It has been shown that the iron content of the urine is negligible except in all but a few conditions, and even in these the amounts excreted are minute, *cf.* Tompsett.[1] The following are typical results:

Normal	<0·01 mg. of Fe per litre
Haemochromatosis	0·16 ,, ,, ,,
Pernicious anaemia	0·19, 1·28 mg. of Fe per litre
Nephritis with gross albuminuria ..	1·72, 2·33 ,, ,, ,,

DIETS—A normal diet contains approximately 10–15 mg. of iron per day. The estimation of such amounts is achieved by colorimetric methods.[1]

FAECES—Normal faeces contains approximately 10–15 mg. of iron per day. The estimation of such amounts is achieved by colorimetric methods.[1] When, however, large amounts of iron have been ingested and since the greater part of this appears in the faeces, it is obvious that such colorimetric methods are inapplicable if accurate results are required. A gravimetric method, in which the iron is precipitated as a complex with cupferron and is subsequently converted into and estimated as Fe_2O_3 has proved to be satisfactory. Faeces is a heterogeneous mixture and contains a considerable amount of calcium phosphate. In respect of this, cupferron has an advantage since it can precipitate iron from acid solution.

Method—A weighed amount of dried faeces is ashed in a silica dish. Finally the ash is moistened with nitric acid and re-ignited. The ash is dissolved in 20% hydrochloric acid and the solution made up to a definite volume. A portion of the solution containing 0·1 to 0·3 g. of iron is taken for analysis and diluted to 150 ml. with 20% hydrochloric acid. A fresh 6% aqueous solution of cupferron is then added with constant stirring until the formation of a white precipitate of nitroso-phenylhydroxylamine indicates that excess has been added. The precipitate is filtered through a No. 42 Whatman filter-paper, washed first with 20% hydrochloric acid and finally with 20% ammonia. The filter paper and precipitate are then dried and ignited in a platinum dish. The resultant ignited precipitate is weighed as Fe_2O_3. The ash content

of the filter paper is allowed for. The method was checked by adding known amounts of an iron salt to dried faeces and completing the determination. The following are typical results:

Iron (Fe_2O_3) added, g. ..	0·0500	0·1000	0·1500	0·2000	0·2500	0·3000	0·4000
" (Fe_2O_3) recovered, g. ..	0·0505	0·1003	0·1502	0·2001	0·2503	0·3003	0·4005

Although certain other metals, *e.g.*, titanium, are precipitated by cupferron, accuracy in determining the amount of iron absorbed is not impaired. Titanium occurs in diets and faeces in traces, and since in balance experiments patients are placed on a constant daily diet the daily intake of titanium is unchanged.

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February, 1946

DETECTION OF MANGANESE WITH AURINTRICARBOXYLIC ACID

THE ammonium salt of aurintricarboxylic acid gives bright red lakes with various metals, *e.g.*, with aluminium, beryllium, yttrium, lanthanum, cerium, neodymium, erbium, zirconium and thorium. All these, except the lakes of beryllium and aluminum, are dissolved or decolorized by addition of a moderate excess of ammonium carbonate. The substance is considered a very sensitive reagent for aluminium, when applied to precipitated aluminum hydroxide. The latter forms with it in acetate solution a bright red precipitate which persists after a mixture of ammonia and ammonium carbonate has been added.

In pursuing some investigations with this reagent we found that it forms with manganese a compound of a deep violet colour, very easily produced, and much more characteristic than that with aluminium. The manganese salt is treated with ammonia, which produces a slight precipitate. The latter is dissolved in a slight excess of acetic acid and an aqueous solution of the ammonium salt of aurintricarboxylic acid is added. Immediately the violet compound separates. It separates even when manganese ions are present at considerable dilution; 0·25 mg. of manganese could be detected in 1 ml. of solution. The violet precipitate is stable in presence of small quantities of ammonium carbonate. A greater excess of this reagent does not dissolve it but turns it red and microcrystalline. Ammonia and sodium hydroxide produce the same change. The violet precipitate dissolves in dilute hydrochloric acid and tartaric acid, but is stable in presence of acetic acid, alcohol or ether.

For the purpose of investigating whether other metal ions give a similar reaction with aurintricarboxylic acid or interfere with the reaction of the manganese ion we treated the acetates of different ions with the reagent and state the result in the following table:

Pb	no reaction; does not interfere with manganese reaction
Cr	" " " " " "
Fe	" " " " " "
Sn	" " " " " "
Cu	forms red colour; " " " "
Zn	no reaction; " " " "
Hg	" " " " " "
Co	" " " " " "
Mg	" " " " " "
Ni	" " " " " "
Bi	" " " " " "
Ag	" " " " " "
Au	oxide insoluble in acetic acid
Sr	no reaction; does not interfere with manganese reaction
MoO_3	oxide insoluble in actic acid
WO_3	" " " " "
Pd	no reaction
Rh	" "
Ru	" "
UO_2	red precipitate which dissolves in ammonium carbonate
Al	" " insoluble in ammonium carbonate

For the detection of manganese in minerals that have been fused with alkali carbonates, a portion of the acid filtrate from the silica is neutralised with ammonia and then acidified with acetic acid and the reagent is added. The presence of aluminum does not interfere; it is, however, optional to follow the routine practice of separation of Al, Zn, Cr ions from Fe, Co, Ni and Mn.

The violet compound, when dried, was found to contain 24·5% of manganese.

METHOD OF TEST—To a few ml. of the test solution ammonia is carefully added until a slight precipitate appears. This is dissolved in a small excess of acetic acid until the solution appears clear again. Thereupon a 0·1% solution of the ammonium salt of aurintricarboxylic acid is added. In a short time when manganese is present the violet precipitate appears or if the quantity of manganese present is very small the solution acquires a violet colour, easily to be distinguished from the colour given by aluminium or the rare elements mentioned.

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March, 1946

A NOTE ON THE DETERMINATION OF NITROGEN IN FOODSTUFFS BY THE KJELDAHL METHOD: DIGESTION CONDITIONS

THE Kjeldahl method for the determination of nitrogen has been the subject of a voluminous literature, and there have been many improvements on the original formula of Kjeldahl[1] using potassium permanganate and sulphuric acid. Bosshard[2] added fuming sulphuric acid or phosphoric acid. Gunning's modification[3] has been generally adopted in some form or other, being essentially the raising of the boiling point of the sulphuric acid by sodium or potassium sulphate. Potassium permanganate was omitted. Arnold[4] introduced the heavy metal catalysts (mercury, copper) and raised the sodium sulphate concentration to a value (15–30 g. Na_2SO_4 to 30–50 ml. H_2SO_4) which, had it been generally accepted would have enhanced the accuracy of the method. Phelps and Daudt,[5] using mercuric oxide as catalyst, laid down a digestion time of 2½ hours, with 10 g. of sodium sulphate to 25 ml. of sulphuric acid for the complete recovery as ammonia of the nitrogen of pyridine zinc chloride.

The Official British Method in the Fertilisers and Feeding Stuffs Act of 1926, copied in numerous textbooks, requires 10 g. of sodium sulphate to 25 ml. of sulphuric acid to be heated for 1 hour after clearing. The A.O.A.C. offers various formulae in which the sodium sulphate concentration may be zero, 10 g. to 15–25 ml. of sulphuric acid or 15–18 g. to 25 ml. of sulphuric acid. The time is rarely laid down, but is usually given as "some time after clearing," or "1 hour after clearing." Normal laboratory practice assumes that complete digestion will have taken place within 2½ hours, though much shorter times are not infrequent.

Recent work (Chibnall, Rees and Williams,[6] Shirley and Becker,[7] Miller and Houghton[8]) has thrown doubt on the accuracy of the method as thus standardised. There is evidence to show that the heterocyclic compounds present in the proteins, and possibly those produced by condensation in the early stages of digestion, require much longer times for complete breakdown.

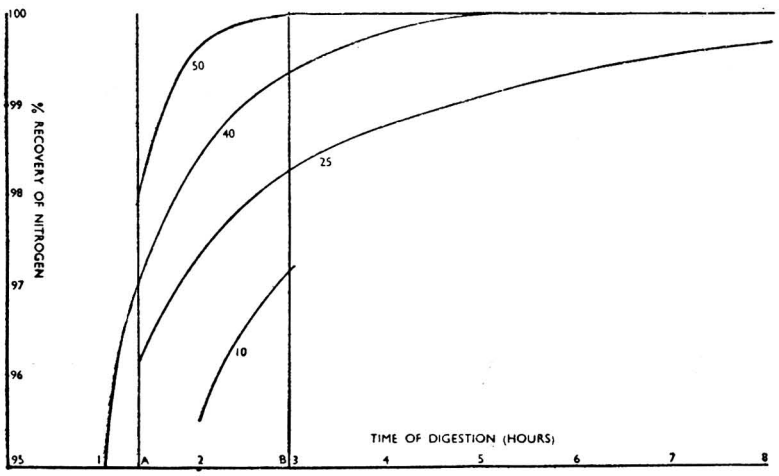
The experiments here described were undertaken to establish the relationship between sodium sulphate concentration (conditioning temperature) and digestion time on the one hand, and nitrogen recovery from typical foodstuffs on the other.

EXPERIMENTAL—Tests showed that, over a wide range, results were independent of catalyst concentration, though, as there was some evidence of nitrogen loss in very protracted (12 hr.) digestions with mercury, copper sulphate was used throughout.

Sufficient of the test material to yield some 25 mg. of nitrogen, 12·5 ml. of sulphuric acid and 0·2 g. of copper sulphate were digested in 300 ml. Kjeldahl flasks, with different quantities of sodium sulphate and for different times. In general the solution was "clear," *i.e.*, pale blue, after 50–60 minutes. The length of time for complete nitrogen recovery was not affected by variations in the clearing time. It is therefore important that a total digestion time should be specified, rather than a time after clearing.

Ammonia was liberated by addition of 40 ml. of 40% sodium hydroxide solution, and distilled into 25 ml. of *N*/10 sulphuric acid, the excess of which was titrated with CO_2-free sodium hydroxide solution, using as indicator methyl red masked by methylene blue (de Wesselow's).

The graph shows a series of determinations made on flour. The vertical axis gives nitrogen found, expressed as a percentage of the maximum (asymptotic) value. The horizontal axis is time in hours. The four curves indicate the effect of four different concentrations of sodium sulphate (expressed as grams per 100 ml. of sulphuric acid).



Similar results were obtained on soya flour, milk powder and desiccated yeast.

The conditions laid down in the standard methods, represented as they are by the area between the vertical lines A and B on the graph, may give recoveries ranging between 96% and 100%. Moreover, as it is not uncommon for directions to allow latitude in the amount of sodium sulphate used, and in the digestion time, determinations following such directions may be subject to a varying error.

The high concentrations of sodium sulphate which reduce digestion time to 3 hours cause deposition

of sooty materials in the neck of the flask and in the fume extractors. The optimum conditions suggested by the findings are therefore:

Sodium sulphate, 40 g. per 100 ml. of sulphuric acid
Copper ,, 1·6 g. ,, ,, ,, ,,
Total digestion time, 6 hours.

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January, 1946

A CARBONATE ESTIMATION APPARATUS

Although many forms of apparatus are available for the estimation of carbonates by the indirect method, the majority, including the familiar Rohrbeck, Geissler and Schrodter types, are top-heavy owing to the disposition of the acid container and the drying unit above the reaction chamber. They have, moreover, an extensive external surface which has to be dried very carefully before weighing. The apparatus described below has been designed to minimise these disadvantages and to satisfy as far as is practicable the following requirements: (1) the centre of gravity of the system to be low; (2) the apparatus to have a small superficial area; (3) the shape of the vessel to allow of quick drying of the external surfaces.

The decomposition vessel A (Fig. 1) is a conical flask (capacity about 150 c.c.) with a ground neck (2·5 cm. diameter) into which fits the combined drying unit and acid container B. The lower part of the drying unit is provided with an inner tube C which is sufficiently wide to take care of any accidental suck-back of the drying liquid. The acid container D is in the form of a pipette with its lower end tapering to a fine jet E and its upper end carrying a small stopcock F. The upper parts of the drying unit and acid container are provided with ground caps G and H, respectively.

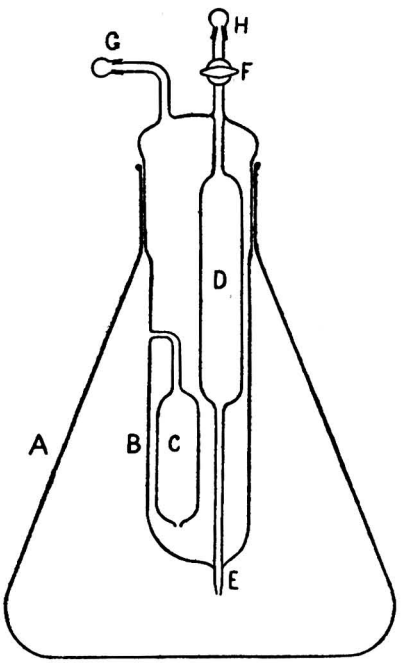

Fig. 1.

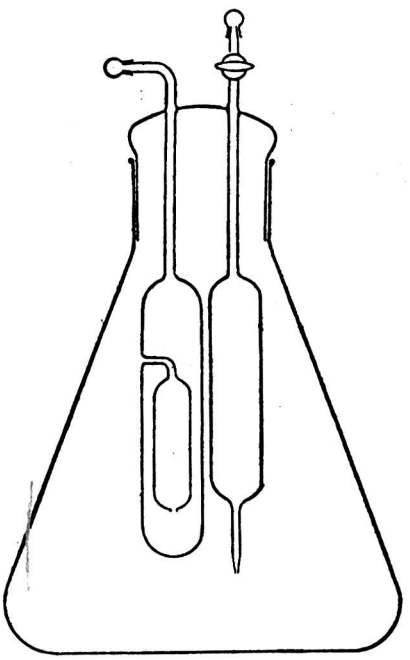

Fig. 2.

The drying unit is charged by introducing a suitable quantity of concentrated sulphuric acid through a small funnel inserted into G, and the acid container is filled by immersing the jet in diluted hydrochloric acid (1+1) and applying suction at H. A known weight of the carbonate is introduced into the flask and the material is covered with water. After inserting the unit B the apparatus is carefully wiped dry and weighed. The carbonate is decomposed by running in the acid at a rate which may be controlled by manipulating the stopcock F. When the reaction is complete, dissolved carbon dioxide is removed from

the solution by warming and shaking the flask while a stream of air is led through the apparatus. After cooling the flask to ordinary temperature, the caps G and H are replaced and the apparatus is dried and weighed in accordance with the usual procedure.

A simple form of the apparatus in which the drying unit and acid container are separately sealed to the stopper of the reaction flask is shown in Fig. 2. Both forms of apparatus are robust and more easily cleaned than the ordinary types.

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February, 1946

THE DETERMINATION OF LIME IN REFRACTORY DEAD-BURNED MAGNESIA

THE oxalate - permanganate procedure commonly used for separating calcium from magnesium involves treating a hot hydrochloric acid solution with ammonium oxalate and making just alkaline to methyl red with ammonia. When the ratio of calcium to magnesium is very small the separation is unsatisfactory unless a very large excess of ammonium oxalate is used to keep magnesium in solution and ensure quantitative precipitation of calcium oxalate.[1] It is apparently generally accepted that the solution from which precipitation takes place should not contain cations other than those of calcium, magnesium and the alkali metals, and that iron and aluminium if present should be removed by preliminary double precipitations with ammonia. Alternative procedures are available, however, which avoid removal of iron and aluminium by precipitating calcium oxalate in slightly acid solution[2] at *p*H 3–4; but under these conditions precipitation of small amounts of calcium may be incomplete.

Hillebrand and Lundell state[3] that although iron is quantitatively precipitated by oxalate in ammoniacal solution more or less of the precipitate is dissolved if washed with cold ammonium oxalate solution. They also state that aluminium when alone is not precipitated by oxalate in ammoniacal solution. According to McAlpine and Soule,[4] ferric iron is not precipitated by oxalates except as reduction to ferrous iron takes place, and oxalates do not precipitate aluminium. It would appear then that in the usual separation of calcium from magnesium prior removal of ferric iron and aluminium is unnecessary, unless they are present in relatively large proportion. Moreover, since the removal of iron and aluminium involves increasing the ammonium chloride content, and since a large amount of this has the effect of retarding the precipitation of calcium oxalate, the procedure, as well as being time-consuming, may be actually harmful.

Precipitation of calcium oxalate without removal of iron and aluminium has been successfully applied by us to the analysis of dead-burned sea-water magnesia, a material of the following average composition: MgO 90–93%, CaO 2–5%, Fe_2O_3 1·5%, Al_2O_3 1·5%, SiO_2 2%, Mn 0·1%. The method adopted, which utilises precipitation in the normal way at a *p*H of about 6, but using a large excess of ammonium oxalate, was developed and tested on synthetic solutions corresponding to magnesias of different lime contents (1–4%). These solutions contained NaCl 11 g., Fe_2O_3 0·0297 g., Al_2O_3 0·0318 g., MgO 0·9627 g., CaO 0·01–0·04 g. The iron, aluminium, magnesium and calcium, here expressed as oxides, were added in the form of soluble salts of analytical reagent quality. The sodium chloride added, also of analytical reagent quality, corresponded in amount to that arising from fusion of refractory magnesia material with sodium carbonate followed by solution in dilute hydrochloric acid. Fusion with sodium carbonate is a necessary preliminary to any analytical separations on this type of magnesia owing to the presence of acid-insoluble silicates.

It was found that precipitation of calcium oxalate in presence of iron and aluminium yielded results as correct as those obtained with solutions from which iron and aluminium had been removed. When less than 0·03 g. of lime was present the amounts found were low, but with this and greater quantities the average recovery was over 98%.

Experimental details of the method follow.

Procedure—Grind the sample to pass B.S. Mesh No. 200 sieve and fuse 1 g. with 10 g. of sodium carbonate. Dissolve the cooled melt in dilute hydrochloric acid, containing 35 ml. of the concentrated acid, and boil with a few drops of concentrated nitric acid to oxidise any ferrous iron.

Add methyl red solution, dilute to 350 ml., and heat to about 90° C. Add 20–25 g. of ammonium oxalate and dissolve with stirring. Precipitate calcium oxalate by dropwise addition of concentrated ammonia solution until the colour changes to yellow, and allow to stand in a warm place for 30 to 60 minutes. Then filter through a No. 30 Whatman paper and wash thoroughly with cold 0·1% ammonium oxalate solution. Dissolve the precipitate with hot dilute hydrochloric acid, containing 10 ml. of the concentrated acid, and wash the paper thoroughly with hot diluted hydrochloric acid (1 in 100).

Add 25 ml. of saturated ammonium oxalate solution, dilute to 250 ml., heat to boiling, and precipitate calcium oxalate as above. Allow to stand in a warm place for 60–120 minutes. Then filter, wash thoroughly with cold water, and finally titrate with $N/10$ permanganate according to the recognised procedure.

Results are compared below with those obtained here by the usual method, which involves double precipitations of ferric oxide and alumina by ammonia before twice precipitating calcium oxalate as described. The same figures within the limits of experimental error are given by both procedures.

Sample	% CaO Fe_2O_3 and Al_2O_3 removed	% CaO Proposed method
1	4·14, 4·18	4·09, 4·14
2	5·14, 5·21	5·12, 5·20
3	5·06, 5·11	5·05, 5·14
4	4·90, 4·97	4·93, 4·96
5	4·67, 4·73	4·62, 4·69
6	4·99, 5·04	4·96, 4·98
7	4·51, 4·54	4·51, 4·52
8	4·53, 4·57	4·53, 4·57

As previously mentioned, when the magnesia contains less than 3% of lime the figures obtained are low. In such cases it is useful for routine work to increase the lime content artificially, by adding known amounts of standard calcium chloride solution, although this procedure is analytically unsound.

We thank the Directors of the British Periclase Co. for permission to publish this note.

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PALLISER WORKS
HARTLEPOOL, CO. DURHAM

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January, 1946

THE EFFICIENCY OF DESICCANTS

SEVERAL investigators[1] have published figures for the comparative efficiencies of various desiccants, the latest and most complete being due to Bower.[2] His method of establishing these relationships was to pass moist air through U-tubes containing the various desiccants arranged in progressive order of efficiency. The efficiency of any particular desiccant is expressed as the number of mg. of water vapour remaining in 1 litre of air after passing through that desiccant. The purpose of this note is to demonstrate that conclusions can be drawn from Bower's table which, if put in practice, can give rise to serious errors in gravimetric analysis.

At least one modern text-book, which has achieved some popularity in this country, quotes a portion of Bower's table and then proceeds to point out that alumina and lime, when used as weighing forms, should never be cooled over calcium chloride—which has a lower efficiency according to Bower—but always over a desiccant of higher efficiency, such as magnesium perchlorate or barium oxide.

These instructions might be acceptable if it had been added that the crucible containing the residue should be covered, even whilst in the desiccator. This important injunction is stressed in text-books written by such authorities as Lundell, Hoffman and Bright,[3] and Kolthoff and Sandell,[4] who also recommend re-ignition and re-weighing, with the weights from the earlier weighing still set on the balance-pan.

Since the impression is given in the instructions referred to above that hygroscopic residues will remain dry whilst being cooled, providing a desiccant of greater efficiency is used in the desiccator, it is necessary to emphasise that Bower's figures can only be a very rough guide as regards desiccants used in a desiccator. Their main value lies in assessing the relative merits of desiccants used in drying-trains, where the conditions are similar to those under which the figures were obtained.

The conditions in desiccators are very different. G. F. Smith[5] was unable to dehydrate magnesium perchlorate dihydrate by exposing it to either barium oxide or phosphorus pentoxide in a desiccator, although the relative efficiencies are as follows:

	Mg. of water remaining in 1 litre of air
Anhydrous magnesium perchlorate	$0{\cdot}002_2$
Barium oxide	$0{\cdot}00065_2$
Phosphorus pentoxide	$0{\cdot}00025_6$

Some further results quoted by Morton[7] are also of interest in this connection. Hydrated copper sulphate was placed in desiccators over alumina, phosphorus pentoxide or calcium chloride. The amounts of water removed by these three desiccants respectively were 42·8%, 41·5% and 40·0% of the total. In a second series of tests, which included barium and magnesium perchlorates, activated alumina proved to be the most efficient desiccant; the perchlorates were the poorest. Similar tests were carried out, using coffee and flour. Morton also quotes results obtained by another method of comparing efficiencies. Catalysts for the conversion of carbon monoxide to carbon dioxide are affected by water. Hence, the amount of conversion to the dioxide after passing through a particular desiccant gives a measure of the water vapour present. The first four in order of efficiency were activated alumina, magnesium perchlorate, calcium chloride and barium oxide. Calcium chloride and activated alumina have efficiencies of 0·36 and 0·005* respectively, according to Bower; the efficiencies of the others are recorded above. Although the method of drying in these tests was similar to that of Bower, insofar as the moist air was passed through the desiccant, the rate of flow was 200 litres per hr., whereas Bower used a rate of flow of 1–5 litres per hr. It will be seen that there is little similarity between the orders of efficiency. From these results it is evident that when choosing a desiccant for a particular purpose any table of desiccant efficiencies is only of real use *if the experimental conditions under which the results were obtained are similar to those for which the desiccant is required.*

Some convincing figures which demonstrate the inefficiency of desiccators have been supplied by Booth and McIntyre.[6] They measured the fall in vapour pressure after admitting air saturated with water-vapour into a desiccator. Several desiccants were tested and the fall in vapour pressure was plotted against time. Their results showed that even after 60 min. there was still an appreciable amount of unabsorbed water-vapour present. These investigators point out that if, on removing the lid from the desiccator, half of the volume of contained air were replaced by atmospheric air, the partial pressure of the water-vapour would only fall by 2 mm. in 10 min. Since a crucible would not be left to cool for much longer than this, a hygroscopic residue could absorb an appreciable amount of water. It might also be pointed

* This figure has recently been revised by Bower and is now reported as 0·001.

out that this could not be remedied by leaving the residue in the desiccator for such time as is necessary for the desiccator atmosphere to become dry. Once the residue has absorbed water it may not relinquish it to a more efficient desiccant, even after long exposure to it in a desiccator. This phenomenon is exemplified by G. F. Smith's results (*supra*) when he attempted to dehydrate magnesium perchlorate dihydrate by means of phosphorus pentoxide.

The procedure recommended by Lundell, Hoffman and Bright, and by Kolthoff and Sandell (*loc. cit.*), namely, to provide the crucible with a well-fitting cover, to weigh as soon as cool, to re-ignite, re-cool and re-weigh with the weights set on the pan, should reduce these errors to negligible proportions. Axiomatically, it might be added that if the residue has to be left for some time before it can be weighed, it should be re-ignited before weighing. These rules are more important than the choosing of a desiccant of high efficiency or even using a desiccator at all.

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DEPT. OF FUEL TECHNOLOGY
THE UNIVERSITY, SHEFFIELD

RONALD BELCHER

January, 1946

THE CONTINUOUS PRODUCTION OF DOUBLY DISTILLED WATER

THE apparatus shown in Fig. 1(*a*) affords a continuous supply of high-quality doubly distilled water. Apart from the reservoir and float system, standard parts with interchangeable ground glass joints are

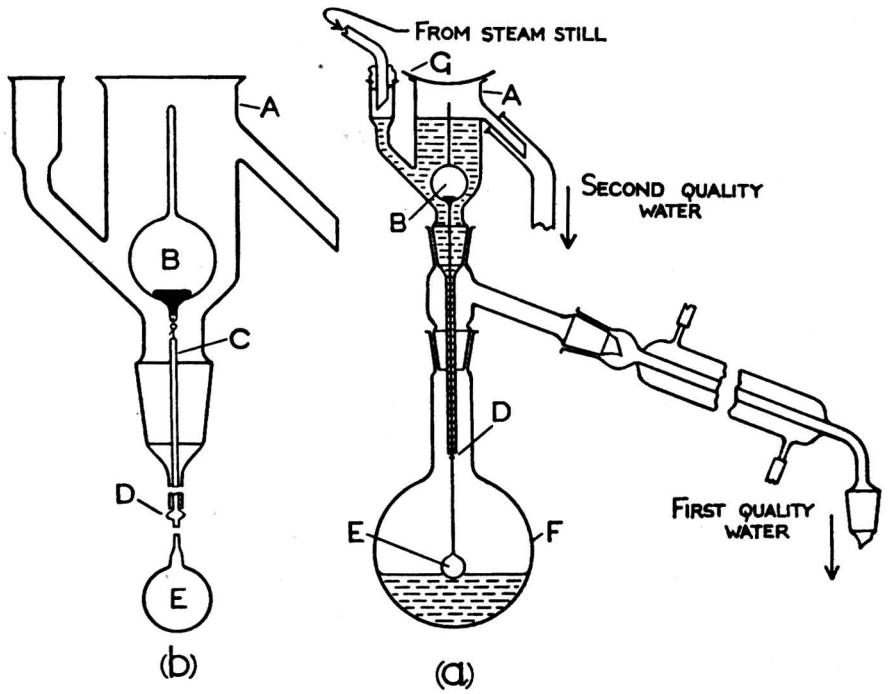

Fig. 1.

employed. Surfaces exposed to water are of Pyrex glass and platinum, so that neither the excess of feed water nor the final distillate suffers contamination. Clock-glass G prevents entry of dust and fumes.

A rapid stream of distilled water from a steam oven still or other source maintains a constant head in reservoir A, the excess overflowing and being suitably collected. Immersed in the liquid is a light float B, 35 mm. in diameter, into the bottom of which is sealed a small loop of platinum wire. Valve rod C is 3 mm. in diameter, and carries at its upper end a small hook of platinum wire which engages with the loop on the float. Details of the float assembly are shown in Fig. 1 (*b*). Valve D, a sphere 7 mm. in diameter formed upon the valve rod, is ground into the extremity of the reservoir stem. Control float E, 24 mm. in diameter, is sealed to the lower end of C. The depth of water maintained in flask F is determined by the position of float E with respect to the bottom of the flask when the valve is closed.

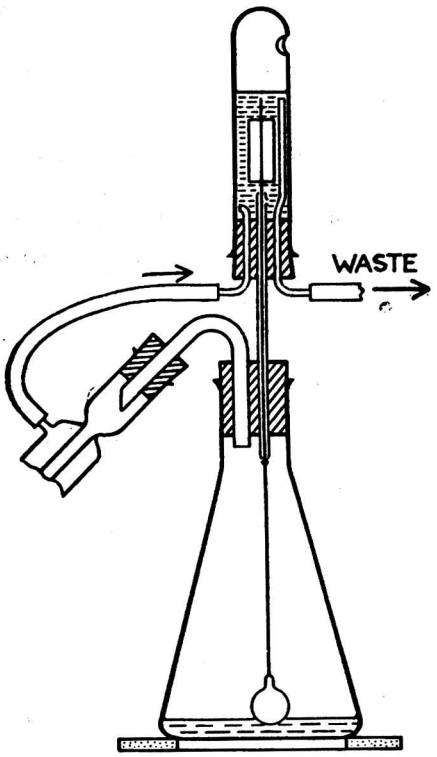

Fig. 2.

To adjust the buoyancy, clamp the reservoir vertically and assemble the float system. Lift the latter to close the valve and fill the reservoir with water. The valve should now remain closed without leak. Introduce mercury or lead shot into float B until the valve first commences to leak and then opens to about 4 mm. Raise a beaker containing water until the surface of the latter touches float E. On raising the beaker a further 5 mm. the valve should close completely. Remove float B and close the tip of the tube surmounting it.

Introduce distilled water into the flask to a little below the working level. Assemble the apparatus and pour a rapid stream of distilled water into the reservoir until overflow occurs. The level in the flask should now rise to its working position, and the valve should close. Having started the steam still, redistillation is commenced by suitably heating the flask.

If the valve is satisfactorily ground in, its leakage is very slight. However, if the apparatus is out of use for several days the reservoir may empty itself into the flask. In such an event, distilled water is poured rapidly into the reservoir until the float system rises. As distillation is proceeded with, the level in the flask rapidly corrects itself.

A simple modification shown in Fig. 2 is useful for the production of distilled water when the usual source is out of action. The reservoir is an inverted boiling tube in which a small hole is blown near the closed end. An overflow device, fed from the condenser jacket, maintains a constant head of warm water in the reservoir. The float assembly resembles that of Fig. 1, but the upper float is a cylinder of sound cork which slides stiffly upon the float rod. Dimensions may be adjusted to suit any type or capacity of flask. For rapid distillation, a 1-litre Pyrex conical flask is particularly suitable. This is heated directly with a large burner, the asbestos ring preventing the flame from rising above the water level and hence cracking the flask. The float system should be set to maintain the level at about 2 cm. Under these conditions, vigorous boiling with the minimum of spurting is achieved.

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HAMMERSMITH, LONDON, W.6

J. T. STOCK
M. A. FILL

February 26th, 1946

A THERMOSTATICALLY-CONTROLLED LOW TEMPERATURE BATH

THE bath described below was developed for the routine examination of alcoholic liquors, such as alcoholic flavouring essences, wines, spirits, etc., on which determinations of specific gravity at 15·5° C. are required. Any predetermined temperature down to about 10° C. below room temperature may be maintained automatically, so that the device is of general use. As in the well-established apparatus for the cryoscopic examination of milk,[1] cooling is effected by the evaporation of ether in a current of air. The rate of evaporation, and hence the cooling effect, is, however, regulated by controlling the air stream.

The bath shown in Fig. 1 is a beaker containing 2 litres of water. It is lagged with a 2-cm. layer of cotton wool, a tinplate canister serving as an outer jacket. The cooler A is a 160 mm. × 28 mm. boiling tube, which is fed from ether reservoir B by way of valve C, so that the volume of ether in A is maintained at approximately 35 ml. Surrounding the cooler is corrugated ring stirrer D. This is operated by the unit originally devised for micro-stirring,[2] the power available being ample.

Air is drawn into the cooler through tube E, and leaves through F after bubbling through the ether. The toluene - mercury regulator G is of the gas-controlling type, but has an additional side tube H, which is connected to a water jet pump. Alternatively, the pump may be connected to the branch of a T-piece inserted into tube F. No alteration to the regulator is then required.

Operation is as follows. At room temperature the drawn-out extremity of tube J dips beneath the surface of the mercury in the regulator stem. The speed of the pump is then adjusted so that air is drawn

through the ether in A at about 150 bubbles per min., fine adjustment being made by a screw clip upon tube F. As the bath cools, the level in the regulator stem falls until the tip of J is uncovered. A path of low resistance thus being opened, air is now drawn in preferentially through J, so that bubbling in A becomes slow or ceases altogether. As the temperature of the bath rises, J is closed by the rising of the mercury column, causing the air stream to be drawn through the ether again. The temperature at which the bath operates thus depends upon the setting of the thermoregulator.

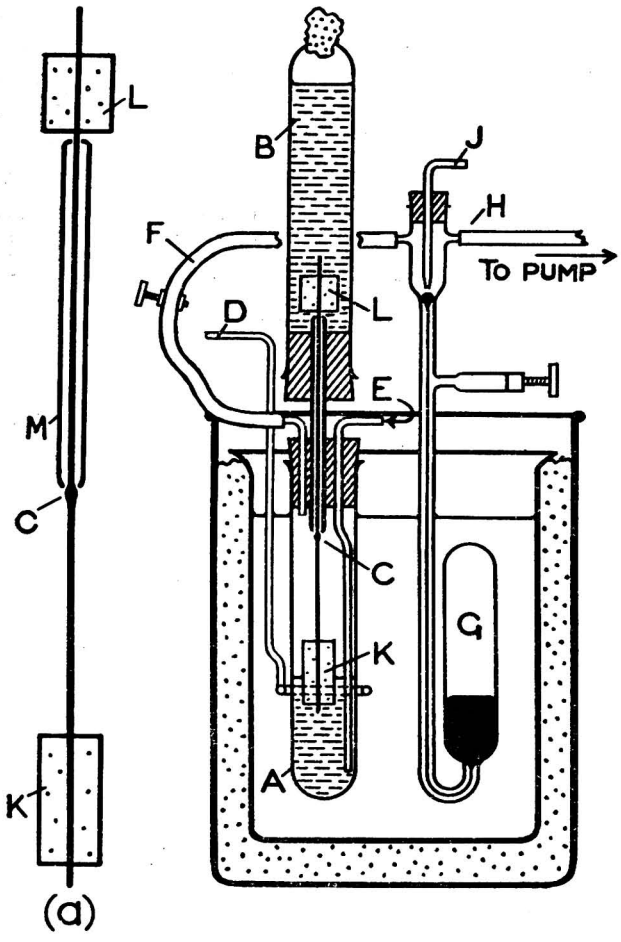

Fig. 1.

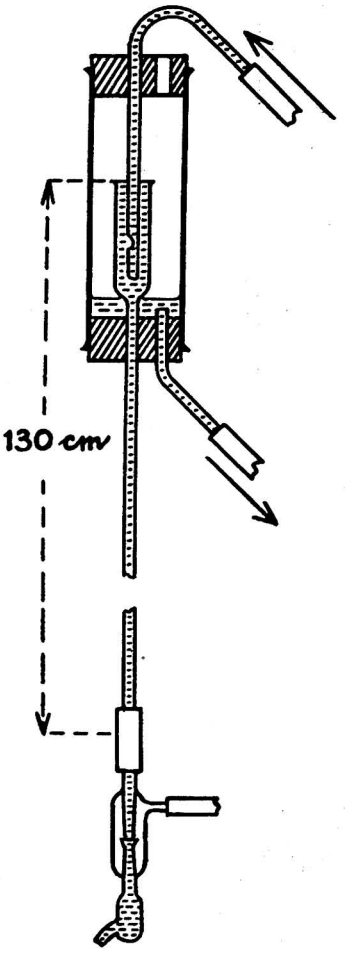

Fig. 2.

In early experiments with the apparatus, attempts were made to control valve C by means of a single float K. Owing to agitation caused by bubbling, very poor results were obtained. The addition of a second, smaller float L, provides a steady upthrust which almost compensates for the weight of the valve system. This allows float K to maintain satisfactory control of the ether level in A. Details of the valve assembly are shown at (*a*) in Fig. 1. Both floats are of sound cork, and slide stiffly upon the stem. Valve C is a 7-mm. sphere formed on the 3-mm. glass stem, and is lightly ground into guide M, the ends of which are constricted to 4 mm. bore.

The rate of bubbling should be reasonably steady. A simple constant-head device applied to the pump as shown in Fig. 2 enables good results to be obtained with widely-fluctuating mains pressures.

On adjusting the regulator to maintain bath temperatures of 15° and 10° C., respectively, the maximum variations during daily runs were found to be ±0·05° and ±0·09° C., respectively. During the observations the laboratory temperature varied from 16° to 23° C.

The rates of consumption of ether are shown in Fig. 3, which also indicates the time required to attain a desired bath temperature (initially 20° C.) with the gentle suction giving the most sensitive control. Obviously it is desirable to pre-cool the bath to within a degree or so of the desired temperature, *e.g.*, by adding ice. Alternatively, the rate of cooling may be greatly increased by temporarily using greater suction. As is clear from Fig. 3, only a small amount of ether (80 to 250 ml. per normal working day) is required to maintain the desired temperature. It is preferable to use dry ether, but the device works quite satisfactorily both with mixtures of ether and light petroleum recovered from fat extractions and with wet ether. Despite the small size of the cooling unit, it can be used to control a much larger bath than that described, provided that the operating temperature is not more than a few degrees below that of the laboratory. Thus a well-stirred bath containing 3 gal. of water was maintained without difficulty at 15° ± 0·05° C. In such cases, it is of course necessary to pre-cool the bath to its working temperature; otherwise wastage of time and of ether is excessive.

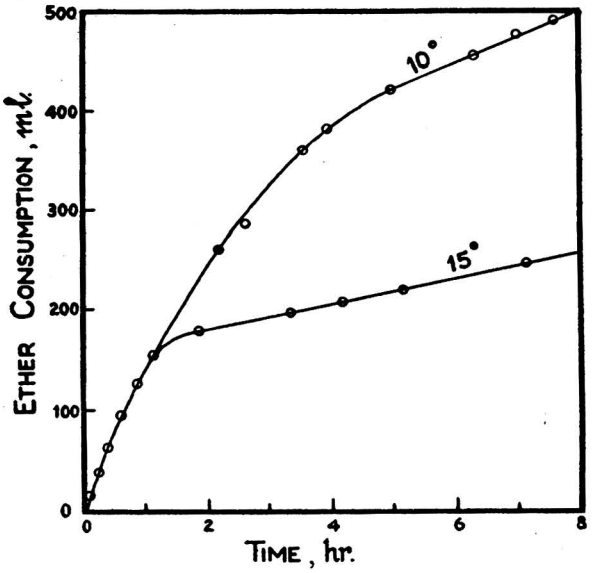

Fig. 3.

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January 12th, 1946

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Chromatographic Estimation of Vitamin A in Mixed Feeds. M. L. Cooley, J. B. Christiansen and C. H. Schroeder (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 689–692)—The adsorbent used is sodium carbonate which allows vitamin A and carotene to pass through the column but retains xanthophylls. Suitable sodium carbonate may be obtained in small quantities or by the barrel from F. W. Kerr Co., 422, West Congress St., Detroit, or in barrel lots from The Wyandotte Chemicals Corpn., Wyandotte, Mich. It is ground to pass a 40-mesh screen and stored to prevent absorption of moisture. Sodium carbonate from other sources did not retain the non-carotene pigments so effectively. To prepare the chromatographic column pack the sodium carbonate above a small plug of cotton tightly into a tube of diam. 3·8 cm. and 25 cm. long to a depth of 18 cm. Attach the tube by means of a rubber stopper to a filter flask having its side arm connected with a suction pump. To 20 g. of the sample in a Waring Blendor add 5 or 10 g. of Hyflo Super Cel and 100 ml. of light petroleum (b.p. 60°–71° C.). Stir the mixture at low speed for 5 min., pour it on to the column, apply suction, rinse the Blendor jar with light petroleum and pass this and additional light petroleum through the column until 100 to 150 ml. have been used for rinsing and elution. If a distinct xanthophyll band passes through the column, submit the eluate to a second adsorption. Evaporate the eluate to slightly less than 100 ml. by mild heat with reduced pressure, pass it through anhydrous sodium sulphate in a funnel, wash the sodium sulphate with light petroleum and adjust the vol. of the combined soln. and washings to 100 ml. Place suitable aliquots in the comparison tube of a spectrophotometer (a Coleman Universal photoelectric spectrophotometer was used in the investigation) and remove almost all the solvent by application of mild heat and reduced pressure. Dissolve the residue in 1 ml. of chloroform and examine the soln. in the spectrophotometer adjusted to the 620 mμ wave band. Add 10 ml. of antimony trichloride reagent from a rapidly delivering automatic pipette, take galvanometer readings and refer them to a standard curve. Correct the readings for the amount of carotene present by means of a curve prepared by treating a standard soln. of carotene with antimony trichloride under the same conditions. The amount of carotene present may be insignificant. Saponified U.S.P. reference cod-liver oil was usually employed for preparing the standard curve for vitamin A, since the unsaponified fish oils used in the investigation gave results agreeing well with this curve. A standard curve may also be prepared by using an accurately assayed oil of the type occurring in the feed. The procedure outlined by Koehn and

Sherman (*J. Biol. Chem.*, 1944, **132**, 527) was used for the saponification, use of an inert gas being omitted.

Investigation of the effect of saponification on the chromatographic separation of the vitamin showed that in some instances the inclusion of saponification caused a loss of 9% of vitamin A. This does not invalidate the method for the practical examination of feeding stuffs for livestock, but saponification should be avoided when practicable. Expts. with mixtures of low carotene content free from vitamin A showed that the antimony trichloride reagent may cause a darkening of the soln. and decreased transmission at 620 mμ. In practice it has been found that saponification is advisable when the vitamin A content is less than one I.U. per g. A. O. J.

2-Phenylazo-*p*-Cresol, a Photometric Standard for Vitamin A. H. R. Kreider (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 694–695)—Taylor (ANALYST, 1942, **67**, 248) suggested the use of benzeneazo-*p*-cresol as a standard for vitamin A because its absorption spectrum resembled that of vitamin A. This suggestion has apparently been overlooked, for no other reference to the use of such a compound has been found. The absorption curve of 2-phenylazo-*p*-cresol was determined with a Beckman spectrophotometer and a Hilger E37 spectrograph, and the curve was found to be virtually identical with that of vitamin A between 290 mμ and 370 mμ. The War Food Administration and the Vitamin Oil Producers' Institute are now studying a reproducible method of evaluating vitamin A in fish liver oils. The proposed method uses the factor $2000 \times E^{1\%}_{1\,cm.}$ at 328 mμ provided that the ratios of $E^{1\%}_{1\,cm.}$ at 300 mμ and at 350 mμ to $E^{1\%}_{1\,cm.}$ at 328 mμ do not exceed 0·73 and 0·65 respectively. For 2-phenylazo-*p*-cresol these ratios are 0·613 and 0·560 respectively, and at 328 mμ $E^{1\%}_{1\,cm.}$ for the dye is 900. Thus, assuming an E value of 1745 for vitamin A, 1 g. of the azo compound is equivalent to 0·515 g. of vitamin A when measured at 328 mμ. To prepare the dye dissolve 8·8 g. of aniline (from sulphate) in 100 ml. of hydrochloric acid and 300 ml. of water, cool to 0° C. and add 8 g. of sodium nitrite in 50 ml. of water over a period of 2 hr. Add 10 g. of *p*-cresol in 50 ml. of 0·5 *N* sodium hydroxide slowly with cooling. Neutralise the resulting soln. with sodium hydroxide to *p*H 6. Recrystallise the pptd. dye from acetone water and alcohol. The product consists of yellow to brown glistening plates melting at 106·5° to 107° C. The solid dye is stable for at least 8 years. A soln. containing 7·5 mg. in 1000 ml. of *iso*propanol showed no change in its absorption curve after exposure to light from a north window for 18 months. After exposure in a quartz cell to direct sunlight for 3 months the extinction coefficient diminished by about 50%. This apparent instability does not seem to be inherent in the compound but is related to the solvent, for a similar soln. in cyclohexane was stable under the same conditions. The compound is stable in maize oil and is soluble in concns. up to 4%, corresponding with a vitamin potency of about 50,000 units per g. The dye will probably not be used as an adulterant because an absorption band in the blue region makes it easily recognisable by its yellow colour even when highly diluted. The best method of using the dye is to prepare a soln. in maize oil in the concn. range of the fish oils being examined and dilute it in the same manner as the samples for spectrophotometric determinations. A fresh dilution may be made for each calibration, or the dilutions may be kept in stoppered bottles and re-used. The dye thus provides a standard that is stable, easily handled and readily available for the calibration of instruments in U.S.P. units or μg. of vitamin A. A. O. J.

Erratum.—February issue, p. 82, col. 1, line 42: *For* "8 g." *read* "18 g." of pure barium hydroxide.

Biochemical

Estimation of Phenylalanine in Protein Hydrolysates with *Leuconostoc mesenteroides* P-60 and *Lactobacillus helveticus*. M. S. Dunn, S. Shankman and M. N. Camien (*J. Biol. Chem.*, 1945, **161**, 643–655)—The basal medium used for the assay of phenylalanine with *Leuconostoc mesenteroides* was the Medium D (Table I) of Dunn *et al.* (*J. Biol. Chem.*, 1944, **156**, 703; ANALYST 1945, **70**, 182), modified to give twice the amount, of the amino acids and with the phenylalanine omitted. The tubes were incubated for 5 days at 35° C. The following modification of Medium D was used for assays with *Lactobacillus helveticus*: *dl*-alanine 200, natural asparagine 200, *l*(+)-arginine monohydrochloride 200, *l*(+)-glutamic acid 200, *l*(−)-histidine monohydrochloride monohydrate 200, *dl-iso*leucine 200, *l*(−)-leucine 200, *l*(+)-lysine monohydrochloride 200, *dl*-methionine 200, *dl*-threonine 200, *l*(−)-tryptophan 200, *l*(−)-tyrosine 200, *dl*-valine 200, *l*(−)-cysteine hydrochloride 275, *dl*-serine 300 mg.; folic acid 6 μg.; water to 1 litre. The mean deviations from the theoretical response of *dl*-phenylalanine were ±2·6% at concentrations up to 30 μg. with *Leuconostoc mesenteroides* and ±3·0% at concentrations up to 50 μg. with *Lactobacillus helveticus*. The results obtained for casein and silk fibroin with both organisms were in good agreement. F. A. R.

Investigations of Amino Acids, Peptides and Proteins. XXIV. The Amino Acid Requirements of *Lactobacillus fermenti* 36. M. S. Dunn, M. N. Camien, and S. Shankman (*J. Biol. Chem.*, 1945, **161**, 657–667). **XXV. Estimation of Histidine in Protein Hydrolysates with *Lactobacillus fermenti* 36. M. S. Dunn, S. Shankman and M. N. Camien** (*Ibid.*, 669–678)—The amino acids arginine, glutamic acid, histidine, *iso*leucine, leucine, methionine, phenylalanine, threonine, tryptophan, tyrosine and valine were found to be essential for the growth of *Lactobacillus fermenti* 36, and three basal media for use in microbiological assays were investigated. A method of assaying histidine with the best of these (Medium C) is described. It has the following composition: *dl*-alanine 400, *l*(+)-arginine monohydrochloride 160, asparagine (natural) 200, *l*(−)-cystine 200, *l*(+)-glutamic acid 240, glycine 200, *l*(−)-histidine monohydrochloride monohydrate 90, *l*(−)-hydroxyproline 200, *dl-iso*leucine 400, *l*(−)-leucine 200, *l*(+)-lysine monohydrochloride 200, *dl*-methionine 200, *dl*-norleucine 200, *dl*-norvaline 200, *dl*-phenylalanine 200, *l*(−)-proline 200, *dl*-serine 200, *dl*-threonine 200, *l*(−)-tryptophan 32, *l*(−)-tyrosine 100, *dl*-valine 400 mg. per litre; glucose 30 g. per litre; adenine sulphate 24, guanine hydrochloride 24, uracil 24, xanthine 24 mg. per litre; sodium acetate 10, ammonium chloride 3 g. per litre; KH_2PO_4 500, K_2HPO_4 500, $MgSO_4.7H_2O$ 200, $FeSO_4.6H_2O$ 10, $MnSO_4.4H_2O$ 10, aneurine hydrochloride 1, pyridoxine 1·6, calcium *dl*-pantothenate 2, riboflavine 2, nicotinic acid 2, *p*-aminobenzoic

acid 0·1 mg. per litre; biotin 5, folic acid 2 μg. per litre. This was used without modification for assaying pure solutions of histidine, but with protein hydrolysates the amounts of the amino acids were increased 1·5-fold. The general procedure was that described by Dunn *et al.* (*cf.* preceding abstract), but growth was found to be better with 3 ml. in 4 in. test-tubes than with 5 ml. in 6 in. tubes. The histidine standard was run at 15 levels and the tests at 5 levels, 6 tubes being used at each. The optimum time of incubation was 2 days and after incubation the tubes were steamed for 10 mins. to remove CO_2. The inoculum consisted of a 21-hr.-old culture re-suspended in sterile saline and diluted not more than 5-fold; more dilute inocula resulted in less acid production. Casein was found to contain 3·0 ± 0·1% of histidine, in good agreement with the results obtained with *Leuconostoc mesenteroides*. The histidine content of silk fibroin was 0·33–0·35%. F. A. R.

Estimation of Ascorbic Acid in Small Amounts of Blood Serum. O. H. Lowry, J. A. Lopez and O. A. Bessey (*J. Biol. Chem.*, 1945, **160**, 609–615)—The method of Roe and Kuether (*J. Biol. Chem.*, 1943, **147**, 399; ANALYST, 1943, **68**, 260) was adapted to the estimation of ascorbic acid in 0·01-ml. quantities of serum. By means of this procedure one worker can analyse 50 samples per day.

Put 0·01 ml. of serum into a small tube and add 0·04 ml. of a suspension of charcoal in trichloroacetic acid (suspend 5 g. of Norit washed with acid and dried as described by Roe and Kuether in 100 ml. of 5% trichloroacetic acid; decant the supernatant liquid, re-suspend in the same volume of 5% trichloroacetic acid and repeat several times to remove finely divided, floating charcoal). Close the tube with a rubber stopper, centrifuge at 3000 r.p.m. for 10 mins. and transfer a 0·03-ml. portion to another tube. Add 0·01 ml. of a 2% dinitrophenylhydrazine - 0·25% thiourea solution in 9 *N* sulphuric acid (this reagent does not keep for more than 1 month at 0° C.), stopper the tube and incubate at 38° C. for 3 hrs. Cool in ice-water, add 0·05 ml. of cold 65% sulphuric acid, with very thorough mixing, and evaluate the colour after 30 mins. at room temperature in a spectrophotometer at 520 mμ. Prepare standard and blanks by adding 4 ml. of the trichloroacetic acid - charcoal suspension to 1-ml. quantities of fresh ascorbic acid solution (1 mg. per ml.) and water respectively; centrifuge and treat 0·03-ml. portions in the same way as the test-solutions. After correction of the blank the colour is directly proportional to the concentration of ascorbic acid. The method may be used for assaying larger volumes than 0·01 ml., simply by multiplying the volumes of the reagents proportionately; it is more convenient than the original method. The average deviation of an individual result was 0·03 mg. per 100 ml. with amounts of ascorbic acid ranging from 0·3 to 1·4 mg. per 100 ml. F. A. R.

Interfering Substances in the Roe and Kuether Method for the Estimation of Ascorbic Acid. J. R. Penney and S. S. Zilva (*Biochem. J.*, 1945, **39**, 392–397)—The method of Roe and Kuether (*J. Biol. Chem.*, 1943, **147**, 399; ANALYST, 1943, **68**, 260) depends on the formation of a red coloration by the action of 85% sulphuric acid on the 2 : 4-dinitrophenylhydrazine derivative of dehydroascorbic acid. Reductic acid, reductone and heated alkaline glucose solutions also give colours with the reagent, but these can be differentiated from the colour given by *l*-ascorbic acid by varying the conditions of the reaction. Thus when the reaction is carried out at 25° C. for 15 mins., reductic acid, purified reductone, and heated solutions of glucose containing Na_2CO_3 and NaOH generated 92, 48, 66 and 45% respectively of the colour produced at 37° C. in 3 hrs., *i.e.*, the conditions employed by Roe and Kuether. *l*-Ascorbic acid, on the other hand, produced only 2·5% of the colour. The presence of reductic acid can therefore be corrected by estimating the amount of "ascorbic acid" under the two sets of conditions and subtracting one result from the other. Similarly, a correction can be applied when reductone is present, though the error is higher than with reductic acid; with a ratio of *l*-ascorbic acid to reductone of 4 : 1 or more, however, this does not exceed 6%, since the colour generated by the ascorbic acid at 25° C. compensates for the incomplete reductone reaction. This method of correcting for interfering substances was less satisfactory with the alkaline glucose solutions where, although the error is reduced, the result may still be misleading. *d*-Gluco-ascorbic acid, *d*-araboascorbic acid and 2 : 3-diketo-*l*-gulonic acid cannot be distinguished from *l*-ascorbic acid in this reaction, so that unless the nature of the interfering substances is known the method of correction now described is inapplicable. F. A. R.

The Free Amino Groups of Insulin. F. Sanger (*Biochem. J.*, 1945, **39**, 507–515)—Attempts have been made in the past to use 2 : 4-dinitrochlorobenzene for the identification of the terminal groups of partial protein hydrolysates, but these met with little success, although it reacts with amino-acids to give bright yellow dinitrophenyl derivatives which can readily be separated chromatographically. Unfortunately, however, reaction does not occur in sodium bicarbonate solution unless the mixture is warmed, and this would result in some hydrolysis of proteins or peptides. It has now been found that 2 : 4-dinitrofluorobenzene (DNFB) reacts readily at room temperature, and the resulting dinitrophenylamino acids can be estimated colorimetrically and separated almost completely from one another by partition chromatography.

Dissolve 0·2 g. of the amino-acid and 0·4 g. of sodium bicarbonate in 5 ml. of water, and add a solution of 0·4 g. (0·28 ml.) of DNFB in 10 ml. of ethanol. Shake for 2 hrs. at room temperature, concentrate to remove ethanol, dissolve the residue in water and extract with ether to remove excess DNFB. Acidify the aqueous solution and allow the oil that separates to crystallise. To separate a mixture of dinitrophenyl-amino acids chromatographically, use a silica gel column as described by Gordon *et al.* (*Biochem. J.*, 1943, **37**, 79; ANALYST, 1943, **68**, 283). No indicator is required, water is used as the stationary phase and chloroform or a solvent mixture such as butanol-chloroform as the mobile phase. Attempts to separate the dinitrophenyl amino-acids by adsorption chromatography were not very successful. To estimate the dinitrophenyl amino-acids, dissolve in *N* hydrochloric acid and evaluate the colour in a photoelectric colorimeter. Dinitrophenyl-phenylalanine is sparingly soluble in *N* hydrochloric acid, and in this instance sodium bicarbonate solution is used. Calculate the results from standard curves, as the colour intensities do not obey Beer's law.

In investigating the nature of the free amino-groups of a protein, this is treated with DNFB, the product is hydrolysed by boiling with 20% hydrochloric acid, and the hydrolysate is extracted with ether. The ethereal extract contains the dinitrophenyl derivatives of most of the amino acids, and the aqueous phase the free amino acids and the dinitrophenyl derivatives of histidine, cysteine, arginine and lysine. The mixtures are separated by partition chromatography and the dinitrophenyl derivatives identified and estimated. F. A. R.

Microtitration Method for the Estimation of Amino Acids. A. E. Sobel, A. Hirschman and L. Besman (*J. Biol. Chem.*, 1945, **161**, 99–103)—In this method the ammonia formed by the action of ninhydrin on amino-acids is determined, after destruction of the ninhydrin by means of hydrogen peroxide, by addition of saturated potassium hydroxide solution, aeration and titration with standard acid.

Put 1 ml. of solution containing 20 to 100 μg. of carboxyl-nitrogen into an aeration tube, add 0·3 ml. of buffer solution of *p*H 2·5 (mix 2·06 g. of trisodium citrate and 19·15 g. of citric acid and prepare a 10% solution of the mixture) and 50 mg. of ninhydrin. Immerse the tube in a boiling water-bath for 2 mins., shake and heat for a further 8 mins. Add 3 drops of 30% hydrogen peroxide, shake, and heat for a further 3 mins. Transfer the tube to the aeration apparatus (*cf.* A. E. Sobel, A. M. Mayer and S. P. Gottfried, *J. Biol. Chem.*, 1944, **156**, 355), add 1 ml. of saturated potassium hydroxide solution (prepared and stored under a 1 in. layer of mineral oil to protect against atmospheric carbon dioxide) and aerate for 40 mins. Collect the ammonia in 1·5 ml. of 2% boric acid - indicator solution (dissolve 20 g. of boric acid in 1 litre of water and add 20 ml. of a solution prepared by mixing 10 parts of 0·1% bromocresol green and 1–2 parts of 0·1% methyl red in 95% alcohol), and titrate with 0·0714 *N* hydrochloric acid with a capillary micro-burette. The amount of amino acid-nitrogen (μg.) = ml. of acid × 1000.

Quantitative results were obtained with most of the 18 amino acids tested, but low results with hydroxyproline, tryptophan, cysteine and cystine. On the whole, the method gave better results than MacFadyen's ammonia method (*J. Biol. Chem.*, 1944, **153**, 507; ANALYST, 1944, **69**, 313) and in some instances results as good as those obtained by the manometric method of Van Slyke *et al.* (*J. Biol. Chem.*, 1941, **141**, 627; ANALYST, 1942, **67**, 104). Moreover the quantities of amino acids required in the present method are much smaller than in the other two methods. F. A. R.

Estimation of Choline in the Liver and Plasma of the Dog. C. Entenman and I. L. Chaikoff (*J. Biol. Chem.*, 1945, **160**, 377–385)—The choline contents of various extracts and fractions of plasma and liver were estimated by slight modifications of the methods of Glick (*J. Biol. Chem.*, 1944, **156**, 643) and Entenman *et al.* (*J. Biol. Chem.*, 1944, **155**, 13). In both methods hydrolysis was effected by means of a 10% solution of $Ba(OH)_2$ instead of a saturated solution as originally recommended. Glick's method was further modified as follows: Put an aliquot of the extract into a 125-ml. conical flask and evaporate to about 5 ml. Add 15 ml. of the barium hydroxide solution and close the mouth of the flask with a glass marble. Heat on the steam-bath for 2 hrs., cool and add 1 drop of thymolphthalein indicator and just sufficient glacial acetic acid to discharge the blue colour. Filter and collect the filtrate in a 30-ml. vial placed inside the filter-flask. Precipitate the choline as described by Glick by adding to the filtrate a solution of Reinecke's salt in methanol. Leave for 2 hrs. and filter through a sintered glass filter. Wash the residue with 2·5 ml. of *n*-propanol and dissolve in acetone, collecting the filtrate in a 15-ml. centrifuge-tube placed inside the flask. Measure the volume and then evaluate the colour in a Klett-Summerson photoelectric colorimeter as in the original method.

The two methods gave identical results with the phospholipid fraction from liver or plasma and with a methanol or alcohol-ether extract of plasma. With an alcohol-ether extract of liver, however, Glick's method gave a lower result than the method of Entenman *et al.*, possibly because the precipitation of compounds other than choline is less likely to occur in an alkaline medium. F. A. R.

Estimation of Oxybiotin in Presence of Biotin. K. Hofmann and T. Winnick (*J. Biol. Chem.*, 1945, **160**, 449–453)—When biotin is treated with dilute potassium permanganate solution it is converted into biotin sulphone which only stimulates the growth of yeasts in presence of aspartic acid. Oxybiotin, on the other hand, is unaffected and can therefore be estimated in presence of biotin by destroying the latter with permanganate and then measuring the growth response in absence of aspartic acid.

To 10 ml. of a solution containing 1 mμg. of *d*-biotin or 4 mμg. of *dl*-oxybiotin, add 10 ml. of 0·01 *N* potassium permanganate and leave for 5–10 mins. at room temperature. Decolorise the excess by careful addition of 0·1 *M* sodium sulphite, dilute to 100 ml. and assay by Hertz's modification (*Proc. Soc. Exp. Biol. Med.*, 1943, **52**, 15) of the yeast growth method of Snell, Eakin and Williams (*J. Amer. Chem. Soc.*, 1940, **62**, 175; ANALYST, 1940, **65**, 524). Biotin added to samples of autolysed yeast was quantitatively recovered and treatment of such samples with potassium permanganate resulted in complete inactivation. Oxybiotin, on the other hand, was not affected, the recoveries in two experiments being 100 and 93% of the theoretical. F. A. R.

Colorimetric Estimation of Blood Acetoin. W. W. Westerfeld (*J. Biol. Chem.*, 1945, **161**, 495–502)—A particularly simple and sensitive method of estimating acetoin in blood is based on the formation of a red colour by addition of creatine and α-naphthol; the reaction is given by both diacetyl and acetoin. When the latter cannot be directly estimated by reason of the presence of interfering substances it is oxidised to diacetyl, which is isolated by distillation and then estimated. Oxidation to diacetyl is also necessary when both compounds are present in solution.

To 5 ml. of a solution containing 1 to 12 μg. of acetoin or diacetyl, add 1 ml. of 0·5% creatine solution, followed by 1 ml. of a 5% solution of α-naphthol in 2·5 *N* sodium hydroxide prepared *immediately* before the addition. Leave at room temperature for exactly 10 mins. when diacetyl is being estimated or 1 hr. with acetoin, and evaluate in an Evelyn photoelectric colorimeter with a 540 filter. Use a blank prepared from 5 ml. of water to set the galvanometer at 100 and include a standard solution containing 5 μg. of acetoin in each series of estimations.

To oxidise acetoin to diacetyl, add 2 ml. of a solution prepared by dissolving 10 g. of ferrous sulphate heptahydrate in 30 ml. of 50% ferric chloride solution, followed by 1 ml. of 10 *N* sulphuric acid, to 7 ml. of the acetoin solution. Stopper the tube lightly and immerse in a boiling water-bath for 30 sec., release the pressure and then stopper tightly and heat for 30 mins. in a boiling water-bath. Cool, transfer the solution to a distillation flask, add a few glass beads and distil carefully until 5 ml. of distillate have collected. Dilute to 10 ml. and estimate the diacetyl in 5 ml. of the solution. The recovery of acetoin by this method was 98% of the theoretical with amounts ranging from 2 to 500 μg. To estimate acetoin in blood, prepare a 1 : 5 tungstic acid filtrate by mixing 2 vols. of water, 1 vol. of blood, 1 vol. of 10% sodium tungstate solution and 1 vol. of 2/3 *N* sulphuric acid. Centrifuge and add a portion of the supernatant liquid to solid sodium chloride (1 g. per 3 ml.) and distil practically to dryness at 150–200 mm. pressure. Oxidise the distillate as described above by means of 2 ml. of the iron solution and 1 ml. of acid per 7 ml. of distillate. The recoveries of added acetoin varied from 85 to 95% of the theoretical. F. A. R.

Determination of Desoxyribonucleic Acid, Ribonucleic Acid and Phosphoproteins in Animal Tissues. G. Schmidt and S. J. Thannhauser (*J. Biol. Chem.*, 1945, **161**, 83–89)—The methods in use for the estimation of nucleic acids are based on certain colour reactions given by their carbohydrate constituents. Although satisfactory results are obtained with nucleosides and nucleotides, difficulties are encountered with the nucleic acids themselves. The method now proposed is based on phosphorus determinations. The phosphorus fraction of ribonucleic acid is separated from that of desoxyribonucleic acid by warming with dilute alkali, which splits the former, but not the latter, into acid-soluble nucleotides. Under these conditions, the phosphorus groups of phosphoproteins are quantitatively liberated as inorganic phosphate. Thus when a mixture of the three substances is warmed with dilute alkali and subsequently precipitated with a strong acid, the total P in the precipitate corresponds to desoxyribonucleic acid, the organic P in the filtrate to the ribonucleic acid, and the inorganic P in the filtrate to the phosphoprotein.

Suspend 0·5–5·0 g. of the minced tissue in approx. 20 vols. of ice-cold 7% trichloroacetic acid, stir for 20 mins. and filter on a relatively large Buchner funnel through a thin layer of filter-aid. Wash liberally with ice-cold 1% trichloroacetic acid until the filtrate is free from inorganic phosphate, then with water until the washings are only faintly acid to litmus and finally with alcohol and ether. Suspend the residue in 30–40 vols. of a mixture (3:1) of alcohol and ether, and boil for a few minutes. Filter and wash with ether. Grind the residue in a mortar, and heat under reflux for 30 mins. with 30–40 vols. of a boiling mixture (1:1) of methanol and chloroform. Filter, wash with ether, and dry in a desiccator.

Transfer the powder quantitatively to a test-tube, add *N*-potassium hydroxide (10 ml. per g. of fresh tissue), close the tube with a rubber stopper, and incubate at 37° C. for 15 hrs. Centrifuge and determine the total P (T_1) in a 1- or 2-ml. aliquot of the solution according to the method of Fiske and Subbarow (*J. Biol. Chem.*, 1925, **66**, 375). Pipette a 5-ml. portion into a test-tube, and add 1 ml. of 6 *N* hydrochloric acid and 5 ml. of 5% trichloroacetic acid. Filter off the precipitate, which contains all the desoxyribonucleic acid, and determine the total P (T_2) in an aliquot of the filtrate by the method of Fiske and Subbarow. Precipitate the inorganic phosphate from a second aliquot by the method of Delory (*Biochem. J.*, 1938, **32**, 1161), and dissolve the washed precipitate in trichloroacetic acid; centrifuge if necessary and determine the inorganic phosphate (T_3) by the method of Fiske and Subbarow. The precipitation is necessary in order to remove protein breakdown products which would otherwise interfere. The difference T_1-T_2 represents the phosphorus of desoxyribonucleic acid, the difference T_2-T_3 the phosphorus of ribonucleic acid, and T_3 the phosphorus of the phosphoproteins. To convert the P values into desoxyribonucleic acid and ribonucleic acid, multiply by 10·1 and 10·6 respectively. It is not possible to give a general conversion factor for phosphoprotein. When known amounts of the two nucleic acids and of casein were added to liver powder, the recoveries were quantitative. F. A. R.

Phosphorus Compounds in Animal Tissues. I. Extraction and Estimation of Desoxyribonucleic Acid and Ribonucleic Acid. W. C. Schneider (*J. Biol. Chem.*, 1945, **161**, 293–303)—Nucleic acids can be quantitatively extracted from animal tissues by heating with 5% trichloroacetic acid after removal of phospholipids and acid-soluble phosphorus compounds, and estimated in the extract colorimetrically.

Mix 1 ml. of a 20% rat liver homogenate with 2·5 ml. of cold 10% trichloroacetic acid and centrifuge. Resuspend the precipitate in 2·5 ml. of cold 10% trichloroacetic acid and centrifuge. The combined extracts contain the acid-soluble phosphorus fraction, the total phosphorus content of which can be estimated by standard methods. Suspend the precipitate in 1·0 ml. of water, add 4·0 ml. of 95% ethyl alcohol and centrifuge. Resuspend the precipitate in 5·0 ml. of alcohol, centrifuge, and boil the precipitate for 3 mins. with three 5·0-ml. portions of a mixture (3:1) of alcohol and ether. The combined alcohol and alcohol-ether extracts contain the phospholipid fraction, the phosphorus content of which can be estimated by standard methods. Suspend the residue in 1·2 ml. of water, add 1·3 ml. of cold 10% trichloroacetic acid and centrifuge. Suspend the residue in 5·0 ml. of 5% trichloroacetic acid, heat for 15 mins. at 90° C., cool, centrifuge, resuspend the residue in 2·5 ml. of 5% trichloroacetic acid and centrifuge. The combined extracts contain the nucleic acids and the residue, which is dissolved in 5·0 ml. of 2% sodium hydroxide solution by heating in a boiling water-bath for 10 mins., the phosphoprotein. Estimate the desoxyribonucleic acid in a 1·0-ml. aliquot of the extract by the diphenylamine reaction (Dische, *Mikrochemie*, 1930, **8**, 4) and in a 0·2-ml. portion by the carbazole reaction (Dische, *loc. cit.*; Gurin and Hood, *J. Biol. Chem.*, 1939, **131**, 211; 1941, **139**, 775) and the ribonucleic acid in a 0·2-ml. portion by the orcinol reaction (Mejbaum, *Z. physiol. Chem.*, 1939, **258**, 117). The diphenylamine reaction is specific for desoxyribonucleic acid, the *E* value of which is 0·0224 per μg. of P, but both nucleic acids react with the carbazole reagent, desoxyribonucleic and ribonucleic acids giving *E* values of 0·152 and 0·0124 per μg. of P respectively. With this reagent, therefore, a correction must be applied for ribonucleic acid. Ribonucleic acid and desoxyribonucleic acid also react with the orcinol

reagent giving E values of 0·135 and 0·0166 per μg. of P respectively, and in this instance a correction must be applied for desoxyribonucleic acid. Calculate the results as follows:

A = Amount (μg.) of desoxyribonucleic acid phosphorus per ml. of solution =

$$\frac{E_{\text{diphenylamine}}}{0{\cdot}0224}$$

B = Amount (μg.) of ribonucleic acid phosphorus per 0·2 ml. solution =

$$\frac{E_{\text{orcinol}} - (0{\cdot}2A \times 0{\cdot}0166)}{0{\cdot}135}$$

C = Amount (μg.) of desoxyribonucleic acid phosphorus per 0·2 ml. solution =

$$\frac{E_{\text{carbazole}} - 0{\cdot}0124B}{0{\cdot}152}$$

Convert the results to μg. of desoxyribonucleic or ribonucleic acid by dividing by 0·099 and 0·095 respectively.

The diphenylamine and carbazole reactions gave very similar results, and the amounts of the two nucleic acids found in animal tissues were in satisfactory agreement with published values.

F. A. R.

Microdetermination of Iodine in Biological Materials with Special Reference to the Combustion of Samples in the Parr Oxygen Bomb. H. Spector and T. S. Hamilton (*J. Biol. Chem.*, 1945, **161**, 127–135)—In this method, which is especially applicable to materials of high carbon content, the sample is ignited in a Parr oxygen bomb and the iodine absorbed in dilute alkali at the bottom of the bomb. The solution is digested with acid permanganate, the iodine liberated with phosphorous acid and distilled in an all-glass apparatus. The iodide in the distillate is oxidised to iodate and titrated with sodium thiosulphate solution, after addition of iodide.

Put a quantity of the sample, containing 2–5 μg. of iodine, into the combustion cup of a Parr bomb and weigh accurately. Put 20 ml. of N-sodium hydroxide into the bottom of the bomb, fill with oxygen at 15 atm. and ignite the material in the usual way, allowing 3 mins. for complete combustion. Release the oxygen slowly and transfer the contents of the bomb to a digestion flask, rinsing out the bomb with 0·1 N sodium hydroxide. To the solution add a few glass beads, 2 g. of recrystallised potassium permanganate and 100 ml. of 18 N sulphuric acid. Fit the flask with a 3-hole rubber stopper carrying a 200° C. thermometer, a tube through which a small stream of compressed air is passed to prevent foaming and a vent to carry off the fumes. Heat over a small flame until the temperature reaches 175° C. Sufficient permanganate should be used so that the digestion mixture does not decolorise before a temperature of 175° C. is reached; if the sample does decolorise it should be rejected. Allow the flask to cool to 100° C., rinse the thermometer with approx. 20 ml. of water and add a further 80 ml. Connect the flask to a distillation apparatus by means of a ground-glass joint and put into the receiver 1 ml. of a solution 2 M with respect to potassium carbonate and 0·2 M with respect to sodium sulphite and adjust the condenser so that the tip dips under the surface. Heat the flask and, as soon as distillation begins, slowly add 5 M phosphorous acid until the solution becomes colourless and then add 2 ml. in excess to liberate the iodine. Continue the distillation until 100 ml. of distillate have collected. Evaporate the distillate to about 5 ml. and transfer to a 50-ml. conical flask with the aid of not more than 20 ml. of water. Again evaporate to 5 ml.

Immerse the flask in a shallow boiling water-bath and add 0·2 M potassium permanganate dropwise until a permanent purple colour is produced. Heat for 3 mins. and add 10 drops of 8 N sulphuric acid. If the permanganate is decolorised, add more immediately. Heat for a further 3 mins. and add 1·5 M sodium nitrite dropwise until the manganese dioxide and excess permanganate are reduced. Add 1 drop of nitrite solution in excess and rotate the flask to ensure that any manganese dioxide adhering to the walls of the flask is reduced. Heat for another 3 mins. and add 5 drops of 5 M urea solution. Heat for 9 mins. longer, and cool to 0° C. Add a crystal of potassium iodide and 2 drops of 1% starch solution and titrate with 0·0002 N sodium thiosulphate from a 1-ml. microburette. One ml. of 0·0002 N $Na_2S_2O_3 \equiv 4{\cdot}23$ μg. of iodine. Subtract from the titration value the value of a blank carried through the same procedure. The recovery of small amounts (5 μg.) of iodine added to body-fluids was 95·2, 100·0 and 97·4% of the theoretical. Satisfactory agreement was obtained in duplicate estimations of various biological materials containing 2 to 15 μg. of iodine per sample.

F. A. R.

Estimation of Copper in Blood Serum. G. E. Cartwright, P. J. Jones and M. M. Wintrobe (*J. Biol. Chem.*, 1945, **160**, 593–600)—Pipette 5-ml. samples of serum or plasma into 15-ml. Pyrex centrifuge tubes, add 1 ml. of water to each and immerse in a boiling water-bath until the solutions become opaque. Cool, add 2 ml. of 20% trichloroacetic acid, stir thoroughly and heat at 90–95° C. for 5 mins. with frequent stirring. Cool, centrifuge at 3000 r.p.m. for 10 mins., and decant the supernatant solutions into 15-ml. graduated centrifuge tubes. To the original tubes add 1 ml. of trichloroacetic acid and 1 ml. of water, break up the precipitates with glass rods and heat at 90–95° C. for 5 mins. with stirring. Cool, centrifuge and decant the supernatant solutions into the tubes containing the first centrifugate. To the residue add 1 ml. of trichloroacetic acid and 1 ml. of water, heat, centrifuge and decant as before. To the graduated tubes, add 1 ml. of a saturated solution of sodium pyrophosphate, 2 ml. of 28% ammonium hydroxide and 1 ml. of a 0·1% solution of sodium diethyldithiocarbamate. Dilute to 15 ml. and evaluate the colour as soon as possible in an Evelyn photoelectric colorimeter, using Filter 440 and a reagent blank to set the galvanometer at 100. Calculate the copper content (μg. per 100 ml.) from the expression:

$$(2 - \log G) \times \frac{100}{\text{ml. of serum used}} \times \frac{1}{K}$$

where G is the galvanometer reading and K is the calibration constant.

Recoveries of copper added to serum ranged from 93 to 101% with an average of 97% of the theoretical.

F. A. R.

Photometric Estimation of Potassium in Biological Materials. R. S. Pereira (*J. Biol. Chem.*, 1945, **160**, 617–629)—In this method, potassium is isolated as the potassium silver cobaltinitrite, and the cobalt is estimated photometrically after addition of dimethylglyoxime and benzidine.

Put a measured quantity of the ammonium- and

halogen-free neutral or slightly acid solution, prepared as described below, into a Pyrex conical centrifuge tube graduated at 5, 7·5 and 10 ml. Add an equal volume of silver cobaltinitrite reagent (Dissolve 25 g. of sodium cobaltinitrite in 150 ml. of a solution containing 50 g. of sodium nitrite. Add, with stirring, 5 ml. of a 40% solution of silver nitrate, dilute to 200 ml. with water, add 2 ml. of a 40% solution of silver nitrate, dilute to 200 ml with water, add 2 ml. of glacial acetic acid and draw air through the cold solution until evolution of gas has ceased. Leave at 4–6° C. for 12–24 hr. and filter. Store at 4–6° C. until required, and filter again immediately before use. The solution keeps for 1 month). Leave for at least 2 hr. at 4° to 6° C., centrifuge for 15 mins. at 3000 r.p.m. and remove as much as possible of the supernatant liquid with the aid of a capillary. Add 1 ml. of water dropwise in such a way as not to disturb the precipitate, and add a further 4 ml. of water. Centrifuge for 10 mins. decant and wash in a similar manner with 50% acetone. Finally wash twice with pure acetone and allow to drain. If the final supernatant liquid is coloured, wash a third time with acetone. Next add 0·1 ml. of hydrochloric acid, sp.gr. 1·19, and evaporate to dryness in an air-bath, the temperature of which is gradually raised to 150–160° C. Cool, add water, heat in a boiling water-bath, again cool, and add 0·5 ml. of a 1% solution of dimethylgloxime in 95% ethanol, followed by 0·2 ml. of a 1% solution of benzidine in 95% ethanol. Dilute to 10 ml. and evaluate the colour in a Zeiss Pulfrich step-photometer. The extinction coefficient is directly proportional to the cobalt or potassium concentration, so that the results can be calculated from the extinction coefficient of a standard solution of potassium treated in the same way.

To prepare biological material for the estimation of potassium by this method, put the sample into a small Pyrex or silica test-tube graduated at 1 ml. and add 0·5 ml. of a mixture of nitric acid, sp.gr. 1·40 (3 vols.) and perchloric acid, sp.gr. 1·67 (1 vol.). Heat in an air-bath, the temperature of which is gradually raised to 135° C. until brown fumes cease to be evolved and the solution is colourless; if the solution is not colourless add more of the acid mixture, 0·2 ml. at a time, and continue the heating. Raise the temperature to 205° C. until white fumes are evolved and, after a white residue is obtained, raise the temperature to 250° C. until excess perchloric acid is removed. Heat the tube vigorously in a micro-burner flame to eliminate ammonium (very slight explosions may occur), cool, add 0·5 ml. of 2 *N* sulphuric acid, evaporate at 130° C. and then heat over a small flame until white fumes appear. Cool, add 0·5 ml. of water, warm gently, cool and dilute to the mark. Treat a standard solution of potassium in exactly the same way. Transfer a portion of the solution to a Pyrex conical centrifuge tube graduated at 5, 7·5 and 10 ml., add a drop of methyl red and a conc. solution of sodium carbonate dropwise until the solution becomes alkaline. Immerse in a boiling water-bath for 5 mins. and, if a precipitate forms, cool, centrifuge and transfer the supernatant liquid to another centrifuge tube. Dissolve the precipitate in a few drops of 2 *N* acetic acid, add 0·5 ml. of water and a drop of methyl red, and repeat the precipitation with sodium carbonate as before. Centrifuge and add the supernatant liquid to the second centrifuge-tube. To this solution add dropwise a measured quantity of 5 *N* acetic acid until the liquid becomes pink. Immerse the tube in a boiling water-bath to remove carbon dioxide, add *N* acetic acid to keep the solution acid, if necessary, and evaporate to about 1 ml. Add 1 ml. of the silver cobaltinitrite reagent and proceed as described above. Treat the standard solution in the same way. In 11 experiments the recovery of potassium added to serum in amounts ranging from 12 to 20 μg. varied from 85 to 110% of the theoretical. F. A. R.

Quantitative Determination of Thyroxine in Iodinated Casein having Thyroidal Activity. E. P. Reineke, C. W. Turner, G. O. Kohler, R. D. Hoover and M. B. Beezley (*J. Biol. Chem.*, 1945, **161**, 599–611)—Thyroxine can be estimated in iodinated proteins by hydrolysis with barium hydroxide solution, extraction from acid solution with *n*-butanol and estimation of iodine. The results compare favourably with those obtained by biological assay.

Hydrolyse 1·0 g. of the iodinated protein with 3·2 g. of $Ba(OH)_2.8H_2O$ and 6·4 ml. of water in a test-tube immersed in a boiling water-bath; the tube is fitted with an air-cooled reflux condenser. Heat for 20 hrs. Add 25 ml. of water to the hydrolysate, leave for a few mins. and decant the clear supernatant liquid into a 100-ml. volumetric flask. Decompose the precipitate of barium salts in the test-tube by warming with 2 ml. of *n*-butanol and exactly 5·0 ml. of 3·5 *N* hydrochloric acid. Transfer the solution to the volumetric flask and make up to volume with water. Transfer 10 or 20 ml. of the solution, containing 2 to 4 mg. of thyroxine, to a separating funnel, add 3 drops of 0·5% bromocresol green solution and titrate with 3·5 *N* hydrochloric acid to a definite yellow colour. Shake with 20 ml. of *n*-butanol and extract the butanol solution first with 1 vol. and then with ½ vol. of 4 *N* sodium hydroxide containing 5% of sodium bicarbonate. Filter the butanol solution through glass wool into a 100-ml. nickel crucible, and evaporate the solution on a steam-bath. Estimate the iodine content by the method of Harrington (*"The Thyroid Gland, its Chemistry and Physiology,"* London, 1933, 192) and multiply the result by 1·529 to calculate the thyroxine content. Recoveries of thyroxine from pure solutions (without hydrolysis) averaged 98% of the theoretical and from solutions containing about 5 times as much diiodotyrosine 99·5%. When the solutions were hydrolysed, the corresponding recoveries were 93 and 94% respectively. The addition of diiodotyrosine to iodinated protein had little or no effect on the thyroxine content as determined by this method. F. A. R.

Chromatography in Aqueous Solution with Mineral Precipitates Insoluble in Water. I. Application to the Separation of Amino Acids by Adsorption on Silver Sulphide. G. C. M. Hamoir (*Biochem. J.*, 1945, **39**, 485–490)—A flocculate of silver sulphide adsorbs irreversibly considerable amounts of silver ions which in turn adsorb ions that form sparingly soluble silver salts. Although the ratio of the substances adsorbed to the adsorbent is extremely low (1:10,000), the adsorbent power of different preparations or of the same preparation at different times is remarkably constant.

To prepare the adsorbent, pass an excess of hydrogen sulphide into 2 litres of silver nitrate solution (equivalent to 12·5 g. of sulphide), leave for a few hours, filter and pulverise the precipitate in a mortar. Leave the precipitate in contact with 1–2% silver nitrate solution for 3 hrs., filter and wash with CO_2-free water until the reaction with

sodium chloride is negative. Transfer the precipitate to a chromatograph tube with a sintered glass plate (permeability 2, 3 or 4) at the base. According to their behaviour on such a column, amino acids can be divided into four groups. The members of group 4, comprising glycine, alanine, serine, valine, threonine, leucine, proline, hydroxyproline, lysine and arginine, are not adsorbed at *p*H 6–7, and 100 ml. of water are sufficient to wash these acids into the filtrate, except with leucine or lysine, for which 150 ml. should be used. Group 3 comprises glutamic and aspartic acids, tryptophan, histidine, phenylalanine and tyrosine. These are adsorbed at *p*H 6, but are readily eluted by 0·001 *M*-acetate buffer of *p*H 5; the ease of elution is in the above order. Members of this group can be separated from one another by varying the *p*H or by addition of salts to the solution. Group 2 comprises methionine only; this amino acid is not eluted by dilute acid, but can be isolated by washing the column with 150 ml. of *N* acetic acid. Group 1 comprises cystine and cysteine, which are adsorbed from *N* acetic acid and have so far not been recovered by elution. The method has been applied with some success to the separation of amino acids, and would appear to be particularly useful for the isolation of methionine. F. A. R.

Organic

Estimation of Free Formaldehyde by Diffusion. M. J. Boyd and M. A. Logan (*J. Biol. Chem.*, 1945, **160**, 571–583)—The method was devised with the object of estimating free formaldehyde in bacterial toxins, without disturbing the amino acid—formaldehyde equilibrium by altering the *p*H or dilution. The formaldehyde is allowed to diffuse from the surface of the solution into a membrane containing phenylhydrazine hydrochloride, and a red colour is developed in the membrane by adding ferricyanide and acid and compared with colours formed in the same way from standard solutions of formaldehyde.

The apparatus consists of a block of resin (Lucite, Plexiglas or Formica), 2·25 × 1 × 12 in., in which 20 holes, 0·25 in. apart, are drilled in 2 rows with a 0·75 in. drill to a depth of 0·3 in. The block is paraffined by filling the holes with molten paraffin and then turning the block upside down to remove the excess. Formica blocks should first be heated to 100° C. until a negative test for formaldehyde is obtained.

Soak a roll of Visking cellulose membrane, 1·875 in. wide (sufficient for 1 day), in a 1·0% solution of phenylhydrazine hydrochloride for at least half an hour. Remove sufficient film to cover a glass photographic plate 14 × 3 × 0·06 in., press it firmly on to the plate and wipe the membrane with a clean cloth. Put 1-ml. quantities of the test-solution, containing 20–1000 μg. of formaldehyde per ml., and 1-ml. quantities of standard solutions, containing 20, 40, 60, etc., μg. per ml., into the holes of the test-block and place the glass plate and membrane over the holes with a small weight to hold it firmly against the block. Leave for 30, 15, 5 and 2 mins. according as the formaldehyde concentrations are 20–100, 100–200, 200–300 and 300–1000 μg. per ml. respectively. Remove the plate and membrane and pour about 200 ml. of 10% potassium ferricyanide solution on to the surface, followed by 200 ml. of 3·5 *N* hydrochloric acid. Wash with a similar amount of acid, and then wipe off the excess reagent and match the colour against those of the standards against a white opaque glass background. With formaldehyde concentrations less than 200 μg. per ml. the error is ± 10 μg. per ml.; with higher concentrations it is ± 5%.

The sensitivity of the method can be increased by using cellulose nitrate plate films instead of the Visking membranes, though they are more troublesome to prepare. Approx. 30 ml. of a mixture in equal proportions of a 10% solution of nitrocellulose in aldehyde-free ethanol and ether (1:1) and a 0·6% solution of phenylhydrazine hydrochloride in aldehyde-free ethanol and ether (9:1) are poured on to a clean thin glass plate, which is turned on edge for 5 secs., and then laid flat and allowed to dry for 5 mins. at 25° C. With this plate film, concentrations of formaldehyde from 0 to 20 μg. per ml. can be estimated with an exposure time 50–75% of that recommended for the cellophane plate. The error with this procedure is ± 2μg. per ml. F. A. R.

Note on the Orcinol Reagent. W. E. Militzer (*Arch. Biochem.*, 1946, **9**, 85–90)—Occasional inconsistent results obtained with the orcinol reagent for the identification of pentoses, pentosans, uronic acids and polyuronides are due to impurities in the reagents and the use of insufficient ferric iron. Consistent results are obtained when the reagent is prepared and used according to the following method Dissolve 1·0 g. of orcinol in 100 ml. of water and just before use add 400 ml. of pure conc. hydrochloric acid and 10 ml. of 10·0% ferric chloride solution. Dissolve 2 mg. of the sugar in 3 ml. of the reagent and heat in a boiling water-bath for 3 mins. Cool and add 8–10 ml. of *n*-butanol. The formation of a blue to blue-green pigment is the only acceptable criterion of a positive reaction. For spectrophotometric comparisons, dilute to 100 ml. with *n*-butanol. F. A. R.

The Kiliani Reaction as a Direct Measure of Reducing Groups. W. E. Millitzer (*Arch. Biochem.*, 1946, **9**, 91–94)—By the application of the Liebig-Dénigès method for the estimation of cyanide, the Kiliani reaction can be used for the estimation of reducing groups in any molecule possessing the aldehyde, ketone, hemiacetal or hemiketal structure.

Weigh out 0·2 g. of the sugar to be tested into a 250-ml. glass-stoppered conical flask, and dissolve in 9·00 ml. of 0·1 *N* acetic acid. Add 10 ml. of 0·2 *N* potassium cyanide, and immediately insert the stopper, which is lubricated with glycerol. Leave in a cool place for 24 hrs., and then add approx. 10 ml. of 6 *N* ammonium hydroxide, shake and add approx. 0·2 g. of potassium iodide. Titrate to the first perceptible turbidity with 0·1 *N* silver nitrate. The difference between the titre and that of a blank carried through the same procedure multiplied by 13 gives the number of mg. of cyanide (as potassium cyanide) consumed by the sugar. For 0·2000 g. of glucose, maltose and arabinose the theoretical quantities are 72·2, 36·0 and 85·1 mg. respectively. Calcium should be removed as oxalate to avoid the formation of a precipitate. Salts of 2-keto-acids usually require more than 24 hrs. for reaction. F. A. R.

Determination of Ethylene. A. W. Francis and S. J. Lukasiewicz (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 703–704)—Bromine solns. and fuming sulphuric acid, as well as conc. sulphuric acid activated with 1% of vanadium pentoxide or 0·6% of silver sulphate, are unsatisfactory for determination of ethylene because they attack paraffins.

A soln. of 3·5% of silver sulphate in 72% sulphuric acid is unreliable because the absorption of ethylene in it is reversible, and 87% sulphuric acid containing 9% of silver sulphate absorbs carbon monoxide and liberates ethylene after use. A soln. of mercuric sulphate in 22% sulphuric acid provides a reagent in which absorption is completely irreversible and is selective for olefine gases with respect to most other gases likely to be present. Dissolve about 57 g. of mercuric sulphate in 200 g. of 22% sulphuric acid (or 41 g. of mercuric oxide in 216 g. of 29% sulphuric acid), filtering if necessary to remove undissolved particles. With an acid concn. below 13% hydrolysis may occur (yellow ppt.), and with an acid concn. above 24% solubility may diminish (white ppt.). The recommended reagent has a sp.gr. of about 1·37. Since with continued use sludge may form it is preferable to use the reagent in a pipette packed with beads or tubes rather than in a Francis autobubbler. The ultimate capacity of the reagent is 5 litres per 200 ml., but after absorption of 3 litres of pure ethylene the rate of solution becomes excessively slow. It is necessary to remove other olefines with appropriate concns. of sulphuric acid (Matuszak, *Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 354). Butadiene is removed as described by Cuneo and Switzer (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 508; ANALYST, 1943, **68**, 342). Carbon dioxide is slowly absorbed by mercuric sulphate and must first be removed by potassium hydroxide soln. Carbon monoxide and hydrogen are not absorbed. Comparison of the reagent with Eberl's silver sulphate reagent (*Id.*, 1942, **14**, 853) showed that with 100 ml. of a mixture of 35% of ethylene and 65% of propane in an Orsat apparatus with Francis autobubblers 10 passes were required for complete absorption of the ethylene in the mercuric sulphate reagent and 15 passes in the silver sulphate reagent. When the fifth sample was passed through the reagents the silver sulphate reagent failed to absorb 2·2 ml. of ethylene, but this residual amount was satisfactorily absorbed by the mercuric sulphate reagent. Similar results were obtained with a mixture of 40% of ethylene and 60% of *n*-butane. A sample of 80 ml. of *n*-butane showed an increase of vol. of 3·2 ml. after 12 passes through the silver sulphate reagent already used with the six samples (*supra*). When the mixture was now passed three times through the mercuric sulphate soln. the vol. was reduced to 80 ml. A fresh sample of *n*-butane showed no change of vol. when passed several times through the mercuric sulphate reagent. A. O. J.

Rapid Method for the Determination of β- and γ- Cellulose. N. S. Lea (*Paper Trade J.*, 1946, **122**, Feb. 7, *T.A.P.P.I. Sect.*, 61–62)—*β- plus γ-Celluloses*—Dilute the filtrate from the α-cellulose determination to 1 litre, and to 100 ml. of this add 5·0 ml. of potassium dichromate soln. (90 g./litre) in a 500-ml. conical beaker. Add 50 ml. of conc. sulphuric acid, boil for exactly 10 min., add 150 ml. of cold water and cool to 20° C. Add 15 ml. of 85% phosphoric acid and 8 drops of an indicator soln. prepared by mixing aqueous solns. of 3·17 g. of barium diphenylamine sulphonate and 5·0 g. of sodium sulphate, diluting to 1 litre and filtering after standing overnight. Titrate with ferrous ammonium sulphate solution (159·9 g./litre); the end-point is the first green colour. *γ-Cellulose*—Neutralise a 400-ml. aliquot portion of the filtrate from the α-cellulose determination with acetic acid, adding about 20 ml. of acid in excess. Heat the mixture on the steam bath for 30 min., cool to 20° C., and filter on an asbestos Gooch crucible (alundum is too slow, and filter paper adsorbs the γ-cellulose). Treat 105 ml. of the filtrate (5 ml. to compensate for the acetic acid used) as described for the β- plus γ- celluloses determination. Then % of β- plus γ- or of γ-cellulose respectively

$= b(a-x)$/wt. of bone-dry pulp taken originally,

where a = ml. of ferrous ammonium sulphate soln. equiv. to 5·0 ml. of potassium dichromate soln.; $b = 61{\cdot}875/a$; x = ml. of ferrous ammonium sulphate required for the β- plus γ- or the γ-cellulose determination, respectively. J. G.

Qualitative Identification of Synthetic Resins J. H. Graff (*Paper Trade J.*, 1946, **122**, Jan. 31, *T.A.P.P.I. Sect.*, 45–47)—A method is given in schematic form for the classification of synthetic resins and sizing materials in or on paper into the following groups: polyvinyl acetates, phenol-formaldehyde resins, ethyl celluloses, urea-formaldehyde resins, melamines and alum, starch and glue. The iodine, biuret and Raspail tests are used for starch, glue and rosin, respectively. To test for polyvinyl alcohol, immerse a strip of the paper in a soln. of 40 g. of ammonium hexanitrate cerate in a mixture of 100 ml. of water and 14 ml. of nitric acid at room temp. for 5 min., blot the strip, and dry it at room temp.; a red colour is a positive reaction, but it is not permanent. For the other synthetic resins, dye the strips for 3 min. at room temp. by immersion in solns. of Neocarmin W, Shirlastain, Colotex B, or Texchrome. Wash and dry at room temp. The colour reactions are given in the table in the original paper, and are permanent. An aid to identification is a determination of the rate of disintegration of the paper in 0·5% sodium hydroxide soln. alone, containing 5% of aluminium sulphate soln. and mixed with acetone. J. G.

Physical Methods, Apparatus, etc.

[Determination of] Lead in Smelter Products. W. and G. R. Hered (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 780–1)—Provided that a suitable supporting electrolyte is used, the polarographic determination of lead in flue dusts, slags, etc., is rapid and probably more reliable than the volumetric molybdate method.

Method—In a 5-inch Pyrex test tube mix thoroughly 0·2 g. of the sample and 2 g. of sodium peroxide and cover with an additional 0·3 g. of the latter. Whilst slowly rotating the tube, cautiously fuse the mixture, which should appear homogeneous after 3 to 5 min. Allow to cool, break the tube into a beaker and add not more than 50 ml. of water. Leave for about 15 min., add rapidly 25 ml. of conc. hydrochloric acid and stir to obtain a clear yellow soln. Add about 3 g. of tartaric acid to prevent pptn. of antimony and dilute to 100 ml.; slight ppt.-formation causes no difficulty. Adjust a suitable aliquot to be 1 *N* in hydrochloric acid (the soln. is initially about 3 *N* in this acid) and add 0·01% of gelatin (as 0·2% soln.). Transfer 15 ml. to the polarographic cell (a special 20-ml. cell is described), and deoxygenate for 15 to 20 min. by passing in nitrogen saturated with water vapour. Polarograph at 25° ± 0·1° C. between −0·10 and −0·60 volt versus the saturated calomel electrode and measure the wave height. When prior deposition of other constituents interferes, a supporting solution which is 0·5 *M* in sodium tartrate and 0·1 *N* in sodium hydroxide is often applicable. The voltage range is then from about −0·50 to −1.00.

A synthetic sample containing 49·81% of lead (balance, oxides of zinc, arsenic, antimony, iron silicon and copper, together with sulphur, carbon and calcium carbonate) gave results reproducible to ±0·3% and accurate to within 1%. Results obtained with other materials, including a slag containing less than 5% of lead, are given. J. T. S.

Test for the Washing Value of Soap. M. Michels (*Melliand Textilber.*, 1946, **21**, 358–360)—Disperse 1 g. of a standard finely-ground ochre earth in 200 ml. of water, by stirring, and add and dissolve sufficient of the sample to produce the concn. appropriate to the test. Dry some standard viscose rayon at 115° C., add 3 g. to the dispersion, and stir for 10 min. Remove the rayon, wash it well with distilled water until no more pigment is removed, squeeze and dry it and determine the ash content; deduct the ash content of the untreated rayon, then the resulting figure is inversely proportional to the washing val. of the soap. In this way soaps may be compared numerically with a standard washing agent. The general conditions controlling the affinity between fibres and pigments dispersed in water or organic solvents were investigated. J. G.

Simplified Method for the Recognition of Damage on Wool. E. Elod and H. Reutter (*Melliand Textilber.*, 1940, **21**, 346–348)—The test depends on the increased affinity, for certain dyestuffs, of the protein after modification by the agents (*e.g.*, acid or alkali) responsible for the damage. A study of the mechanism of the test indicates that such damage probably results in the widening of the inter-micellar spaces, or in changes in the micelles themselves; it is unlikely that the principal valency chains of the protein substance are ruptured. Wash the sample with water to remove neutral salts, dry it, weigh out 0·5 g., moisten this, and add it to the dye bath at 70° ± 0·5° C. The bath contains 10 ml. of about 0·05 *N* hydrochloric acid (*p*H 1·3), 8 ml. of 0·50% crystal ponceau soln. (*p*H 1·3) and 3 ml. of Leonil-O (*p*H 1·3). Make the test in triplicate. Stir the wool well in the bath, and decant off the 3 liquids after 3, 4 and 5 min. (10 min. for very thick samples), respectively. The colours of the resulting solns. are approx. inversely proportional to the degree of damage undergone by the fibre, but the test is only really satisfactory when the comparison is made against solns. obtained with a piece of the same sample known to be undamaged. The test is preferable to the Pauly reaction, but it is essential that the conditions are strictly standardised (especially the temp. and *p*H); the *p*H of the constituents of the bath may be adjusted, if necessary, by means of hydrochloric acid or ammonia. J. G.

Compression Test of Fibreboard Shipping Containers. Anon. (*Paper Trade J.*, 1945, **121**, Nov. 29, *T.A.P.P.I. Sect.*, 215–216)—In the T.A.P.P.I. Tentative Standard Method described (T804 m-45) a standardised procedure for sealing the flaps of the containers is described. The sample is then conditioned, weighed, and subjected to a steadily increasing load between the platens of a compression tester; the corresponding deformation is measured until the max. load or failure is reached. The results are expressed in terms of a load-deformation curve. J. G.

Reviews

THE BRITISH PHARMACEUTICAL CODEX, 1934, Supplements I–VII bound into one Volume. London: The Pharmaceutical Press. 1946. Price 21s.

Owing to the war, normal plans for the production of a new edition of the Codex had to be abandoned although progress was maintained in the development of new drugs and in methods of administration; also many medicinal preparations were modified owing to difficulties appertaining to the supply of important ingredients. In these circumstances it became necessary to issue Supplements and in the following enumeration of them the figures in brackets indicate the number of text pages: First, 1940, Standard Dressing (14); Second, 1941 Formulary (40); Third, 1942, New Monographs (97); Fourth, 1942, Amendments to Monographs (78); Fifth, 1942, Quinine Preparations (13); Sixth, 1944, Additions and Amendments (30); Seventh, 1945, Additions and Amendments with Cumulative Index (134).

The First Supplement contains formulae and standards for fifteen surgical dressings and a monograph on euflavine lint; analysts will be interested to know that a procedure for determining the active constituent of the latter is included. The contents of the next Supplement comprise additions and amendments to formulae for galenicals and other preparations in Part III of the Codex and authorises the use of alternatives for those preparations in which certain of the ingredients were in short supply or reserved for more important purposes. The Third Supplement contains sixty-seven monographs additional to those in Part I of the Codex and concerns substances described in the first four Addenda to the British Pharmacopoeia, 1932, and also medicaments which have come increasingly into use since the publication of the Codex in 1934 but which had not at that time been included in an Addendum to the Pharmacopoeia. Herein is much useful information, including analytical standards and methods of assay. Of particular interest are the notes on the physiological action and uses of these relatively new drugs, while a supplementary list, occupying twenty-one pages, of proprietary trade names is a boon to all concerned with the marketing of medical products.

The Fourth and Sixth Supplements mainly consist of amendments rendered necessary by the incidence of wartime conditions and the Fifth Supplement is concerned with alterations

in the formulae for quinine preparations rendered necessary by the restrictions imposed on their sale in 1942. However, here and there in these Supplements there are interesting new monographs adding welcome relief to the amendments.

The Seventh Supplement contains new monographs on substances defined in the Seventh Addendum to the British Pharmacopoeia, 1932, but not previously described in the British Pharmaceutical Codex, together with fourteen monographs on medicaments which have recently come into use but which have not been included in an Addendum to the Pharmacopoeia. Standards are proposed for the latter group of substances together with summaries of the pharmacopoeial requirements wherever these apply. A notable addition, included in this Supplement, is a series of revised specifications for tablets and solution-tablets wherein analytical procedures for determining the active constituents are given; many of these are compound preparations and all are additional to those defined in the Seventh Addendum to the Pharmacopoeia.

Suggestions for making alterations in works of authority demand extended and constructive discussion, hence it is clearly inappropriate to indulge in criticism here. It is sufficient to observe that, taken together, these Supplements epitomise a vast amount of painstaking effort, undertaken voluntarily by enthusiastic helpers serving on the various Codex Committees. This handsomely bound book, so packed with useful information, is indeed a worthy memento of all that work carried out in those uncomfortable wartime days.

N. L. ALLPORT

FORENSIC CHEMISTRY AND SCIENTIFIC CRIMINAL INVESTIGATION. By A. LUCAS, F.R.I.C. 4th Edition. Pp. 340. London: Edward Arnold & Co. Price 25s.

Twenty-five years ago the author published a small book on Legal Chemistry, and when this was reviewed in THE ANALYST it was suggested that it should be expanded into a work on Forensic Chemistry. This advice was adopted, and since then there has been a steady demand for the work and successive editions have appeared at regular intervals. Unfortunately the author has not lived to see the issue of the present edition, as he died in Cairo early this year. His work as Director of the Chemical Department in Egypt and, later, his archaeological research for the Egyptian Government gave him exceptional opportunities of studying materials often of a type different from those that have to be investigated in this country. The present edition has been brought up to date and includes a description of many striking cases which occurred between 1940 and December, 1944, such as, for example, the deciphering of charred documents, typewriting and handwriting, erased inscriptions and numerous shotting cases.

The arrangement of the book follows that of previous editions, each of the separate sections being arranged under alphabetical headings. At the end of each section there is a valuable list of references to the literature relating to each subject, and these now number about 200 more than in the previous edition. The index, which fills 8½ pages, is very full and covers both authors and subjects.

Lucas made many notable contributions to the subject of criminal investigation, most of which were published in THE ANALYST and the results of which are incorporated in this book. It thus becomes a fitting memorial to his memory.

C. A. MITCHELL

BIOLOGICAL METHODS GROUP

A MEETING of the Biological Methods Group will be held at 6 o'clock on Monday, May 27th, in the rooms of the Chemical Society, Burlington House, Piccadilly, London, W.1, when the following papers will be read—

"Genetical Aspects of Bio-Assay with Higher Organisms," by K. Mather, D.Sc.

"The Genetics of Micro-organisms used for Biological Assays," by G. Pontecorvo.

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