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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

Obituary

SYDNEY EMSLEY

SYDNEY EMSLEY was born on September 17th, 1889. His father was Walter Emsley, the well-known Manchester artist and portrait painter, one-time pupil of Herkomer. His first school was a little private one in Bushey run by two maiden ladies, and from here he went to the Manchester Cathedral Choir School where, in 1903, he won a scholarship to the Cheetham Higher Grade School. Here he distinguished himself in chemistry and mathematics, and in 1907 passed his matriculation in the First Division. He then proceeded to the Owens College, University of Manchester, where he graduated with honours in chemistry in 1911.

His first appointment was as assistant to Mr. F. W. Arnaud, then Borough Analyst for Portsmouth and now County Analyst for Kent. He next became a research chemist at Woolwich Arsenal, and in 1913 a chemist to the London, Brighton & South Coast Railway, whence, after a short stay, he obtained a position as assistant to Mr. Brierley, the Borough Analyst for the County Borough of Southampton. He passed his Associateship of the Institute of Chemistry in Food and Drugs in 1915, and became a Fellow in 1919. From 1916 to 1918 he was called upon to do war work in the Soda Ash Laboratories of Brunner, Mond & Co., at Northwich, but returned to Southampton in 1919, and on the retirement of Mr. Brierley in 1922 he was appointed Borough Analyst and Agricultural Analyst for Southampton. Also, when Dr. Angell, the Public Analyst for the County of Southampton, the Isle of Wight and the City of Winchester, died in 1930, he was appointed Analyst for the Isle of Wight and the City of Winchester. He held these appointments until he died on December 20th last at the early age of 55. On November 6th, 1940, he was injured in a daylight bombing raid on the Civic Centre, and whether this initiated his illness or merely aggravated it will never be known, but it is certain that he never enjoyed good health from that time.

Such in bare outline are the facts of Emsley's life, and it is the privilege of the writer, who had been associated with him ever since he became Borough Analyst, to indicate the nature of the man. Tall, quiet and retiring, clever, with a very good memory, never robust, his activities were governed by the frailness of his body, and his nature was more passive than active. He was a firm believer in the League of Nations, and was greatly disappointed when it failed. His recreations included in turn, tennis, golf, wireless, chess (for some years he was match secretary for the Southampton Chess Club), skating, and more latterly gardening (particularly fruit tree culture) and nature study, which he enjoyed with his wife in their country home—an ideal site for this latter study. These hobbies were pursued with all the vigour and interest of which he was capable. A kindly nature, he impressed with his friendliness all with whom he came in contact, and, although his circle of friends was not extensive, he will be sadly missed by those who were privileged to be of it. He married Mary Schofield in 1919, and was devotedly nursed by her in his last illness.

R. WATRIDGE

ERNEST VICTOR SUCKLING

ERNEST VICTOR SUCKLING died suddenly at Gidea Park on the 16th November, 1944, in his 52nd year. His death will be deeply regretted by all concerned with safeguarding the purity of water supplies.

Born at Chelmsford, he was educated at King Edward VI Grammar School and, while continuing his studies, was a pupil assistant in the laboratories of Dr. J. C. Thresh from 1910 to 1914. Early in the last war he joined the Royal Army Medical Corps and served in France as a non-commissioned officer in the capacity of laboratory assistant, being mentioned in dispatches and promoted lieutenant, R.A.M.C. In France, and subsequently as officer in charge of No. 4 Analytical Laboratory, British Army of Occupation in Germany, he had a wide and varied experience in the control of water supplies.

On demobilisation he continued his studies at the London Hospital Medical School and University College, London, taking his M.R.C.S., L.R.C.P. in 1924 with the premier prize for medicine, and graduating, M.B., B.S. (Lond.) in 1925. He took the diploma in Public Health of the Royal Colleges of Physicians and Surgeons in 1927.

After qualifying in medicine, Suckling became a partner with Drs. Thresh and Beale in the Counties Public Health Laboratories, and following upon the death of the former and the retirement of the latter he became the sole director in 1938. This position involved the control of a widespread practice as consulting bacteriologist and water examiner to numerous water undertakings, county and local authorities and Government departments. His assumption of the sole directorship followed closely upon the episode in his career which brought him most prominently before the public, but which was in reality merely one instance of his methods and the standard of practice which he invariably strove to maintain. This episode was the Croydon epidemic of typhoid fever in 1937, and his investigations and general testimony at the Inquiry might well serve as a model for all engaged in similar investigations and generally in the prevention of water-borne diseases.

Suckling's strict adherence to the highest standards of practice, even in situations where this might not be easy, his steadfastness and his direct approach to difficult problems were sources of inspiration, and his personality was such that he commanded not only the respect, but also the adherence of those whom he was called upon to advise. There can be no doubt that, had he lived, his influence upon the quality of domestic water supplies both in this and other countries would have been profound; it was already great, for he was joint author of the 4th Edition of *"The Examination of Waters and Water Supplies,"* and had recently completed the preparation of the 5th Edition despite the loss of most of his manuscripts when his laboratories were destroyed by enemy action in 1941. This work is generally recognised as the standard text book on its subject.

He held the appointment of bacteriologist to the Essex County Council between 1938 and 1942, he was a professional assistant of the Institution of Water Engineers, a member of the British Waterworks Association, a Fellow of the Society of Medical Officers of Health, a Member of the Society of Public Analysts and other Analytical Chemists and, in 1943, was elected a Fellow of the Royal Institute of Chemistry and was appointed the Institute's Examiner in water supply and the treatment of sewage and trade effluents.

Suckling was above all a most approachable man, his experience was vast and his counsels sound. His loss is to be deplored. E. F. W. MACKENZIE

The Determination of Mono- and Tri-Ethylamine in Commercially Pure Diethylamine

BY H. N. WILSON AND A. E. HERON

(*Read at the Meeting of the North of England Section on November* 11, 1944)

INTRODUCTION—Diethylamine used for the preparation of pure chemicals must have a high degree of purity, and refined methods of analysis are necessary. The principal impurities are mono- and tri-ethylamine, and the results for each must be accurate to 0·02%. We have devised adequate methods for both these impurities.

A. Monoethylamine

As no available technique for carrying out Hoffmann's reaction was sufficiently accurate, we sought some alternative. Those considered were:

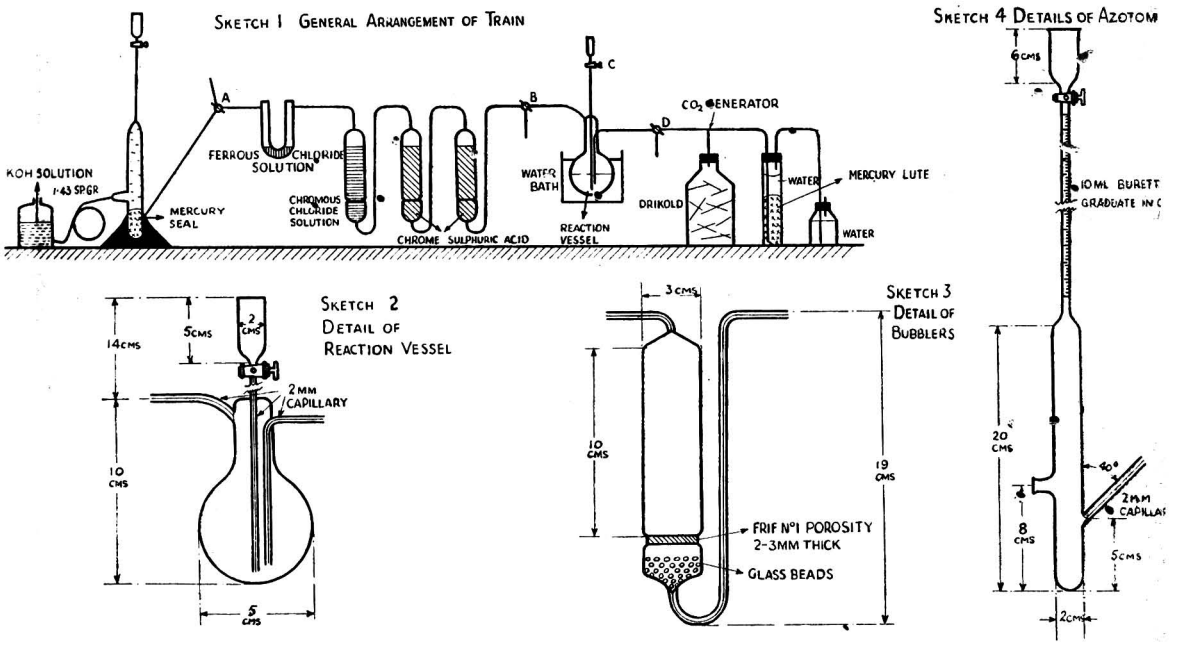
(1) *The carbylamine reaction*—This is exceedingly sensitive. According to Meyer,[1] 0·5 μg of primary amine can be detected in 1 ml, but the reaction cannot be made quantitative.

(2) *Colorimetric reaction with nitroprusside*[2]—The purple colour given by monoethylamine is quite different from the orange colour given by diethylamine, but we did not succeed in finding conditions under which the reaction could be used quantitatively.

(3) *Mustard oil reaction*—Although primary and secondary amines differ in their reactions with carbon disulphide followed by desulphurising agents, the reaction cannot be used for determining minor amounts of monoethylamine.

(4) *Metaphosphates* of the three amines are said to have widely differing solubilities, but all are too soluble for our purpose.

We were then forced to reconsider Hoffmann's reaction. Because of the very small volumes of gas we should have to measure, we decided against apparatus of the Van Slyke type, and used an azotometer of small size, sweeping out the nitrogen with carbon dioxide. Preliminary expts. indicated that very efficient washing of the gas stream was essential, and, after trying several types of absorbers, we finally evolved the apparatus described below.



APPARATUS—The azotometer has a capacity of 10 ml. The three gas-washing tubes should have sintered glass discs of No. 1 porosity. Sintered discs are essential to ensure complete removal of oxides of nitrogen. The U-tube should be packed with broken glass rod or beads. Some form of carbon dioxide generator is required. That illustrated is for use with Drikold; any form of generator suitable for the micro-Dumas procedure could be used, but it must give a pressure equiv. to *ca.* 5 cm of mercury.

REAGENTS—All reagents must be made up from air-free water. The distilled water used should be boiled for an hour or so and rapidly cooled.

(1) *Sodium nitrite solution*—Dissolve 400 g of sodium nitrite (purest obtainable) in boiling water, filter, and dilute to 1 litre. (2) *Acetic acid*—Glacial. (3) *Chromic-sulphuric acid*—Dissolve 67 g of chromium trioxide in *ca.* 500 ml of water, add 100 ml of conc. sulphuric acid, and dilute to 1 litre. (4) *Chromous chloride soln.*—This is prepared by reduction of chromic chloride and the spent reagent is regenerated in the same way. Half fill a 500-ml separating funnel with a known weight of granulated zinc. The zinc must now be amalgamated with between 1% and 1·5% of its weight of mercury. Weigh out the appropriate weight of mercuric nitrate, dissolve in diluted nitric acid (1 + 1), and dilute with water to 250 ml. Cover the zinc in the funnel with the mercuric nitrate soln. and leave with occasional shaking for 15–20 min. Then run off the spent soln. and wash with water until all turbidity is removed. (The reductor must be kept full of water when not in use.) Dissolve 80 g of hydrated chromic chloride in 150 ml of water, add 10 ml of conc. hydrochloric acid and boil for 10 min. Cool to *ca.* 40 to 50° C., dilute to 200 ml with water, and transfer to the prepared reductor. Run a layer of paraffin over the surface to prevent atmospheric oxidation, and leave in a warm place until reduction is complete (12 to 18 hr.). If kept out of contact with oxygen, this soln. is fairly stable. To regenerate the spent reagent, add 10 ml of conc. hydrochloric acid to 200 ml, boil and treat as above. The reagent may be regenerated at least 3 times. (5) *Ferrous chloride solution.*—Dissolve 40 g of hydrated ferrous chloride in 85 ml of water and 15 ml of hydrochloric acid. Add a few iron turnings or some iron wire to reduce ferric ions. This soln. is only used as an indicator for any "oxides of nitrogen." If nitric oxide passes the

chromous chloride washing tube the ferrous chloride soln. darkens in colour and the run must be repeated. (6) *Potassium hydroxide soln.*—For azotometer, density 1·13.

PROCEDURE—Fill the gas absorption tubes to within 1 in. of the top. Before filling the chromous chloride tube, sweep out the air with carbon dioxide, and then by means of gentle suction charge it from the chromous chloride stock bottle, using a siphon tube, after which stopper the ends until it is required for use. After filling the chromic acid tubes, sweep out the air with carbon dioxide, connect to the chromium chloride tube, and connect this in turn to the ferrous chloride indicator tube and the azotometer *via* the 3-way tap A. Next connect the reaction vessel to the train and pass a current of carbon dioxide, closing tap B against the passage of CO_2, and leaving tap C open. Then close C, open B to the atmosphere, and after a few min. turn B so that the carbon dioxide passes into the train and not out to atmosphere. Tap A should be open to atmosphere. After 5 min. close A to the atmosphere and open it to the azotometer, so that it may be observed whether all air is displaced.* If all air is not removed, continue the passage of carbon dioxide until it is. When "micro" bubbles are obtained, open B to the atmosphere and reaction vessel. Close D. Introduce 4 ml of glacial acetic acid and then 10 ml of water into the reaction vessel and 2·0 ml of sample, rinsing the funnel after the last 2 additions with 2 ml of water. Great care must be taken not to admit air during these operations; if any is admitted, it must now be swept out through B. Finally add 5 ml of sodium nitrite soln. and again rinse the funnel. No air must be admitted at this point. Close tap C, open B to the train, open D and adjust the gas rate by means of A to 60 or 70 bubbles per min. in the U-tube. Close D against both the reaction vessel and the atmosphere.

Place a beaker of water at 70° C. round the reaction vessel, and keep the temp. of the bath between 70 and 80° C. until the end of the run. After 15 min. again open D (the pressure of CO_2 should be such that it is escaping through the lute) and sweep out the train until "micro" bubbles are again produced. This usually takes 15 to 20 min. Close A against the nitrometer and, after an interval of 10 min., measure the vol. of nitrogen.

Carry out a "blank" run, using a sample of diethylamine which is free from monoethylamine, on each batch of sodium nitrite solution.

CALCULATION—Vol. of sample 2·0 ml. Let A = ml of nitrogen collected; p = barometric pressure; t = temp.; B = ml of nitrogen at N.T.P. collected during "blank" determination. Then A/50 is "Pregl's Correction," *i.e.*, the correction to be applied for tension of aqueous vapour over potassium hydroxide soln. of 1·43 density, and also for the adherence of potassium hydroxide soln. to the walls of the azotometer.

From the equation $\underset{(45\text{ g})}{C_2H_5NH_2} + HNO_2 = C_2H_5OH + H_2O + \underset{(22{\cdot}4\text{ litres})}{N_2}$

1 ml of nitrogen at N.T.P. is produced from 2·016 mg of $C_2H_5NH_2$ on reaction with nitrous acid.

$$\text{Hence } \left[(A - \frac{A}{50}) \times \frac{273}{273 + t} \times \frac{p}{760} - B\right] \times 0{\cdot}1008 = \text{g } C_2H_5NH_2 \text{ per 100 ml of sample.}$$

RESULTS—To check the accuracy of the method, numerous determinations were made on the same samples of diethylamine, to which known amounts of monoethylamine were added, with results as follows. In 18 determinations the average recovery was 98·2%, the max. deviation for a single determination being +9% on mixtures containing from 0·06 to 0·93 of the mono compound.

The following are representative duplicate analyses of actual samples.

Sample No.	1	2	3	4	5	6	7	8	9	10
Results, %	0·010	0·096	0·083	0·055	0·032	0·041	0·077	0·174	0·28	0·190
	0·010	0·094	0·078	0·053	0·042	0·035	0·076	0·174	0·28	0·192

These results indicate the excellent reproducibility of the results.

DISCUSSION—The reaction between nitrous acid and amines has been used for years in analysis, but there has always been a disturbing feature—the "blank." Thus Van Slyke[3] states that the magnitude of the "blank" varies from batch to batch of nitrite, and that it tends to diminish on keeping the soln. Having settled the procedure on general lines, we made very many expts. to decide on the best conditions, such as temp. and concn. of reagents, time of reaction, etc., in the course of which we confirmed that nitrogen is always evolved during the reaction between sodium nitrite and acetic acid and that it is necessary to determine

* With a new train, CO_2 should be passed for at least 2 hr. to remove all adsorbed air from the glass frits.

the "blank" with each batch of reagent. The evolution of nitrogen is not due to ammonia as an impurity in the reagents; nitrogen is produced when AnalaR acetic acid and very pure sodium nitrite (99·9% $NaNO_2$) are used, and the amount cannot be related to impurities in the nitrite. In the reaction of the quantities of acetic acid and nitrite given above (PROCEDURE) at 70° C. for 30 min., an average of 0·72 ml of nitrogen (at N.T.P.) is produced. Other expts. showed that the "blank" is approximately proportional to the amount of nitrite present.

During an analysis, however, conditions are somewhat different. Nitrous acid reacts fairly rapidly with diethylamine and the "blank" arises for the most part from the excess of nitrite. This excess amounts to 0·64 g of $NaNO_2$, equiv. to 0·24 ml of nitrogen. In fact however, the blank" differs from this figure, partly because the acidity of the soln. changes during nitrosification, partly because nitrosodiethylamine itself very slowly decomposes under the conditions of the reaction. This was proved by preparing pure nitrosodiethylamine, and treating 2 g in the apparatus for 30 min. with acetic acid and sodium acetate (equiv. to the acetic acid and nitrite used) at 75° C. The vol. of nitrogen evolved (average of 3 expts.) was 0·03 ml, which, added to 0·24 ml from excess nitrite, amounts to 0·27 ml.

To check this experimentally we made use of diethylamine of a very high degree of purity, specially prepared for the purpose. Monoethylamine could not be detected by the carbylamine reaction; hence, if present, the amount must have been exceedingly small (probably <5 parts/10^6). Replicate determinations of the nitrogen evolved during analyses of 2 ml gave the following results (at N.T.P.), using the procedure given above, 0·32; 0·33, 0·30; 0·39; 0·27; 0·26; 0·25 ml; average 0·30 ml. This differs by 0·03 ml from the figure 0·27 ml arrived at above, but is within experimental error.

The *design of the apparatus* calls for one or two comments, as do the reagents used in the purifying train. The problem is to remove completely preponderating amounts of oxides of nitrogen, of which NO is difficultly soluble.

Various reagents were tried, including acid and alkaline (Na_2CO_3) permanganate, chromous acid in aqueous solution, CrO_3 in sulphuric acid, and ferrous sulphate, but chromic acid in dil. sulphuric acid, followed by chromous chloride to remove NO, was most satisfactory. Ferrous chloride seems to be the most suitable indicator for the presence of NO in the purified gas stream.

The bubblers should have the minimum of free space, and must have sintered glass frits to ensure thorough washing. The reaction vessel is all glass, and joints should be butt to butt. If the U-tube is of the type with stoppers, they must be cemented with Krönig cement or they will probably be blown out.

The azotometer may be made from a 10-ml burette (graduated in 0·05 ml) or a "semi-micro" 8-ml azotometer may be used. It should be calibrated.

B. Triethylamine

We had to determine accurately triethylamine of the order of 0·1% for which the usual method (distillation with caustic soda of the residue from the action of nitrous acid) is insufficiently precise. Results were erratic and high. Expts. showed that nitroso diethylamine is decomposed to some extent by boiling with caustic soda soln.; 1 g of nitroso compound evolves about 4–5 mg of diethylamine during 30 min. boiling with dil. sodium hydroxide soln. Attempts to improve the method by extraction of the nitrosamine with benzene, chloroform or carbon tetrachloride were unsuccessful; considerable difficulty was experienced in detecting the end-point of the subsequent titration. This may be due to nitric oxide in the aqueous soln., which is evolved and partly oxidised by air during the distillation of the tertiary amine.

It became apparent that some entirely fresh approach was desirable, so reactions given only by tertiary amines were sought. The characteristic reaction is the formation of a quaternary compound on treatment with alkyl halides, but methyl iodide reacts very rapidly with mono- and di-ethylamine also, converting them ultimately to quaternary compounds. It occurred to us, however, that if the secondary (and primary) amines were acetylated, they would not then react. This proved to be the fact, and a very satisfactory method, using methyl iodide, for small quantities of triethylamine was evolved.

APPARATUS—250-ml flasks with ground glass joints; Liebig condensers to fit flasks, as reflux condensers; swan-neck adaptors, to fit flasks and condensers.

REAGENTS—*Ethyl acetate*—redistilled; should be practically free from water. *Acetic anhydride*—redistilled. *Methyl iodide*. *N*/20 *Silver nitrate*. *N*/20 *Potassium thiocyanate*.

PROCEDURE—(N.B. Methyl iodide must not be pipetted by suction applied by the

mouth.) Measure 25 ml of ethyl acetate into a flask, add 10·0 ml of the sample, and then 15 ml of acetic anhydride, keeping the mixture cool. Boil gently under reflux for 10 min. Cool somewhat and add down the condenser 2·0 ml of methyl iodide. Boil under reflux for a further 30 min. Then distil off 30 ml. Cool the residue, add 50 ml of water, 5 ml of diluted nitric acid (1+5) and lastly excess of $N/20$ silver nitrate. Back-titrate excess of silver nitrate with $N/20$ potassium thiocyanate, using ferric alum as indicator.

1 ml of $N/20$ $AgNO_3 \equiv 0{\cdot}00505$ g of $(C_2H_5)_3N$.

Thus (ml of $N/20 \times 0{\cdot}00505 \times 10$) $- 0{\cdot}02 =$ % w/v of triethylamine.

RESULTS—To three samples of diethylamine varying quantities of triethylamine were added, with the following results, which are typical of many.

TABLE I

Sample No.	Triethylamine added, mg	Triethylamine found, mg	Triethylamine found, % w/v	Recovery of added triethylamine, %
1	2·8	3·1	0·031	110
	21·0	20·2	0·202	96
2	*42	46·5	0·47	99
	*84	88·9	0·89	101
3	nil	46	0·46	—
	*29	76·7	0·77	106
	*56	106	1·06	107
	*77	116	1·16	91
	*102	141	1·41	93
	*126	155	1·55	86

* In these expts. the amount of triethylamine added was unknown to the analyst.

DISCUSSION—The reactions of the amines with both methyl iodide and acetic anhydride are undesirably violent, and some diluent is necessary. It is known that the rate of methylation[4] is much influenced by the solvent, alcohols being most favourable, and hydrocarbons least. Alcohols being inadmissible, we tried methyl acetate, ethyl acetate, and toluene; whilst, generally speaking, no great difference was noticed, the best results were obtained with ethyl acetate, toluene tending to give rather low results. Ethyl iodide also was tried as alkylating agent. Its reaction, however, is far too slow. A very considerable excess of CH_3I over the stoichiometric amount is necessary. With 0·5 ml of methyl iodide instead of 2 ml results from 25 to 40% too low were obtained. As will be seen, even 2 ml is insufficient to methylate quantitatively more than 100 mg of triethylamine. (See last 3 results in Table I above.)

Varying proportions of CH_3I and acetic anhydride and varying times of reaction were investigated, the manipulative details being as given in the procedure. The best results were obtained with the quantities recommended.

We advise that if this method be used for material containing more than 1% of triethylamine, smaller samples should be taken. This will never occur in the examination of the commercial pure diethylamine.

Considerable quantities of monoethylamine are without effect, as one would expect. Our expts. proved that up to 2% is without influence, and we think that even very large percentages would introduce no error.

The following table of representative duplicate determinations indicates the precision of the method.

Sample No.		1	2	3	4	5	6	7
Triethylamine, %	{	0·06	0·07	0·03	0·02	0·05	0·05	0·13
		0·07	0·07	0·04	0·02	0·05	0·04	0·15

The constant 0·02 to be deducted in calculating the % present must be explained. In examining a large number of samples, we never obtained a result of less than 0·02% of triethylamine, although in many instances there were very strong reasons to suppose that it would be absent. It was found that this is a systematic error, due to very slight methylation of diethylamine, as was shown by substituting NN-diethylacetamide for diethylamine.

The diethylacetamide was prepared from diethylamine which apparently contained 0·03% of triethylamine, and 100 ml were acetylated with 150 ml of acetic anhydride. After reaction had ceased 250 ml of water were added, and the mixture was shaken thoroughly. The soln. was extracted five times with benzene, the united benzene layers (containing the

diethylacetamide) were washed with dil. sulphuric acid to remove any triethylamine and finally several times with water. The benzene layer was dried with anhydrous Na_2SO_4, filtered, and distilled under reduced pressure, the fraction (39·9 g) distilling at 75° C. (8 mm of mercury) being collected. At 760 mm this boiled at 186° C. (*Beilstein* gives 185–186° C.).

Then, 11·5 g (≡ 7·1 g of diethylamine = 10 ml of diethylamine approx.) of diethyl acetamide were mixed with 25 ml of ethyl acetate, and 5 ml of acetic anhydride, which is equivalent to the excess of anhydride usually present. This mixture was heated under reflux for 10 min. and then treated with 2 ml of methyl iodide as usual. The amount of ionisable iodine produced was equiv. to 0·15 ml of $N/10$ in one expt. and 0·16 ml in the second. This is equivalent to 0·02% of triethylamine, when 10 ml of diethylamine are taken for analysis.

NOTE—In the use of our method for triethylamine, if water is present the quantity of acetic anhydride must be correspondingly increased. This will not occur in the examination of commercial samples.

SUMMARY—Methods are described for the analysis of commercially pure diethylamine for mono- and tri-ethylamine. Monoethylamine is determined by reaction with nitrous acid, the nitrogen evolved being swept forward by carbon dioxide, purified in a train of sintered glass bubblers containing chromic acid in dil. sulphuric acid, and chromous chloride soln. The decomposition of nitrous acid under the experimental conditions is discussed in connection with the "blank" of the method. Numerous experimental results showed that the method is accurate, the average deviation being 3·3% (on monoethylamine contents from 0 to 0·9%). Triethylamine is determined by reaction with methyl iodide in an ethyl acetate soln., to form the quaternary compound after acetylating secondary (and primary) amines with acetic anhydride. Excess of methyl iodide is distilled off and, after dilution with water, the quaternary iodide is titrated with silver nitrate. The accuracy is about the same as in the determination of primary amine.

In conclusion, we would like to express our thanks to Dr. F. F. Snowden for preparing extremely pure samples of mono-, di- and tri-ethylamine for our use; also to Dr. M. P. Applebey (Research Manager) and to Mr. W. C. Hughes (Chief Analyst), Billingham Division, for continued interest and suggestions during this investigation.

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I.C.I. LTD., BILLINGHAM DIVISION
ANALYTICAL SECTION, RESEARCH DEPARTMENT

September, 1944

Photometric Determination of Silicon in Aluminium and its Alloys

BY W. H. HADLEY

SINCE the publication of my papers on the determination of silicon in aluminium and its alloys, using the Pulfrich Photometer,[1] there have been several communications dealing with difficulties encountered with particular alloys and with the adaptation of the method for use with the Spekker Absorptiometer.[2,3,4] In addition, the British Aluminium Company have received a number of communications on the subject. In order to remove any misapprehensions as to the applicability and technique of the method, I should like to discuss the more important suggestions and criticisms, particularly those of Stross.[4]

The general method, as modified by Stross, is practically identical with that put forward in my former papers, except that interference by manganese is prevented by filtration instead of by reduction with sulphurous acid and no heating is employed to dissolve alumina. To avoid possible losses due to adsorption, and the additional manipulation, it is preferred, however, in these laboratories not to filter the solution, unless necessary.

LIGHT-FILTER COMBINATIONS—In applying the method to the Spekker Absorptiometer, the following combinations of light-source and colour-filters have been found suitable.

Light source	Colour filters
Mercury vapour	(*a*) Calorex* (H.503) and Wratten 50
	(*b*) Calorex* and Chance 7†
Tungsten filament	Chance 6§ and Wratten 36

With either light source, serious fluctuations are encountered with the gelatin filters (Wratten 36 and 50), probably owing to heat effects, but these may be readily overcome by fixing two clips on the side of the calibrated aperture remote from the lamp and sliding the gelatin filter under the clips. (It would be a great advantage if the Spekker Absorptiometer were fitted with a slide in this position to take the filters; this would also facilitate the changing of filters.) The left-hand filters are not affected in this way, since they are protected by the 1-cm water cell. With the mercury vapour lamp the all-glass combination (*b*) has been found preferable, as the heating effect is negligible.

INITIAL ATTACK ON ALLOY—On the purely chemical side of the process the only alloys which, in these laboratories, have caused trouble are the Mg-Si types. This appears to be due to incomplete decomposition of the Mg_2Si constituent during the initial caustic attack, with subsequent loss of silicon after acidification. This can be readily prevented by evaporating the caustic soda soln. of the alloy to a very small bulk (1–2 ml), as already suggested by Scott[3]—a process that requires only 5–10 min. (the addition of hydrogen peroxide is unnecessary). This procedure is to be preferred to that of Stross for the lower range of silicon contents, since any increase in the amount of caustic soda tends to increase the blank value of the reagents, owing to the silica content of the sodium hydroxide. We cannot confirm Stross's observation that silica tends to accumulate on the surface of the nickel crucible, but the use of stainless steel beakers or crucibles to avoid the transference of the solution to glass beakers is certainly advantageous.

*p*H VALUE—More recent *p*H determinations for maximum colour development, using a Cambridge *p*H meter, are in agreement with those quoted by Stross, but for practical purposes the working range is the optimum range for maximum colour development in terms of ml of acid, which remains as published originally.[1]

RESULTS—The chemical technique outlined above has been employed for more than 60,000 silicon determinations in the Company's laboratories during the past two years. The alloys included the following: L33, L34, L46, D.T.D. 213, D.T.D. 420, D.T.D. 310B, D.T.D. 424, D.T.D. 428, B.A. 35, B.A. 42, 5L3. Some typical results with the Spekker Absorptiometer are given in Table I.

TABLE I

SILICON DETERMINATIONS: SPEKKER ABSORPTIOMETER

(*a*) Mercury vapour lamp: Filters, H.503 and Chance 7.

	Silicon content, %	
Alloy	Standard Regelsberger method	Colorimetric method
D.T.D. 479	0·59	0·59, 0·59, 0·60, 0·61, 0·58, 0·59, 0·56, 0·58
D.T.D. 424	4·69	4·71, 4·78, 4·61, 4·50, 4·78, 4·66
D.T.D. 240	11·9	12·1, 11·5, 11·7, 11·8
	12·8	12·7, 13·0, 12·9, 13·0, 13·2 12·9, 13·0

(*b*) Tungsten filament lamp: Filters, Chance 6 and Wratten 36.

	Silicon content, %	
Alloy	Standard Regelsberger method	Colorimetic method
5L3	0·50	0·48, 0·49, 0·50, 0·50, 0·50, 0·49
5L3	0·78	0·75, 0·76, 0·77, 0·78, 0·78, 0·80
L34	0·40	0·39, 0·40, 0·40, 0·40
L34	0·20	0·19, 0·20, 0·21, 0·21, 0·21, 0·22

* Now designated Chance Heat-absorbent ON 13. † Now designated Chance Blue OB 1.
§ Now designated Chance Blue-green OB 2.

The estimated standard error is ±1·5 to 2·0% of the silicon content being determined.

The method used by Vaughan,[5] in which the yellow molybdate complex is reduced to the blue compound, has not shown any higher degree of accuracy than the photometric method based on the yellow complex, and, for routine analysis, has the disadvantage that the time required is nearly doubled.

SUMMARY—Criticisms of my photometric method of determining silicon in aluminium alloys[1] are discussed. Filter combinations for use with the Spekker Absorptiometer are given. The method, with the slightly modified attack given above, has been applied satisfactorily under routine conditions in the analysis of more than 60,000 samples of twelve different alloys.

I am indebted to my colleague, Mr. F. Pitts, for some of the results quoted above, and to Mr. A. G. Coates for helpful interest and criticism. The work described above was carried out in the laboratories of the British Aluminium Co., under the general direction of Dr. A. G. C. Gwyer. I have to thank the company for permission to publish the communication.

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May 1944

Determination of Water by means of Calcium Hydride

BY P. W. PERRYMAN

ROSENBAUM and Walton[1] and Notevarp[2] have used the reaction with calcium hydride to determine water in several organic liquids. More recently a general method has been described by Elitzur,[3] in which powdered calcium hydride is used in conjunction with anhydrous ethanol as intermediary liquid. Perkin and Pratt,[4] however, have shown that calcium hydride reacts with alcohols to give the calcium alkyloxide and hydrogen.

My expts. have shown that on bringing excess of calcium hydride and abs. ethanol into contact at room temp. a slow evolution of hydrogen occurs, leading to the formation of calcium ethoxide, and the use of ethanol is therefore unsatisfactory. In the modification here described anhydrous dioxan has been used in place of ethanol as water extractant and suspending medium in conjunction with calcium hydride for a rapid micro-determination of water. The method was originally designed for the determination of water in blood, but has been extended to some solutions and to powders.

APPARATUS—A water-jacketed 50-ml graduated gas-burette is connected to a levelling bulb by pressure tubing. Mercury is the enclosing fluid. At the upper end of the gas-burette a 3-way tap connects to the air *via* a calcium chloride guard-tube, and to a side-arm to which is attached 12 inches of pressure tubing terminating in a standard conical ground-glass joint fitting the reaction flask. A set of six interchangeable conical glass reaction flasks are used, each having a capacity of 15 ml. For quantities of water less than 25 mg each flask is charged with 0·2 g of powdered calcium hydride and 40 to 50 glass beads are introduced to facilitate mixing of specimen and reagent. The dioxan, containing the water to be determined, is introduced into the reaction flask in a special bulb; this is easily made by blowing a very thin bulb on the end of a piece of quill tubing of about 4 mm bore, flattening the end slightly, and cutting off to make a miniature flat-bottomed flask holding about 0·5 ml of fluid. Such bulbs must be made sufficiently thin to fracture easily when shaken vigorously with the glass beads. A small bung is used to close the mouth of the bulb during weighing, etc.

Specimen bulbs, prepared reaction flasks, and all pipettes are stored over phosphoric

anhydride when not in use. A calcium chloride guard-tube is interposed between the mouth and the pipette to prevent contamination by moisture when the pipette is used.

For the dioxan extraction of samples, 2-ml glass-stoppered bottles have been found satisfactory; where larger volumes are required, 25-g glass-stoppered weighing bottles may be used.

MATERIALS—The dioxan used throughout was of highly purified grade. A supply was maintained anhydrous by keeping 50 ml in a flask over 5 g of calcium hydride powder.

The calcium hydride was ordinary commercial "Hydrolith," finely powdered. It contained traces of carbide, nitride and oxide, but was quite satisfactory.

PROCEDURE—Place a weighed sample of the substance under test, in powdered or comminuted form if solid, in a weighed quantity of anhydrous dioxan (1 to 2 ml). Shake the tube and set aside for one-half to 24 hr. depending on the nature of the sample. Then transfer 0·5 ml of the dioxan extract to a specimen bulb, and determine its weight. Next detach the reaction flask rapidly from the gas burette, introduce the specimen bulb without bung, and re-connect the flask. Level the mercury at the zero mark of the burette. Then shake the flask to smash the bulb and mix the contents with the calcium hydride powder. As the gas is rapidly evolved, lower the mercury reservoir to keep pace with the evolution and so minimise risk of leakage. Shake the reaction flask at 5-min. intervals until the vol. of gas is constant; usually 40 to 60 min. suffice. Read the gas volume V, temperature t° C. and barometric pressure. Find the vapour pressure of dioxan at t° C. from Table I and calculate the vol. of gas at S.T.P.

TABLE I. VAPOUR PRESSURE OF DIOXAN

(Calculated from Herz and Lorenz[5])

t° C.	V.P., mm	t° C.	V.P., mm
0	6·2	19	25·6
10	11·8	20	27·1
15	19·6	21	28·6
16	21·5	22	30·2
17	22·9	23	31·8
18	24·2	24	33·6

Next make an identical expt., using the same vol. of anhydrous dioxan in a specimen bulb and another standard reaction flask, and determine the small vol. (V_B) due to gas adsorbed on the calcium hydride powder. Correct for this, and from the true vol. of gas equiv. to the water in the dioxan extract thus found calculate the amount of water in the original sample.

It has been found preferable to calibrate the apparatus with small weighed quantities of water rather than to use the stoichiometrical equivalent (0·804 mg of water per ml of gas at S.T.P.).

In many routine examinations, if a constant technique is used V_B is constant; at the same time the rate of reaction of calcium hydride with water in the sample is, in general, so much greater than the rate with any other reactive constituent that extraction can be dispensed with altogether. In the examination of blood, for example, a 20 cu. mm sample is added directly to 0·1 ml of anhydrous dioxan in a specimen bulb. This is then placed in the reaction flask and the water present is determined directly.

RESULTS—Ten determinations were made on 20-mg quantities of distilled water and 0·1 ml of anhydrous dioxan. The mean equivalent found was 0·841 ± 0·007 mg of water per ml of gas.

Similarly, 20-mg quantities from a sample of normal oxalated human blood gave the following figures:—79·7%, 79·7%, 80·5%, 80·3%; mean = 80·05 ± 0·45% of water. Two g of the same sample dried at 105° C. for 6 hr. gave 79·6% of water by loss of weight.

Strong solutions of serum were prepared from M.R.C. desiccated serum by weighing. Duplicate analyses were made, on 20-mg samples by the direct calcium hydride technique, and on 5-g samples by drying at 105° C. for 6 hr. Similar expts. were made with solns. of urea and sodium chloride. Table II shows the results.

A number of solutions of different sugars were tried and the results by the direct action technique were all rather high, probably on account of some reaction of the solutes with calcium hydride; for such samples the longer dioxan extraction technique must be used.

In Table III the results of the analyses of eight different samples of oxalated blood from normal subjects, using 20 mg, are compared with those found by drying 2 g at 105° C. for 6 hr.

TABLE II

(Water, g per 100 g of solution)

	(1) As prepared	(2) Found by CaH_2	Difference (1)–(2)	(3) Found by drying at 105° C.	Difference (1)–(3)
Serum solns.	90·1	89·7	0·4	90·3	−0·2
	86·9	87·3	−0·4	86·6	0·3
	83·2	82·3	0·9	82·2	1·0
	79·0	78·8	0·2	77·4	1·6
	75·2	75·1	0·1	73·6	1·6
Urea solns.	48·8	48·4	0·4	49·2	−0·4
	77·2	76·6	0·6	77·5	−0·3
	88·7	88·5	0·2	88·8	−0·1
Sodium chloride soln.	76·8	76·1	0·7	76·7	0·1

TABLE III. NORMAL BLOOD-WATER

Haemoglobin, % (Haldane)	Water by hydride %	Water by drying at 105° C., %	Difference
95	79·7	79·7	0·0
94	79·2	78·8	−0·4
90	79·1	79·3	+0·2
90	79·6	79·3	−0·3
90	79·9	79·4	−0·5
90	80·4	80·1	−0·3
90	81·2	80·7	−0·5
90	81·1	80·4	−0·7

The method has been extended to dioxan extracts of solid powders. In each test 0·2 to 0·5 g of the powdered sample and 1 to 2 ml of anhydrous dioxan were used for the extraction. Some results are shown in Table IV.

TABLE IV

	Extraction time, hr.	Water by CaH_2, %	Water by drying at 105° C. %
Dried-egg powder ..	1·5	4·61	5·10
Dried milk-powder ..	2·5	2·85	3·93
Dehydrated carrot ..	3·0	3·32	3·50
Dried serum ..	1·0	0·64	0·93
	30·0	0·72	

From all these expts. it will be seen that as the saving of time is considerable and no elaborate apparatus is required, the dioxan-CaH_2 method outlined may be of value in several fields where the determination of water in small samples is being undertaken.

It should be noted that a difficulty might arise in the use of dioxan as water extractant when *large* amounts of lipoidal substances, such as fats, are present in a sample, as these, being partly or wholly extracted together with the water, would lead to low results. The effect is in most substances negligible, but where appreciable it might be overcome by using a strictly uniform technique in conjunction with a calibration curve made by plotting the gas volume at S.T.P, against % of water as found by a standard reference method, such as vacuum drying.

I wish to thank Dr. C. F. Selous, pathologist at this hospital, for the provision of facilities in his laboratory.

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Notes

STANDARD SOLUTIONS FOR THE ESTIMATION OF RIBOFLAVIN

WHEN first attempting the estimation of riboflavin in dried egg powder by the method of Hodson and Norris[1] we were disturbed at being unable to reproduce the calibration curve relating readings on the Coleman Electronic Photofluorometer with strengths of pure riboflavin solution, and came to the conclusion that the "standard" solutions were at fault.

Comparisons of two samples of the vitamin—one bought from a reputable firm, and the other kindly supplied by the Director of Biological Standards, Medical Research Council—did not suggest the presence of impurities; but we noticed the deposition of minute golden specks from our stock soln. of 50 mg of riboflavin per litre.

The only information regarding the solubility of riboflavin was found in *"The Chemistry and Physiology of the Vitamins"* (Rosenberg, Interscience Publishers, Inc., 1942), which gave 12 mg per 100 ml at 27·5° C. and 19 mg per 100 ml at 40° C., and in *"The Newer Knowledge of Nutrition"* (McCollum *et al.*, Macmillan, 1942), which gave the solubility as 25 p.p.m. at 25° C.

This discrepancy, and the latter information in particular, was disquieting, for the widely used Hodson and Norris method calls for a standard stock soln. of 50 μg per ml (= 50 p.p.m.). To investigate the problem, riboflavin was weighed out into a series of volumetric flasks so as to give nominal concns. of about 8, 16, 32, 64, 128 and 256 p.p.m., distilled water was added to the mark, and the flasks were shaken for two min. and placed in a dark cupboard fitted with a maximum-minimum thermometer. They were taken out and shaken, in dim light, twice each day for 7 days, during which period the temperature varied between the limits of 16° and 21° C.

At the end of this period, the solns. were centrifuged and diluted to practically the same strength. The fluorescence of each diluted soln. was measured on the Coleman instrument, and the scale reading multiplied by the dilution factor so as to give a figure equivalent to the fluorescence of the original soln. if fluorescence had been proportional to concn. over the whole range. In other words, the "equivalent fluorescence" quoted is a measure of the actual strength of the solution.

TABLE I

Weight of riboflavin per 50 ml (mg)	Parts per million	Dilution factor for fluorescence measurement	Fluorescence of dil. solution	Fluorescence equiv. to full strength soln.	Fluorescence per 1 p.p.m.
0·3695	7·4	25	85	21 × 100	2·8
0·7710	15·4	50	75	37 ,,	2·4
1·5850	31·7	125	68·5	86 ,,	2·7
3·1510	63·0	200	70	140 ,,	2·2
6·3780	127·6	400	46	184 ,,	1·4
12·7780	255·6	714	35	250 ,,	1·0

Table I gives the figures involved, and a graph showed that at low concns. all the riboflavin dissolves, giving a straight line relationship between concn. and fluorescence. (It is in this region that the fluorescences of the diluted solns., referred to above, were measured.)

From this it is clear that the straight line might continue to about 50 p.p.m., but it might be dangerous to work above 30 p.p.m. under the conditions outlined here. Since we did not previously shake twice a day for a week, the fact that our results were variable is not surprising, and we thought the point might well be of interest to others using standard riboflavin solutions of the same order of concn.

We thank Mr. Rothwell, microchemist of the Organic Chemistry Department, Liverpool University, for weighing the riboflavin, and the Government Chemist for permission to publish this note.

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ARSENIC IN DRIED PARSLEY

A PROSECUTION was instituted recently at Greenwich for the sale of dried parsley containing an excess of arsenic. Although the amount found was unduly large, it may be of interest to record some results obtained during the last year for the arsenic content of dried parsley of known origin intended for retail sale.

The samples were examined by 3 methods: (i) ashing with magnesia, (ii) nitric acid wet combustion, and (iii) sulphuric acid destruction in a reducing atmosphere.[1] In both laboratories the magnesia method tended to give low figures compared with the other methods, the results with which were generally in fair agreement. Method (iii) is recommended as being the most rapid for the analyst.

	Source	Arsenic content p.p.m.
(*a*)	Hawkley, Hants. (no fertiliser or spray since sowing. Dried in electric oven)	2·5
(*b*)	Foxfield, Hants. (conditions as (*a*))	2·0
(*c*)	Castle Donnington, Derby (market garden)	3·5
(*d*)	Apsley, Lancs.	4·0
(*e*)	Powdered stalk, autumn gathered	3·3
(*f*)	Private garden, Nottingham	3·0
(*g*)	,, ,, ,,	3·2
(*h*)	,, ,, S. London	2·5
(*i*)	,, ,, N. London	4·0

The source of the contamination, if it is such, and not a trace element required in the plant metabolism, was not explained; a sample of powdered dried conium, which is of the same family, was found to contain 2·6 p.p.m. of arsenic. Soil from the place of growth contained low arsenic contents, and the possibility of contamination by arsenical sprays or the fumes of the drying ovens is discounted, since specimens of parsley grown in private gardens and known not to have had arsenic sprays and which were subsequently dried in electric ovens in the laboratory contained arsenic in the amounts found in commercial products. Specimens (*h*) and (*i*) were also examined when freshly gathered and were found to contain proportional amounts of arsenic. The high arsenic content of parsley has been noted by Rowson and Waterhouse.[2]

Commercial samples of unknown origin examined during the present year contained arsenic as follows:

	Range of arsenic contents (p.p.m.)				
	below 2·0	2·0–4·0	4·1–6·0	6·1–8·0	above 8·0
No of samples ..	0	15	4	2	1

Hence, compared with clean specimens of other herbs, it is our opinion that clean dried parsley will unavoidably contain in most instances more arsenic than the usually accepted limit of 1·4 p.p.m., and that some latitude should be allowed in the purity requirements of this commodity in respect of its arsenic content.

Thanks are due to Messrs. Newball & Mason, Ltd., Nottingham, for authentic commercially prepared specimens.

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THE IDENTIFICATION OF MALT VINEGAR IN PICKLES

A DETERMINATION of the oxidation, iodine and ester values of vinegars by the well-known procedures of Edwards and Nanji[1] or of Illing and Whittle[2] provides a positive means of distinguishing between malt, spirit and artificial vinegars. An estimation of the bisulphite-binding substances in vinegar was shown by Whitmarsh[3] to be a further aid to the differentiation.

Employing the methods of the above authors, expts. have been made to determine whether it is possible to distinguish malt vinegar from spirit or artificial vinegars in pickles. In so far as attention has been confined only to a limited range of pickles, however, this investigation must be regarded as essentially preliminary.

EXPERIMENTAL—Expts. were carried out on about 100 jars of malt, spirit and artificial vinegar pickles made from fully fermented brined onions, cauliflower and marrow. The vegetables were pickled separately and also after mixing in varying proportions. Although the acidities of the finished pickles (2·8–4·1%) were determined as acetic acid, it should be noted that acids due to fermentation and treatment of the vegetables also contribute to these values.

RESULTS—In order to indicate the very marked difference between the malt vinegar pickles and the others, only the maximum and minimum figures obtained for each type of pickle liquor are given. Columns (1) and (2) refer to the figures obtained by the respective procedures of Edwards and Nanji and Illing and Whittle. All results have been calculated with reference to a liquor of 4·0% acetic acid.

Liquor	Oxidation values A		Iodine values B		Ester values C		Bisulphite-binding compounds
	(1)	(2)	(1)	(2)	(1)	(2)	
Malt	600–1000	1000–2800	570–670	500–800	8·6–11·4	24·0–36·0	5·1–13·5
Spirit	280–350	550–1000	20–40	35–90	6·2–14·6	11·0–25·6	0·0–3·8
Artificial ..	80–360	70–1000	15–40	18–50	1·4–4·0	0·0–11·0	0·0–1·3

During the course of the expts. some rather surprising observations were made. It was seen from individual results that the vegetable was not making a simple additive contribution to the oxidation and iodine values. For example, the oxidation values obtained for the pickled onion spirit vinegar and the artificial vinegar liquors were practically the same. Furthermore, it was noted that the amount of reducing substances in the distillates from the pickle liquors was much greater than the amount derived from the vegetable plus the vinegar when distilled separately. It will be seen, also, from the table of results, that the values obtained in columns A, B and C vary according to which procedure was used.

CONCLUSIONS—Within the limits of the investigation it may be tentatively concluded that:

(1) It is possible to identify a malt vinegar pickle by determining the oxidation, iodine and ester values and bisulphite-binding compounds in the pickle liquor.
(2) Identification of a spirit or artificial vinegar may also be possible.
(3) The numerical results obtained for the three types of vinegar liquors do not bear the same relationship to each other as is found with ordinary vinegars.

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A FATAL CASE OF SODIUM NITRITE POISONING

FATAL cases of sodium nitrite poisoning are rare, the only ones hitherto recorded as having occurred in the British Isles being in 1936. These involved 3 accidental deaths in one family in Middlesbrough and 1 death by suicide in Hull, sodium nitrite being discovered on the premises in each case, quite apart from that actually found in the organs.

In a recent case in Leeds, involving the suicide of a medical practitioner, there was no poison suggestive of the cause of death immediately to hand, and the comparative suddenness with which death supervened was particularly mystifying in the absence of confirmatory symptoms of cyanide poisoning. The circumstances, moreover, were peculiar in that the victim had himself summoned by telephone a heart specialist in the early hours of the morning to the bedside of his wife, who, on the specialist's arrival 15 minutes later, was found to be suffering from nothing worse than hysteria. Nothing was seen or heard of the husband until he fell shortly afterwards in a moribund state outside the bedroom door, succumbing 5 minutes later to a poison which, it was estimated, had been taken within an hour of death.

The stomach contents (about 8 fl.oz.), received later in the day, had no unusual smell, but, instead of being acid, were faintly alkaline to litmus paper. Acidification with tartaric acid resulted in the formation of oxides of nitrogen, recognised initially by their smell, and subsequently, following distillation, by the reactions characteristic of nitrous acid. After removal of the organic matter (present in a measured portion of the stomach contents) with basic lead acetate, followed by precipitation of excess lead with potassium sulphate, the nitrite was estimated by means of (*a*) 0·1 *N* potassium permanganate + sulphuric acid, and (*b*) Griess–Ilosvay's tintometric method. As a result, the presence of 35 grains of nitrite (calculated as sodium-nitrite) in the total stomach contents was indicated, this amount therefore representing the minimum quantity of poison taken.

In the Middlesbrough and Hull cases the amounts found ranged from 16 to 100 grains, the maximum medicinal dose being 2 grains.

In the Leeds case it transpired that a few days before his death deceased had complained of pain across the chest, suggestive of angina pectoris. Apart from the normal signs of asphyxia, however, and a slight inflammation of the stomach wall, the post-mortem examination showed that all the organs were in a sound condition. If suicide had been contemplated at this time, such a statement was somewhat significant in view of the pharmacological action of sodium nitrite.

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PEROXIDE VALUES OF MOTOR SPIRITS

IN "*Standard Methods of Testing Petroleum and its Products*" (4th Ed.) there is a tentative method for the determination of peroxides in light petroleum. Some time ago we made a comparison of three methods for this determination in motor spirit fractions and, in view of the fact that the results varied considerably with the method employed, a brief account of this work may be useful.

The first two methods studied (A and B) were adaptations of procedures used for determniation of peroxides in fatty oils.

Method A—To 10 ml of the spirit add 20 ml of a mixture of equal vols. of chloroform and glacial acetic acid followed by 1·0 ml of sat. potassium iodide soln. Leave for the requisite time in the dark and in an atmosphere of carbon dioxide, then add 100 ml of water and titrate the liberated iodine with thiosulphate, using starch as indicator.

Method B was that devised by Marks and Morrell (ANALYST, 1929, **54**, 503). Mix 10 ml of spirit with 20 ml of glacial acetic acid and 1·0 ml of 50% sulphuric acid and add 2·0 ml of a sat. soln. of potassium iodide. Leave in the dark and in an atmosphere of carbon dioxide for the requisite time, then add 100 ml of water and titrate the liberated iodine as in Method A.

Method C was a modification of that described by Yule and Wilson (*Ind. Eng. Chem.*, 1931, **23**, 1254) for peroxides in motor spirits. Add 50 ml of ferrous thiocyanate soln. (prepared and stored as described in the original publication) to 10 ml of the spirit, leave in the dark and in an atmosphere of carbon dioxide for the requisite time, and titrate the resulting ferric thiocyanate with titanous chloride soln. It is difficult to ensure that the standard ferrous thiocyanate is completely reduced to the ferrous state by iron and we found it advantageous to substitute zinc for this purpose. This soln. and the standard titanous chloride soln. were stored under hydrogen.

All standard solns. were standardised immediately before use, and each determination was systematically corrected by a blank determination. Each method suffers from the defect that the reaction mixture consists of more than one phase.

As shown in Table I, a series of determinations indicated that the peroxide value varies with the time of standing before titration. Moreover, when the results on Spirits III (*a*, *b* and *c*) were plotted graphically it could be clearly seen by the flattening of the curves that the increase in the peroxide value with time of standing became decidedly less after a period of 3 to 4 hr. with Methods A and B, whilst with Method C the time of standing had apparently little effect on the peroxide value.

In subsequent determinations it was decided to use the standard time of 4 hr. standing for each method.

The investigation was extended to the determination of peroxide values by the three methods on (*a*) a sample of cracked spirit kept in a clear Winchester-quart bottle and exposed to diffuse light in the laboratory, and (*b*) a sample of the same material kept under the same conditions but having in solution the auto-oxidation inhibitor catechol in amount equiv. to 0·006 g per 100 ml of spirit. Determinations

were made at weekly intervals over a period of seven weeks and a final examination, using Method A only, was made after one year. The results are given in Table II and show that with the same spirit highest values are obtained by Method B, intermediate values by Method A, and lowest values by Method C. No explanation can be offered for the curious fall in the values determined at the sixth week. It may have been due to lighting conditions. Conceivably, brighter conditions may have caused decomposition of some of the peroxides in the spirit. The influence of the inhibitor is clearly indicated in the comparisons.

TABLE I

PEROXIDE VALUES AS MG OF OXYGEN PER LITRE OF SPIRIT

	Method A			Method B		Method C
Spirit*	I	II*a*	III*a*	II*b*	III*b*	III*c*
Time. 5 min.	373	17·7	17·7	95	114	21·1
15 ,,	421	18·7	19·8	125	165	20·7
30 ,,	477	21·8	20·8	131	196	20·2
1 hr.	421	18·7	23·9	164	230	21·1
2 ,,	550	25·0	35·4	265	295	20·7
3 ,,	410	25·0	41·6	278	472	19·4
4 ,,	438	25·0	37·4	250	518	20·7
6 ,,	—	—	42·6	—	—	—
16 ,,	—	—	62·4	—	711	29·5
24 ,,	—	—	81·1	—	806	27·3
48 ,,	—	—	—	—	989	—

* Spirit I = Old cracked spirit containing some pptd. gum.
Spirits II*a* and II*b* = Old cracked spirits without gummy deposit.
Spirits III*a*, III*b* and III*c* = Artificially aged cracked spirits.

TABLE II

PEROXIDE VALUES AS MG OF OXYGEN PER LITRE OF SPIRIT

	Method A		Method B		Method C	
Spirit*	IV	V	IV	V	IV	V
Time 1 week	21	—	37	—	24	—
2 weeks	84	—	332	—	62	—
3 ,,	209	—	454	—	92	—
4 ,,	429	9	634	22	138	10
5 ,,	404	—	642	—	154	—
6 ,,	350	—	598	—	139	—
7 ,,	478	54	684	64	195	17
52 ,,	875	99	—	—	—	—

Spirit IV = Cracked spirit.
Spirit V = Cracked spirit plus 0·006 g of catechol per 100 ml.

We wish to thank Scottish Oils, Ltd., for supplying samples of cracked spirit for this investigation and the Governors of the Heriot Watt College for laboratory facilities.

HERIOT WATT COLLEGE
EDINBURGH

J. RISBEY
H. B. NISBET

September, 1944

Official Appointments

GOVERNMENT CHEMIST

THE Lords Commissioners of His Majesty Treasury have appointed PROFESSOR G. M. BENNETT, M.A., Sc.D., PH.D., F.R.I.C., Professor of Organic Chemistry at King's College, London University, to be Government Chemist in succession to the late Sir John Fox, C.B., O.B.E., F.R.S., D.Sc., F.R.I.C.

Professor Bennett's appointment will not become effective until he vacates his tenure of the Chair of Chemistry at the end of the academic session in the late summer of this year. Until then Dr. A. G. Francis, Q.B.E., D.Sc., F.R.I.C, the present Deputy Government Chemist, will act as Head of the Department.

FERTILISERS AND FEEDING STUFFS ACT, 1926

THE Ministry of Agriculture and Fisheries has notified the Society of the following change in the appointments of Agricultural Analysts which has taken place since July 14th, 1944 (ANALYST, 1944, **69**, 247).

R. F. WRIGHT, B.Sc., A.R.C.S., F.R.I.C., Agricultural Analyst for Hastings County Borough, *vice* G. M. Norman, B.Sc., A.R.C.S., F.R.I.C., retired.

Order in Council

REGULATION 60 CAA OF THE DEFENCE (GENERAL) REGULATIONS, 1939*

1. THE PUBLIC HEALTH (PRESERVATIVES, ETC., IN FOOD) REGULATIONS, 1925.

An Order in Council adding Regulation 60 CAA to the Defence (General) Regulations, 1939, has been made (S.R. & O. 1944, No. 1311). Paragraphs (1) to (7) of the new Regulation relax the prohibitions contained in Regulations 4 and 11 of the Public Health (Preservatives, etc., in Food) Regulations, 1925, to allow:—

(1) the importation, under and in accordance with the terms of a licence granted by the Minister of Food, of oranges in wrappers treated with diphenyl, and their subsequent sale;

(2) the importation or manufacture under licence issued by the Minister of Food, and subsequent sale of (i) meat which contains sulphur dioxide; (ii) margarine which contains borax; (iii) bacon which contains borax; (iv) dehydrated vegetables which contain sulphur dioxide; and (v) jam which contains sulphur dioxide in excess of the proportion specified in Part I of the First Schedule of the Regulations.

The amendments made under paragraphs 2(i), (ii) and (iii) above had already been effected by Ministry of Food Statutory Rule and Order (*i.e.*, the Meat (Addition of Preservative) Order, 1941, No. 1395; the Margarine (Addition of Borax) Order, 1940, No. 982; and the Bacon (Addition of Borax) Order, 1940, Nos. 547 and 1833 and 1942, No. 1372. These have now been revoked.

With regard to the relaxation in respect of dehydrated vegetables, the sulphur dioxide content will be limited, the limits being specified either in the licence issued by the Minister of Food or in the authorisation to import. Further information regarding the limits to be prescribed will be circulated for the information of Food and Drugs Authorities.

In the case of jam, licences issued by the Minister of Food will authorise a sulphur dioxide content of not more than 100 parts per million.

2. THE PUBLIC HEALTH (CONDENSED MILK) REGULATIONS, 1923.

Paragraphs (8) and (9) of the new Regulation contain a relaxation of certain requirements of the Public Health (Condensed Milk) Regulations, 1923, already effected by the Condensed Milk (Milk Content) Order, 1940, No. 1896, which has now been revoked.

3. THE AGRICULTURAL PRODUCE (GRADING AND MARKING) ACT, 1928.

Paragraphs (10 and (11) of the new Regulation relax certain requirements of the Agricultural Produce (Grading and Marking) Act, 1928. Paragraph (10) exempts from section three of the Act (which provides that it shall not be lawful to sell or expose for sale any preserved eggs unless they are marked in the prescribed manner)—

(*a*) any hen or duck eggs in shell which have been laid in the United Kingdom; and (*b*) any hen or duck eggs in shell which have been imported into the United Kingdom from the United States of America or from any of the Dominions, except eggs which have been marked with the word "cooking" in pursuance of any law of the Union of South Africa relating to the export of eggs.

Paragraph (11) of the new Regulation suspends Section four of the Act which provides for the registration of premises used for the cold storage or chemical storage of eggs and for the marking of British eggs which have been kept in such premises.

Ministry of Food

STATUTORY RULES AND ORDERS†

1944—No. 1436. Order, dated December 21, 1944, amending the Flour Order, 1944. Price 1d.

The amending Order makes two alterations to the Flour Order from December 31, 1944, namely,

(1) A decrease in the rate of extraction of national flour, which now becomes 80% instead of 82½%, and a consequent alteration in the definition of "W" flour.

(2) Increases (*specified*) in the maximum retail prices on sales in weights of 140 lb. or more of (*a*) national and imported flour; (*b*) self-raising flour.

— **No. 1443. Order, dated December 22, 1944, amending the Feeding Stuffs (Regulation of Manufacture) Order, 1944.** Price 2d.

This Order, which comes into force on January 15, 1945, increases the proportion of certain compounds, concentrates and livestock mixtures which may be manufactured, alters the composition of National compounds by reducing the quantity of barley included, and makes compulsory the use of maize. The maximum fibre content of National compounds is also slightly reduced.

THE LABELLING OF FOOD (No. 2) ORDER, 1944‡

Following the decision already announced to postpone the date of operation of the Labelling of Food Order as regards labels until May 1, 1945 (with a further three and six months' dispensation to permit wholesalers and retailers to dispose of their stocks), the Order has been re-enacted as the Labelling of Food (No. 2) Order, 1944, S.R. & O., No. 1447.

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* P.N. 3493, Jan. 4, 1945.

† Obtainable from H.M. Stationery Office. Italics signify changed wording.

‡ P.N. 2489a, Dec. 30, 1944.

Other amendments to the Order which experience in advising traders as to their labels has shown to be desirable have been made as follows.

(1) The statement of composition on a label must indicate the true nature of the ingredient or ingredients of which the food is composed.
(2) Where a complete quantitative disclosure of the ingredients is made, the order in which they are disclosed need not be that of the proportion by weight in which they were used.
(3) The statement of the minimum quantity of the food must be expressed in terms of net weight or of measure.
(4) Foods packed in advance by retailers on the premises on which they are sold are exempt from the full labelling provisions of the Order, provided the label bears no words referring in any way to the food other than such words or other markings as are reasonably necessary to identify the food and a statement of the quantity or price.
(5) As already announced, the use of a plain liner is permitted where it is not removed from the outer wrapper.
(6) When the presence of a mineral is disclosed as an ingredient in accordance with the requirements of Article 2 (3) and no other claim or suggestion is made as to its presence, it need not be substantiated by a declaration of the minimum quantity of the mineral present.
(7) Liquid milk, shell eggs and fish are exempt from the special requirements applicable to foods in which the presence of vitamins or minerals is claimed.
(8) As a result of amendments to the First Schedule
   (*a*) Wrapped or banded sausages and sausage meat are required to be labelled with a name and address or trade mark.
   (*b*) The ingredients which may be designated as flavourings without further specification are restricted to those products which are used in food primarily for flavouring purposes. (A definition of flavourings is inserted.)
   (*c*) Food colourings when pre-packed for sale as such may be labelled without disclosing the identity of the colouring ingredient, but if the latter is of synthetic origin, the word "colour" must be qualified by either "synthetic" or "artificial."
   (*d*) When any product which is the subject of a monograph in the British Pharmacopoeia or the British Pharmaceutical Codex is used as an ingredient of a food it may be named without disclosure of its constituents.
   (*e*) In common with sweetened and modified dried milk, it is sufficient disclosure of the quantity of compounded dried milk if the label bears a statement of the equivalent pints.
   (*f*) National flour is exempt from the necessity to state the ingredients and a name and address, or trade mark, only if it is labelled "National Flour."
   (*g*) Speciality flour is exempt from the disclosure of ingredients which are not present in greater quantities than the quantities in which they are customary in National Flour.
   (*h*) When used as ingredients, deodorised fatty oils may be designated as "edible Oil" or "edible Fat."
(9) The requirements in regard to the declaration of the quantity of vitamins or minerals in a food where their presence is claimed come into force on January 1, 1945, so far as advertisements are concerned. The Second Schedule to the Order indicates the synonymous terms which may be used in the declaration of the vitamin content.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determining Volatile Bases in Fish. Comparison of Precision of Certain Methods. M. E. Stansby, R. W. Harrison, J. Dassow and M. Sater (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 593–596)—The determination of volatile bases in fish has been widely used as an index of the freshness of the fish, but, since results obtained by different methods do not always agree, comparative results obtained by some of them have been studied. Samples of fish of varying degrees of freshness were prepared from 55 eviscerated silver salmon procured when 4 hr. out of the water. Twenty-five were stored at room temp. and 30 in crushed ice. Five were dressed immediately and the flesh, freed from skin and bone, was ground twice in an electric meat grinder, mixed thoroughly, packed in 0·2-kg cans, frozen immediately and stored at *ca.* −15° C. At suitable intervals the fish stored at room temp. or in ice were sampled by treating five fish in the same way, and it was assumed that no increase of volatile base occurred after freezing. For the separation of the volatile bases from proteins, extraction methods and methods depending upon precipitation of protein from press juice were tried. The very gummy ppt. obtained when trichloroacetic acid was added to the press juice was difficult to wash and probably adsorbed considerable amounts of volatile bases. Of the extraction methods, that with 60% ethanol as described for meat by Allen (*"Commercial Organic Analysis,"* 5th Ed., IX, p. 324) gave the most consistent results, especially when the fish was finely disintegrated in 60% ethanol in a liquidiser. The Waring Blendor with an aluminium container closed by a screw lid is recommended for this purpose. For determination of the volatile bases the methods examined were the micro-diffusion method of Conway and Byrne (*Biochem. J.*, 1933, **27**, 419) as modified for fish by Beatty and Gibbons (*J. Biol. Board Can.*, 1936, **3**, 77), distillation of the alcoholic extract with alkali at atmospheric pressure and aeration of the flesh suspended in water (*"Methods of Analysis of the A.O.A.C.,"* 5th Ed., 1940) or finely disintegrated in 60% ethanol. The micro-diffusion method has the advantages of convenience and conditions that minimise decomposition of nitrogenous matter and the disadvantages of lack of high precision, the use of special apparatus and the need for great care, owing to the small amount of sample used. The distillation procedure is rapid and precise, but

requires much attention to prevent frothing and, although it is the most suitable method for determining total volatile bases, it cannot be used for determining tertiary bases unless allowance is made for its tendency to give high results, especially with fresh fish. Although the aeration method has certain advantages and gives moderately precise results, it is cumbersome and is not recommended. The recommended procedure is as follows. Place 40 g of fish in a liquidiser with a tightly fitting lid with 100 ml of 60% ethanol and mix for 5 min. Rinse the product into a 250-ml centrifuge bottle with 60% ethanol, centrifuge for 10 min. and decant the supernatant liquid into a 250-ml flask. Stir the residue with 25 ml of 60% ethanol, centrifuge, decant and repeat the procedure with 25 ml of 60% ethanol. Finally dilute the combined extracts with 60% ethanol to 250 ml. For the tertiary volatile base determination, pipette a 2-ml aliquot into the outer section of a Conway unit add 2 ml of neutral formalin, pipette 1 ml of 0·005 *N* hydrochloric acid into the centre section and then, with the lid almost in place, add 1 ml of sat. potassium carbonate soln., quickly slide the lid, with its ground-glass surface previously greased, into place, mix the contents of the dish by a slight rotary motion and incubate at 40° C. for 3 hr. Make blank determinations simultaneously with 2 ml of 60% ethanol in place of the sample. Titrate the excess of acid using a micro-burette and a mixed indicator (methyl red with either methylene blue or bromocresol green) previously adjusted to the neutral point. Make all determinations in triplicate. The same procedure can be used for determination of total volatile bases, the addition of formalin being omitted and 1 ml of 0·02 *N* acid being used in the centre dish, but the distillation method is to be preferred because of the advantages already mentioned. Place the contents of the 250-ml flask (after removal of the aliquots for tertiary base determination) in a 500-ml Kjeldahl flask with 4 glass beads and 5 g of powdered borax. Connect the flask quickly with the still and collect 100 ml of distillate in 50 ml of 0·05 *N* hydrochloric acid. A few drops of capryl alcohol may be added to prevent frothing, but excess must be avoided. Titrate the excess of acid with standard alkali to the methyl red neutral point and correct the result by means of a blank determination with 60% ethanol in place of the sample. Results may be expressed as mg of nitrogen per 100 g of fish.

A. O. J.

Determination of Maltose in Presence of Glucose. H. H. Browne (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 582–583)—The method is based upon the fact that the optical rotation of glucose can be reduced to zero by addition of a sufficient amount of sodium bisulphite, the rotation of maltose and dextrins being only slightly affected. The optical rotation of lactose and other reducing sugars (but not that of the sugar alcohols) is also lowered by sodium bisulphite, and the method cannot be applied to mixtures of maltose and glucose with other carbohydrates (*e.g.*, malt syrup and corn syrup). Prepare a series of 7 solns., each containing 10 g of a mixture of maltose and glucose, the ratios of glucose to maltose in the different solns. being 10:0, 8:2, 6:4, 5:5, 4:6, 2:8 and 0:10, and each soln. containing not less than 75 ml of water. Place the solns. in 7 sugar flasks graduated at 110 ml, each containing 30 g of sodium metabisulphite (or an equiv. amount of sodium bisulphite). Shake the flasks to dissolve the salt, cool to 20° C., make up to 110 ml with water and determine the optical rotation at 20° C. Plot the observed rotations against % of maltose, thus obtaining a curve which is almost linear and from which % of maltose corresponding with intermediate polarimetric readings may be obtained. To apply the method to an unknown mixture, first determine the total sugars by an accepted method. To each sugar flask used add 10 g of the mixture (or the amount of its soln. containing 10 g), dilute to *ca.* 75 ml, and proceed as already described, reading the % of maltose from the standard curve. Since the curve is, for practical purposes, a straight line, the tangent of its angle of slope may be used as a factor to convert polarimetric readings to % maltose. With 5 mixtures of known composition the max., min. and aver. deviations from the known values were 0·28, 0·04 and 0·20% of maltose, respectively, calculated on the mixtures.

A. O. J.

Determination of Sodium Acetate in FD & C Blue No. 1. R. N. Sclar and G. R. Clark (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 472–474)—The Coal Tar Colour Regulations (S.R.A.F.D.C.3) limit the quantity of sodium acetate permitted in FD & C Blue No. 1 to not more than 3%. The tentative method for the determination of sodium acetate in FD & C Blue No. 1 ("*Methods of Analysis of the A.O.A.C.*," 1940, 255) is tedious and gives varying results. A procedure based upon Freudenberg's method for determination of acetyl groups (*Ann.*, 1923, **433**, 230; 1932, **494**, 68) gives more satisfactory results. The sodium acetate is esterified to ethyl acetate in presence of *p*-toluenesulphonic acid and silver *p*-toluenesulphonate (to remove halides from the reaction), the ester is saponified with a measured excess of standard sodium hydroxide soln., and the unused alkali is titrated with standard acid. The apparatus consists of a 125-ml acetylation flask with a 24/40 standard taper joint carrying a graduated tap funnel and connected with a 300-ml receiving flask through a splash head and a vertical water condenser. Place the receiving flask containing 20 ml of abs. alcohol in an ice-bath and connect it with the apparatus. In the distilling flask place 2 g of the dye, 4 g of *p*-toluenesulphonic acid, 4 g of silver *p*-toluenesulphonate (prepared by dissolving freshly pptd. and washed silver oxide in *ca.* 10% excess of *p*-toluenesulphonic acid, evaporating to dryness and drying at 135° C. for 8 hr.) and 40 ml of abs. alcohol, marking the level of the contents. Distil from a vigorously boiling water-bath, and 15 min. after the distillate begins to enter the receiver add 20 ml of abs. alcohol. Distil for 10 min., add enough alcohol to bring the contents to the original vol. and continue distillation for 15 min. Disconnect the receiving flask and saponify the contents under reflux for 10 min. with a measured excess of 0·1 *N* sodium hydroxide. Rinse the condenser with water, remove the flask, dilute the contents with an approx. equal vol. of water, cool and titrate with 0·1 *N* hydrochloric acid to the phenolphthalein end-point. Make a blank determination in the same manner, omitting the dye. Each ml of 0·1 *N* sodium hydroxide required for saponification = 0·0082 g of sodium acetate.

A. O. J.

Determination of Quinacrine Hydrochloride [Mepacrine] by Absorption Spectrophotometry. J. Carol (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 360–365)—A rapid method is needed for determining

quinacrine hydrochloride (mepacrine) in tablets and other forms in which it may be dispensed, the assay of the U.S.P. XII being inconvenient for rapid work with large numbers of samples. The proposed spectrophotometric method is based upon the bright yellow colour of the aq. soln. To test the applicability of the Beer–Lambert law, absorption measurements were made of a series of solns. of 1·0–5·0 mg of quinacrine hydrochloride in 100 ml of 0·1 *N* hydrochloric acid at 425*mμ*, the wavelength of the principal peak absorption. The linearity of the graph obtained by plotting absorption against concn. showed that the Beer-Lambert law is satisfied within experimental error. The effect of change of *p*H of the soln. was determined by measuring the absorption relative to the solvent at 425 *mμ* of solns. of quinacrine hydrochloride in *N* and 0·1 *N* hydrochloric acid, in water and in 0·1 *N* sodium hydroxide. The results show that absorption is unaffected by *p*H changes below 7, but that alkaline solns. precipitate the base and are unsuitable for spectrophotometric analysis. The following method is applicable to quinacrine hydrochloride preparations containing no other water sol. substance having an absorption band at 425 *mμ*. To an accurately weighed or measured sample containing *ca.* 100 mg of quinacrine hydrochloride in a litre flask add *ca.* 100 ml of *ca.* 0·1 *N* hydrochloric acid and heat the mixture on the steam-bath until the quinacrine hydrochloride has all dissolved. Cool, dilute to 1 litre with 0·1 *N* hydrochloric acid, filter and dilute an aliquot containing 2–3 mg of the salt to 100 ml with 0·1 *N* hydrochloric acid. Determine the absorption (E) relative to that of 0·1 *N* hydrochloric acid at 425 *mμ*. Determine also the absorption of a standard soln. containing 2·5 mg per 100 ml of pure quinacrine hydrochloride. Mg of quinacrine hydrochloride in the aliquot $= \frac{\text{E (sample)}}{\text{E (standard)}} \times 2{\cdot}5$. The purity of the standard specimen may be established either by the U.S.P. assay or by determination of its nitrogen content by the Kjeldahl method.

A. O. J.

Estimation of Total H_2S Metals in [Pharmaceutical] Iron Salts. H. Rosenblum and J. Rosin (*Amer. J. Pharm.*, 1944, **116**, 296)—The determination of heavy metals as impurities in salts by the usual sulphide method cannot be applied to ferric salts, since sulphur is liberated by the reaction between the ferric iron and hydrogen sulphide. Pptn. of the ferric hydroxide by ammonia introduces errors by the adsorption of lead on the ppt. and, although copper and lead, the two most usual foreign metals present in iron salts may be determined separately, other heavy metals may be present and, moreover, two separate determinations are tedious. A method is described whereby ferric iron is reduced to the ferrous state with hydroxylamine hydrochloride, a precise quantity of ammonia is added to partially neutralise the free acid produced and the heavy metals are determined with hydrogen sulphide. Using 0·5 g of the ferric salt for the test, the following volumes of 10% ammonia soln. are suitable—$FeCl_3.6H_2O$, 0·50 ml; $Fe_2(SO_4)_3$ (*ca.* 20% H_2O), 0·55 ml; $Fe_2(SO_4)_3.(NH_4)_2SO_4.24H_2O$, 0·25 ml.

Water-soluble ferric salts (including such insoluble cpds. as ferric oxide after evaporation with hydrochloric acid)—Add 0·4 g of hydroxylamine hydrochloride to a soln. of 0·5 g of the salt in *ca.* 35 ml of water and shake until dissolved. Gently swirl the soln. and add dropwise the requisite amount of 10% ammonia soln. (see above), then warm on a steam-bath until practically colourless and boil gently for 1 min. Rapidly cool, dilute to 40 ml, add 10 ml of a saturated soln. of hydrogen sulphide and compare the colour produced with controls prepared from standard cupric sulphate soln. and to which has been added 0·5 ml of 0·1 *N* hydrochloric acid and 0·4 g of hydroxylamine hydrochloride. A correction should be made for the colour of the ferrous salt by omitting the sulphide and comparing the intensity of the colour with cupric sulphate standards. This correction may amount to *ca.* 5–10 p.p.m. *Ferric ammonium citrate*—Carbonise 0·5 g of the soln. by heating with 1–2 ml of conc. sulphuric acid. Add 4 ml of conc. nitric acid a few drops at a time, heating for 30–60 sec., after each addition, then heat until fumes of SO_3 appear, cool, cautiously add 2 ml of water, and evaporate almost to dryness. Cool, add 50 ml of water and 0·5 g of hydroxylamine hydrochloride, heat until practically colourless, cool, add 10% ammonia dropwise until a slight turbidity is produced and boil gently until the soln. is clear and practically colourless. Immediately dilute the mixture to 80 ml, tak 40 ml of this and add 10 ml of saturated hydrogen sulphide water. Compare the colour produced with cupric sulphate standards similarly treated. Correction should be made for the colour due to the ferrous iron. The test may conveniently be applied to exsiccated ferrous sulphate, since this invariably contains a little ferric iron. In this test 0·05 g of hydroxylamine hydrochloride is used for 0·5 g of the salt. A table is reproduced showing satisfactory recoveries of added lead and copper from various ferric salts.

J. A.

Biochemical

Determination of Blood Plasma Iron. G. Kitzes, C. A. Elvehjem and H. A. Shuette (*J. Biol. Chem.*, 1944, **155**, 653–660)—Pipette 3 to 5 ml of blood plasma, containing 3 to 9 μg of inorganic iron, into a 15-ml Pyrex centrifuge tube, add 3 ml of redistilled water and mix with the plasma. At the same time prepare a blank consisting of all the reagents. Immerse the tubes in boiling water for 2–3 min., or until the soln. becomes opaque, and then transfer to cold water. Add 2 ml of 25% trichloroacetic acid soln. and stir thoroughly. Immerse the tubes in a water-bath at 90–95° C. for 3 min. with stirring, again cool and centrifuge at 2000–3000 r.p.m. for 5 min. Decant the supernatant liquid into a 15-ml graduated centrifuge tube. To the original tube add 4 ml of redistilled water and 1 ml of trichloroacetic acid, break up the ppt. and stir well, and then immerse in water at 90–95° C. for 3 min., stirring each soln. once. Cool, centrifuge for 5 min., and decant the supernatant liquid into the tube containing the first decantate. Add one drop of 0·1% *p*-nitrophenol soln., followed by 6 *N* ammonia, drop by drop, until the soln. becomes yellow. Add 1 ml of a buffer soln., *p*H 4·58 (27·2 ml of glacial acetic acid and 33·4 g of anhydrous sodium acetate in 250 ml), and dilute to 15 ml with water. Transfer 5 or 10 ml of the soln., containing 2 to 3 μg of iron, into an Evelyn colorimeter tube and add 2 drops of thioglycollic acid. Dilute to 10 ml, if necessary, and determine the centre setting for each soln. in the colorimeter. Add 1 ml of 0·2% αα′-dipyridyl soln. in 5% acetic acid, and measure the colour of each soln. in the colorimeter with the respective centre settings previously determined. Calculate the

amount of iron in the soln. in each tube by reference to a standard curve or from the expression $\frac{\mu g \text{ of iron}}{\text{aliquot}} = 40{\cdot}6 \times (L_{aliquot} - L_{reagent\ blank})$, where the L values are calculated from the galvanometer readings. Recoveries of added iron ranged from 92 to 104%. F. A. R.

Determination of Free Choline in Animal Tissues. R. W. Luecke and P. B. Pearson (*J. Biol. Chem.*, 1944, **155**, 507–512)—The micro-biological method of Horowitz and Beadle (*J. Biol. Chem.*, 1943, **150**, 325; ANALYST, 1944, **69**, 158) with minor modifications gave results for the free and total choline content of tissues in good agreement with those obtained by chemical methods. Grind a 2 to 3-g sample of the fresh tissue in a mortar, and transfer it to a 125-ml conical flask with the aid of 50 ml of 1% sodium acetate soln. adjusted to *p*H 4·6. Heat at 80° C. for 1 hr., centrifuge and decant the supernatant liquid. Transfer a 5-ml portion to a 15-ml centrifuge tube containing 10 ml of acetone, immerse in an ice-bath for 2 hr., and then centrifuge to remove the pptd. lecithin. Transfer the clear soln. to a small beaker, and remove the acetone by evaporation. Dilute the aqueous soln. to 50 ml and run 10 ml through a column of Decalso (1 g), activated by the method of Hennessy (*Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 216). Elute with 10 ml of 5% sodium chloride soln. and estimate the choline micro-biologically, using the medium described by Horowitz and Beadle. One μg of biotin per litre was found to give as good a response as the 5 μg recommended in the original medium. To estimate total choline, autoclave a 0·2-g sample of the finely minced tissue with 10 ml of 3 *N* hydrochloric acid at 15 lb. pressure for 2 hr. Neutralise with sodium hydroxide, adjust to a convenient vol. and adsorb on Decalso as before. The average recovery of choline chloride added to tissue was 98%. The results were in excellent agreement with those obtained by the methods of Engel (*J. Biol. Chem.*, 1942, **144**, 701; ANALYST, 1942, **67**, 398) and of Marenzi and Cardini (*J. Biol. Chem.*, 1943, **147**, 363; ANALYST, 1943, **68**, 258). F. A. R.

Manometric, Titrimetric and Colorimetric Methods for Measurement of Urease Activity. D. D. Van Slyke and R. M. Archibald (*J. Biol. Chem.*, 1944, **154**, 623–642)—Jack bean is a much richer source of urease than the soya bean used in earlier work, and therefore with jack bean the amount of extract must be reduced, or so much urea will be hydrolysed that the *p*H will rise above 7·4 and reduce the activity of the enzyme. However, with these smaller amounts not enough jack bean protein is present to stabilise the enzyme; it has been found that a certain concn. of protein is necessary to protect the enzyme from partial inactivation by the high concn. of urea used in the standardisation. The presence of protein is also desirable when the Van Slyke–Neill chamber is used, as it inhibits the inactivation of the enzyme by mercury. To overcome this difficulty, it is recommended that jack bean urease be dissolved in a 5% soln. of egg albumin, and that the Van Slyke–Neill chamber be rinsed with albumin soln. before each analysis.

Manometric procedure A—Rinse the gas chamber with 1 ml of 10% egg albumin to remove mercury ions, put 0·5 ml of 0·67 *M* phosphate buffer *p*H 6·4 (5·66 g of K_2HPO_4 and 4·49 g of $NaH_2PO_4H_2O$ in 100 ml) into the cup of the Van Slyke–Neill chamber, and run the soln. into the chamber until the cup is empty but the capillary below is filled with soln. Run 1 drop of 0·1% bromothymol blue and then a drop of caprylic alcohol, into the capillary, and put 0·5 ml of mercury into the cup and run 5 ml of 3% urea soln. into the chamber. Seal the capillary by filling it with part of the mercury in the cup, close the cock and mix the soln. by lowering and raising the mercury in the chamber three times. In the same way introduce into the chamber 1 ml of 0·1% urease soln. in 5% egg albumin, so that the urease forms a layer above the denser substrate soln. Seal the capillary with mercury and close the cock. Note the time by a stop-watch, mix the contents of the chamber, and put 1 ml of 2 *N* lactic acid into the cup. After 2½ min. note the temperature in the water-jacket of the manometric chamber and, after 5 min., run in 0·5 ml of 2 *N* lactic acid, so stopping the action of the urease. Seal the capillary and cock with mercury and extract carbon dioxide from the acidified soln. by evacuating and shaking. After 2 min. extraction adjust the gas vol. to 2 ml and record the manometric reading, p_1. Open the cock leading to the levelling bulb and add 0·5 ml of 5 *N* sodium hydroxide under slight negative pressure to absorb the carbon dioxide. Adjust to the 2-ml mark and record the manometric reading, p_2. Make a blank determination in which the urease soln. is replaced by 5% egg albumin soln. The difference between the readings p_1 and p_2 is the blank correction c. The results are calculated in Sumner units of urease per mg of urease powder from the expression $\frac{5 \times Pco_2 \times A}{\text{mg} \times \text{min.}}$, where $Pco_2 = p_1 - p_2 - c$ and A is a factor depending on the temperature. The value of A for different temperatures is given in a table in the original paper.

Manometric Procedure B—Insert a 1-hole rubber stopper in a 25-ml conical flask, and plug the hole with a glycerol-lubricated glass rod. Bring all the reagents to room temp. (t_1) and put into the flask 10 ml of 3% urea soln., 1 ml of 0·65 *N* phosphate buffer *p*H 6·4 and 1 drop of bromothymol blue soln. Add 2 ml of the urease soln., stopper the flask, mix the reagents and set a stopwatch. When the indicator becomes bluish-green (4–8 min.), remove the glass plug, add 2 drops of 18 *N* sodium hydroxide and immediately replace the plug. The alkali stops the action of the urease and absorbs the small amounts of carbon dioxide that escape into the gas space of the flask during the digestion. Mix and note the time. Transfer 5 ml of the soln. to the manometric chamber of the apparatus and add 0·7 ml of 2 *N* lactic acid and a drop of caprylic alcohol. Extract the carbon dioxide and note the values of p_1 and p_2 as before. Record the temp. of the water-jacket (t_2). Determine the value of c, using 2 ml of 5% albumin soln. instead of 2 ml of urease-albumin soln. The Sumner units per mg $= \frac{10 \times Pco_2 \times B \times C}{\text{mg} \times \text{min.}}$, where B and C are constants, recorded in a table in the original paper, depending on the temperatures t_2 and t_1 respectively.

Aeration-titration method—Put 5 ml of urea-buffer mixture of *p*H 6·8 (3 g of urea, 1·1 g) of K_2HPO_4 and 0·85 g of $NaH_2PO_4H_2O$ in 100 ml of water) into each of the two aeration tubes (Van Slyke and Cullen, *J. Biol. Chem.*, 1914, **19**, 211), one for the digestion and the other for the blank. Put 25 ml of 4% boric acid soln. and 2 drops of 0·1% bromocresol green soln. into each of two receiving tubes, add 2 drops of caprylic alcohol to the aeration tubes and 1 drop to each of the receiving tubes. To the

aeration tube to be used for the digestion, add 1 ml of 0·2% urease soln. in 5% egg albumin soln., mix, note the time and allow the digestion to proceed for exactly 15 min., immersing the tube in a constant temp. bath. After 15 min. remove the stopper and introduce quickly 10 ml of saturated potassium carbonate soln. (90 g per 100 ml). Replace the stopper and connect the tube to the receiving tube. Add 10 ml of saturated potassium carbonate soln. to the blank, followed by 1 ml of 0·2% urease soln., and connect this tube also to the receiving tube. Aerate the ammonia formed in both tubes into the boric acid soln. by passing about 75 litres of air, freed from ammonia by passage through 5% sulphuric acid, through the apparatus. To facilitate the determination of the end-point, prepare a control soln. from 25 ml of 4% boric acid soln. and 25 ml of water and add 2 drops of bromocresol green soln. Titrate the solns. in the receiving tubes with 0·01 *N* sulphuric acid or hydrochloric acid until the colour approaches that of the control, then dilute the solns. to 50 ml and continue the titration until the colour of the soln. matches that of the control. Sumner units per mg

$$= \frac{0{\cdot}0467 \times C \text{ (acid minus blank)}}{\text{mg}},$$

where C is a factor depending on the temperature.

Colorimetric timing method—This method is based on the observation, shown to apply to urease, that enzyme concn. is inversely proportional to the time required for a given amount of product to form. The urease is allowed to act on urea in a phosphate buffer soln. to form ammonium carbonate, which reacts with the acid phosphate in the buffer, thereby increasing the *p*H. The time required to attain a definite *p*H is inversely proportional to the concn. of enzyme. Put 5 ml of buffered urea soln., *p*H 6·7 (3 g of urea in 10 ml of *M* NaH_2PO_4, 10 ml of *M* K_2HPO_4 and dilute to 100 ml with water) into a test-tube, and 5 ml of control, *p*H 7·7 (1 ml of *M* NaH_2PO_4 and 7 ml *M* K_2HPO_4 diluted to 80 ml) into a similar tube, and add 2 drops of 0·1% phenol red soln. to each. Immerse the soln. in a standard temp. bath, add 0·5 ml of the urease soln. to be tested (approx. 10%) to each, mix and note the time. Allow the digestion to proceed and note the time taken for the colour of the urea plus enzyme soln. to reach the same red shade as the control. Fifty mg of urease, containing 0·2 unit per mg, required 5 min. at 20° C. If the colours match in less than 2 min. repeat the determination after diluting the urease soln. to V vols. with water. Sumner units per mg = $\frac{\text{Factor} \times V}{\text{time in min.}}$. The factor varies with the temperature and has been determined experimentally from urease preparations of known activity. The original paper includes a table giving the value of this factor at different temps. F. A. R.

Study of Various Procedures for the Estimation of Tryptophan. M. X. Sullivan and W. C. Hess (*J. Biol. Chem.*, 1944, **155**, 441–446)—A modification of the procedure of May and Rose (*J. Biol. Chem.*, 1922, **54**, 213), in which the development of the blue colour with *p*-dimethylaminobenzaldehyde is accelerated by addition of hydrogen peroxide, gave a value of 2·4% for the apparent tryptophan content of casein. A further modification of the method, due to Bates (*J. Biol. Chem.*, 1937, **119**, vii), in which sodium nitrate is used as accelerator, gave a value of only 1·25%. Even lower values have been reported by other workers. It has now been found that the variable values are due to the fact that the colour develops more quickly with free tryptophan than with unhydrolysed casein, and also to the relative instability of the tryptophan colour as compared with the casein colour. Low or high values will therefore be obtained according as the comparison is made at the maximum readings of the tryptophan standard and the casein soln., or at the later relatively stable levels. Thus when hydrogen peroxide was used as accelerator, and the maximum colours of the sample and the standard were compared, a value of 1·15% was found for the tryptophan content. Bates's method gave an almost identical result (1·17%): Treat 50 mg of casein in 2 ml of 0·1 *N* sodium hydroxide with 0·5 ml of *p*-dimethylaminobenzaldehyde reagent, 2 mg of sodium nitrate and 25 ml of conc. hydrochloric acid, and, after it has stood for 15 to 20 min., dilute the soln. to 50 ml with 17·5% hydrochloric acid (instead of with water as in the original method). Shaw and McFarlane's method (*Canad. J. Res., Sect. B.*, 1938, **16**, 361) gave a value of 1·26%; To 1 ml of a 0·5% soln. of casein in *N* sodium hydroxide and to a standard tryptophan soln., containing 0·1 mg, add, after cooling them in ice water, 0·5 ml of freshly prepared glyoxylic acid soln. and 0·4 ml of 0·4 *M* copper sulphate soln., and water to 3 ml. Cool the mixtures in ice, add 5 ml of conc. sulphuric acid, 0·5 ml at a time and, after 10 min., heat them for 5 min. in a boiling water-bath. Cool, dilute to 10 ml with approx. 65% sulphuric acid, and deduct from the readings the colour of a casein blank in which the glyoxylic acid has been omitted. The method of Folin and Ciocalteu (*J. Biol. Chem.*, 1927, **73**, 627) gave a value of 1·28%. Accordingly the tryptophan content of casein is assumed to be 1·2 to 1·3%. In view of the good agreement obtained by these four methods, other proteins were analysed by the Bates method, with the following results: squash seed globulin 1·69, egg albumin 1·23, pepsin, 2·25%. F. A. R.

Thiamine [Aneurine] Determination. M. A. Eppright and R. J. Williams (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 576–579)—The following procedures have been compared:—(1) The yeast-growth method described by Williams, McMahan and Eakin (*Univ. Texas. Pub.*, 1941, **4137**, 31), measuring the yeast growth turbidimetrically. (2) The yeast fermentation procedure described by Schultz, Atkin and Frey (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 37), using a commercial fermentometer. (3) The thiochrome method of Hennessy (*Cereal Chemist's Bull.*, 1942, **2**, No. 2) measuring the fluorescence with a commercial fluorophotometer. The thiochrome method appears to give satisfactory results for natural (*e.g.*, plant and animal tissues) and processed (*e.g.*, roasted pea-nuts and yeast extract) materials. Results on extracts, however, are low in comparison with those by the other two methods owing to the presence of substances which prevent quantitative adsorption of the vitamin on an activated column. The yeast-growth method gives the highest results (for processed materials the results are valueless), but the specificity of this method can be increased by the use of an adsorption technique which separates thiamine from other materials active in yeast growth. This modification is not suitable for wheat products. Results obtained by the yeast fermentation method agree satisfactorily with thiochrome results. The removal of the excess of sulphite used in this method by the addition of hydrogen peroxide appears partially successful; attempts to eliminate the

sulphite treatment by a modification involving adsorption were wholly unsuccessful. Results on alkali-treated samples determined by the three methods differ widely. C. F. P.

Photometric Destruction of Riboflavin in Milk, and Losses during Processing. J. A. Ziegler and N. B. Keevil (*J. Biol. Chem.*, 1944, **155**, 605–606)—The riboflavin in milk is known to be rapidly destroyed by sunlight, and it was therefore considered of importance to ascertain whether riboflavin was similarly lost during pasteurisation or on irradiation to increase the vitamin D content. Riboflavin determinations were made, before and after processing, by the microbiological method; recoveries of known amounts of riboflavin added to milk were always within 94 to 102% of the theoretical. The results indicated the loss of 9 to 16% during pasteurisation, 5 to 8% during irradiation, and 3 to 5% during bottling and in the brief storage prior to delivery. F. A. R.

Estimation of Pyridine Content of Pyridine-Acetic Acid Mixture used in Riboflavin Determination. J. H. Lanning and C. A. Roszmann (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 583–584)—In the method of Conner and Staub (*Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 385; ANALYST, 1941, **66**, 504) for determination of riboflavin a mixture of pyridine and acetic acid is used to elute the riboflavin from the adsorbent. When a number of determinations are made much of the used mixture is collected, and the pyridine (together with water and acetic acid) may be recovered by distillation, but, unless the concn. of these components is known, the mixture is worthless. The acetic acid content is readily determined by titration, and it was found that the pyridine could be separated by means of strong sodium hydroxide soln. After distillation of the mixture pour a 20-ml portion of the distillate into a graduated 25-ml glass-stoppered cylinder, add 5 ml of 20 *N* sodium hydroxide, shake vigorously, leave for 15 min. and note the vol. of the upper layer. The pyridine thus recovered is not pure, and to determine the relation between the crude pyridine recovered and that originally present in the mixture, two series of mixtures were prepared with reagent grade pyridine (b.p. 114–116° C.) and water and acetic acid. The first series consisted of mixtures of pyridine and water differing in pyridine content. The second series consisted of mixtures of pyridine, water and acetic acid, varying in pyridine content but with a constant acetic acid concn. of 0·3 *N*. The mixtures were treated as already described and also with the substitution of 15·3 *N* sodium hydroxide for 20 *N* sodium hydroxide. The results showed that when the concn. of acetic acid was 0·3 *N* the amount of crude pyridine recovered was, for all practical purposes, the same as if no acid was present. When the concn. of the sodium hydroxide was reduced, measurable differences were observed, hence the sodium hydroxide soln. should be maintained at *ca.* 20 *N*. By plotting the vol. of crude pyridine recovered (ordinate) against the pyridine content of the original mixture, a curve was obtained from which the original pyridine content of an unknown mixture could be read from the apparent recovered pyridine. Since the curve is practically linear, the following co-ordinates selected from the results are sufficient to define it, the first co-ordinate being the abscissa (% of pyridine in the original mixture)—(16·1, 19·0); (20·1, 23·8); (24·1, 29·0) A. O. J.

Chemical Determination of the Tocopherols in Distilled Concentrates. H. W. Rawlings (*Oil and Soap*, 1944, **21**, 251)—The method of Emmerie and Engel (*Rec. trav. chim.*, 1938, **57**, 1351) has been modified by carrying out the reactions in absence of light and by using hydroquinone as a basic standard. *Method*—Transfer a weighed sample of the vitamin E bearing substance to a graduated flask with abs. alcohol, previously distilled from potassium hydroxide and potassium permanganate, and dilute the soln. so that finally it may be expected to contain between 0·05 and 0·3 mg of tocopherol per ml. Transfer 1 ml of this dilution to a 50-ml opaque bottle, add 1 ml of a 0·1% soln. of ferric chloride ($6H_2O$) in redistilled abs. alcohol and 1 ml of a 0·25% soln. of $\alpha\alpha'$-dipyridyl in redistilled abs. alcohol, dilute to 25 ml with redistilled abs. alcohol, close the bottle with a glass stopper and leave for 10 min. from the time of adding the ferric chloride soln. at normal room temp. Prepare a blank consisting of 1 ml of ferric chloride soln., 1 ml of $\alpha\alpha'$-dipyridyl soln. and 23 ml of redistilled abs. alcohol and use this soln. to adjust a photoelectric colorimeter for 100% transmission, using a 520 $m\mu$ filter. Replace the blank with the test soln. and rapidly note the galvanometer reading; correlate this with the tocopherol content of the soln. under test by means of a graph made with pure tocopherol or with hydroquinone. It is stated that the reaction is substantially complete in 1 min., but more precise results are obtained if the longer period is used. If oxidisable impurities are present in the sample to be assayed, a more reliable reading may be obtained after 1 min. In presence of light the ferric chloride tends to oxidise the abs. alcohol, with the production of a spurious colour. It has been found that use of an opaque bottle is sufficient protection and is more convenient than working in a dark-room. Theoretically, hydroquinone is equiv. to tocopherol for the reduction of ferric iron to ferrous iron, and a graph is reproduced in which the same straight line is obtained on 2-cycle logarithmic paper by plotting the galvanometer reading against equiv. weights of α-tocopherol, of β- and γ-tocopherols and of hydroquinone. This method has been used for the determination of tocopherols in distilled concentrates containing between 15 and 80% and many other products containing as little as 0·5% of tocopherols may be directly assayed, but it is pointed out that the Emmerie and Engel method measures total reducing power and should not be applied to crude materials. J. A.

Extension of the Thunberg Technique for Measurement of Dehydrogenase Activity. A. C. Walker and C. L. A. Schmidt (*J. Biol. Chem.*, 1944, **155**, 683–684)—Dehydrogenase activity is normally measured in presence of excess of substrate by the rate of oxygen uptake in presence of methylene blue (Warburg technique), or by the decolorisation time of methylene-blue in absence of oxygen (Thunberg technique). The latter has certain advantages, but requires the addition of a constant amount of methylene blue and complete removal of oxygen. These disadvantages are avoided in the following method. Seal on to a Pyrex test-tube, 14 × 125 mm, a standard outer ground-glass joint and a side-arm fitted with a glass stopcock in such a position that it will not interfere with the use of the test-tube in the colorimeter. Seal off the arm of a standard inner joint so as to form a small bulb and bend the tube at right angles. Put 1 ml of 5% substrate into the bulb and heat to boiling for a

few sec. Put 5 ml of 0·002% methylene blue soln. into the main tube and boil this also. Cool both tubes to 37° C. and then add 0·5 ml of tissue extract or enzyme preparation and 0·5 ml of codehydrogenase preparation to the main tube. Attach the bulb to the main tube and evacuate the apparatus to a pressure of 40–50 mm of mercury. (It is unnecessary and undesirable to remove all the oxygen.) Close the stopcock, mix the contents of the tubes and insert the main tube in a colorimeter with a No. 66 red filter. After 5 to 15 min., the residual oxygen becomes exhausted and the colour of the methylene blue decreases linearly with time. The rate of decrease of the colorimeter reading is a measure of the dehydrogenase content. Calibrate the apparatus with known amounts of methylene blue dissolved in 7 ml of water. The apparatus has been used successfully for the estimation of glutamic, glucose, succinic, citric and alcohol dehydrogenases in various liver preparations.

F. A. R.

Bacteriological

Preparation of Clarified Malt Wort for Culture Media. J. Tošić and T. K. Walker (*J. Inst. Brewing*, 1944, 295–6)—Malt wort prepared and clarified by the usual method sometimes develops turbidity on standing. To prevent this, mash the malt and maintain it at 67° C. until the wort ceases to give a positive reaction with iodine (*ca.* 1 to 2 hr.). Strain through muslin, boil for 1 hr., filter through a loose pad of absorbent cotton-wool and sterilise in steam at 100° C. on 3 consecutive days. When required for use, add to 100 ml of this stock at 45° to 50° C. 5 ml of egg albumin soln. (1 of powdered egg albumin to 5 of water), steam for 30 min. at 100° C. and leave overnight. Then filter, steam again for 30 min. and again filter, introduce into tubes and sterilise in steam at 100° C. on 3 consecutive days. It is claimed that this method gives a brilliant wort which remains clear on prolonged storage; bacterial growth is detected by the appearance of even slight turbidity.

D. R. W.

***Acetobacter* Infection [in Beer]: *Acetobacter mobile* (N.S.) J. Tošic and T. K. Walker** (*J. Inst. Brewing*, 1944, 296–300)—The micro-organism described was isolated from bottled ale produced in a London brewery. Its characteristics render its allocation to the genus *Acetobacter* in the American classification as well as in the Natural classification of Kluyver and van Niel a matter of no doubt, and it is a species which has not hitherto been described. It produces markedly deleterious effects when inoculated into beers, and its description is therefore a contribution to the study of beer-disease bacteria. In view of its exceptional motility it has been named *Acetobacter mobile*. A detailed description of its morphology and cultural characteristics is given. Very briefly, it is a short bacterium, up to 1·6 μ long by 1·0 μ broad, with tendency to form pairs of cells (a photomicrograph is included); it is Gram-negative and shows capsule formation in 3 days old cultures. On malt wort agar at 25° C. it forms colonies visible to the naked eye in 48 hr., and in malt wort growth is evident after 18 hr.; it will grow in malt worts containing 6% v/v of ab. alcohol and is resistant to hop antiseptic; it produces acid but no gas in malt wort. Its optimum *p*H range for growth is between *p*H 6·0 and 4·0; at *p*H 3·1 growth was evident in 30 hr. and at *p*H 2·6 slight growth developed in 17 days. When grown in yeast water containing 2% w/w of acetic acid 28·4% of this acid was utilised in 14 days at 25° C. It does not liquefy gelatin, gives no Voges-Proskauer reaction but gives a positive catalase reaction.

D. R. W.

Agricultural

Determination of Extractable Gossypol in Cottonseed and Cottonseed Meal. C. H. Boatner, M. Caravella and L. Kyame (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 566–572)—Gossypol reacts with antimony trichloride in chloroform to form a soluble red product having an absorption curve with a broad max. at 510–520 $m\mu$. The magnitude of the extinction at the max. is directly proportional to the concn. of gossypol. The absorption curves of the antimony chloride compound with extracts of cottonseed differ from those obtained with pure gossypol, but it was found that the reaction product exhibits initially an absorption curve identical with that obtained with pure gossypol, the reaction evidently taking place more rapidly with gossypol than with the interfering substances. The orange pigment in some hydraulic press-cake meals and in some raw cotton-seed can be converted into gossypol by treating the extract with conc. hydrochloric acid. The absorption curve for pure gossypol has two maxima, a broad one in the visible range at 510–520 $m\mu$ and a sharper one in the near ultra-violet at 380 $m\mu$ and a minimum at 430 $m\mu$. At these wavelengths a linear relation exists between the extinction coefficient and the concn. of gossypol for concns. ranging from 0·004 to 0·016 g per 100 ml of soln. The existence of these three clearly defined points on the curve enables the specificity of the reaction for gossypol in cottonseed extracts to be established, the value of the ratio of the extinction ($\log_{10} I_0/I$) at 520 $m\mu$ to the extinction at 430 $m\mu$ being 2·68 ± 0·23 and the ratio of the extinction at 520 $m\mu$ to the extinction at 380 $m\mu$ being 1·22 ± 0·07 for pure gossypol in the antimony trichloride reaction. Expts. to determine the conditions under which gossypol is extracted from cottonseed showed that complete extraction from ground seed or hydraulic pressed meal can be effected by simple equilibration with chloroform in 2 hr. if the amount of orange pigment present is negligible, and in 24 hr. if there is a considerable concn. of the pigment. Ether may also be used, but very erratic results are obtained, with ether or chloroform, if the seed has previously been defatted with light petroleum (Skellysolve F), despite published results that no gossypol can be detected in the petroleum extracts. It is apparent that accurate results can be obtained only by extraction of untreated seed or meal with chloroform or ether. Treat a weighed sample of ground cottonseed kernels or meal with a measured vol. of chloroform in a stoppered flask for 24 hr. with occasional shaking. Withdraw a filtered aliquot by means of a pipette tipped with a cottonwool plug or a sintered glass suction funnel, shake it with conc. hydrochloric acid (10 drops per 5 ml) and allow the mixture to stand for 24 hr. Pipette 1 ml of the extract into a glass-stoppered absorption cell, add 1 drop of acetic anhydride (to remove moisture haze) and 5 ml of a sat. soln. of antimony trichloride prepared by washing 30 g of the anhydrous salt with chloroform, adding 100 ml of chloroform, warming the suspension and allowing it to cool to room temp. Within 10 to 40 min. after adding the reagent measure the transmission of the liquid against that

of a blank soln. prepared with 1 ml of chloroform in place of the sample. The concn. of gossypol (%) in the original material is given by $\frac{\log I_0/I \times V/W}{E^{1\%}}$ where $\log I_0/I$ is the extinction at 520 $m\mu$ of the test soln., I_0 is the transmission of the reagent blank at 520 $m\mu$, and V is the vol. of solvent used to extract a wt. W of the sample. $E^{1\%}$ is the extinction coefficient of gossypol at 510–520 $m\mu$ calculated for 1% gossypol in the original soln. before reaction with antimony trichloride. With the absorption cell of depth 1·27 cm used in the Coleman double monochromator spectrophotometer

$$E^{1\%}_{1\cdot27} = 65\cdot5 \pm 1\cdot9.$$ A. O. J.

Moisture Content of Distillers' By-products. W. Baumgarten, L. Stone and C. S. Boruff (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 425–430)—The materials investigated were distillers' dried grains, *i.e.*, the dried residue obtained in the manufacture of alcohol and distilled liquors after removal of the alconol, and distillers' dried solubles, *i.e.*, the dried water-soluble portion of the residue obtained by removing the coarse particles and centrifuging. The moisture contents were of the order of 2 to 5%. The original raw material consisted of rye and barley malt with wheat or maize or with both. Three drying processes were examined, *viz.*, drying in air at elevated temp., drying *in vacuo*, with or without drying agents, and the Bidwell–Sterling procedure ("*Methods of Analysis of the A.O.A.C.*," 1940, 353). Higher drying temp. always led to higher results, with decomposition and darkening of the sample after long periods of drying. In vacuum drying the initial loss of wt. is great, but curves indicating the progressive loss flatten out after several hr. Where no decomposition was observed, drying *in vacuo* had to be prolonged for several hundred hr. before drying for an additional 72 hr. produced no further change in wt. Determination of moisture by the Bidwell–Sterling distillation procedure has similar limitations. The use of higher boiling solvents increases the rate of distillation of the water but may cause decomposition. To compare the effects of various solvents in causing decomposition of dried distillers' solubles, the *p*H of the aqueous distillate was determined as well as its nitrogen content, with the following results, the first figure being the *p*H and the second the nitrogen content of the distillate (mg per 100 g of sample)—Chloroform, 4·5, 0·14; carbon tetrachloride, 4·0, 0·62; benzene, 4·0, 0·30; toluene, 9·4, 19·0; xylene, 8·6, 23·0. To determine the nitrogen, the distillate was evaporated to dryness with conc. sulphuric acid, the residue was decolorised with conc. hydrogen peroxide, and the colour formed with Nessler's reagent was measured in a Coleman spectrophotometer. Distillers' dried grains with solubles are more resistant to decomposition than are dried solubles. A sample of wet syrup (*i.e.*, evaporated soluble stillage containing *ca.* 35% of solid matter) was distilled with xylene and the distillate appeared to be free from decomposition products. The use of chloroform and carbon tetrachloride as solvents in the distillation procedure has the advantage that the low b.p. reduces the risk of decomposition, and the more complete suspension of the sample in these solvents prevents charring by contact with the sides of the flask and promotes contact between solvent and water. The moisture content of distillers' dried grains with solubles is best determined by drying *in vacuo* at 49° C. over phosphorus pentoxide. Distillation with toluene gave a higher figure and decomposition products appeared in the distillate. Distillation with benzene was not accompanied by decomposition, but the figure was slightly lower than that found with phosphorus pentoxide, the correct figure being probably between the two. Distillation with benzene (12 hr.) is preferable for routine analyses because of the time required for drying *in vacuo* (300 hr.). Comparison of the moisture content of distillers' dried solubles obtained by different methods indicated that drying over phosphorus pentoxide *in vacuo* at 25° C. gives the best results and is recommended as the method for standardisation of dried solubles, but distillation with chloroform in a Bidwell–Sterling apparatus is most satisfactory for laboratory work. No definite conclusion was reached as to the condition responsible for the susceptibility of the products to decomposition. The fact that wet syrup can be distilled with xylene without decomposition, whereas dried solubles show considerable decomposition, may indicate that the heat to which dried solubles are subjected during drying is responsible. A. O. J.

Organic

Sulphuric Acid Extraction in Hydrocarbon Type Analysis. C. C. Allen and H. W. Duckwall (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 558–560)—The use of sulphuric acid extraction to separate unsaturated from saturated hydrocarbons in mixtures has been objected to on the generally valid grounds of inaccuracy due to incomplete removal of unsaturated compounds, solubility of saturated compounds in the acid extract or chemical attack by the acid. Earlier work indicated that a two- or three-step extraction with strong acid in varying proportions might yield linearly related volume and physical constant data and thus simplify analysis. Expts. showed that acid strong enough to remove aromatic compounds completely from a kerosene fraction also reacted with the saturated compounds present, but that the action of the acid on the saturated hydrocarbons could be allowed for by the use of several different ratios of acid to oil and extrapolation to a hypothetical zero acid to oil ratio. The principle underlying the use of sulphuric acid in type analysis (Thomas, Bloch and Hoekstra, *Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 153) is the determination of a sufficient number of physical properties of a mixture before and after reaction with the acid to enable the proximate composition to be calculated by combination of the data. For routine purposes a procedure giving % of paraffins with naphthenes, the paraffin-naphthene ratio, % of olefines with aromatic bodies and an independent determination of olefines may be considered a type analysis. If accurate values of % of saturated hydrocarbons with density and n_D^{20} of the saturated bodies can be obtained by the use of acid, then, with molecular wt. and bromine number determinations on the original mixture, the composition can be expressed by established methods. A single saturated fraction of light kerosene was used in all tests and mixtures were made with xylene, mono- and diamyl benzene, mono- and diamyl naphthalene, cyclohexane and di-isobutylene. The acid used was 101% sulphuric acid containing 4·4% w/w of free sulphur trioxide, and the extractions were made in graduated bottles similar to Stoddard solvent bottles (Amer. Soc. Testing Materials, Specification D484–40), but made to contain 10 ml additional vol. Optical measurements were made with an

Abbé refractometer and density determinations in 2- or 3-ml pycnometer bottles. The effect of acid extraction on the aniline point was also observed. The extractions were made by the method of Thomas, Bloch and Hoekstra (*loc. cit.*) at ice-water temp., three acid-oil ratios being used, *viz.*, 4:1, 3:1 and 2:1. After measurement of the vol. of hydrocarbon absorbed, the extracts were discarded and the raffinates washed with sodium carbonate soln. and dried over sodium carbonate. The density and n_D^{20} of the washed and dried raffinates were determined and extrapolated graphically to a fictitious 0 : 1 acid-oil ratio. The results show that with paraffin-naphthene-benzene or paraffin-naphthene-naphthalene mixtures, where the concn. of aromatic hydrocarbons is 20% or less, the accuracy of the extrapolation method for saturated hydrocarbons is as follows—vol. %, ±0·2%; density, ±0·005; n_D^{20}, ±0·0005; aniline pt., ±0·8° F. When the mixture contains olefines at 12–20% concn. the inaccuracy is approx. doubled. Density and n_D^{20} are approx. vol.-additive properties in higher-boiling hydrocarbon mixtures, hence

$$F_u^v = \frac{d_m - d_s}{d_u - d_s} = \frac{n_m - n_s}{n_u - n_s}$$

where F_u^v is the volume fraction of unsaturated hydrocarbons as determined by extrapolation, d is density, n refractive index, and the subscript letters m, s and u denote that the physical constant is that of the original mixture, the saturated hydrocarbons (as extrapolated) and the unsaturated hydrocarbons respectively. The agreement of calculated and observed values averaged *ca.* ±0·005 for density and *ca.* ±0·004 for n_D^{20}. Calculation of unsaturated aniline pts. was unsatisfactory. From the results it was evident that the accuracy of the extrapolation method is seriously reduced by olefines at high concn. and that large proportions of olefines (above 20%) can make extrapolation indefinite. For hydrocarbon mixtures containing 20% or less of unsaturated compounds it is a safe guide to regard the extrapolation method as failing when a linear continuation of the data to zero acid-oil ratio is not immediately apparent. The method appears to have the disadvantage of giving as unsaturated any substance having at least one unsaturated group per mol. This, however, is not always a disadvantage because the density and n_D^{20} of the extracted unsaturated material can be determined with sufficient accuracy to give a general idea of the nature of the unsaturated bodies, especially if determinations of bromine value and optical dispersion are made as well. A. O. J.

Systematic Procedure for Identification of Synthetic Resins and Plastics. T. P. G. Shaw ((*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 541–549)—The resin is first separated from vehicles, plasticisers, dyes and pigments by a procedure determined for the particular substance by test-tube expts. Thus it may take the form of pptn. of the resin from solution by a non-solvent (*e.g.*, ligroin), Soxhlet extraction in alundum thimbles with suitable solvents, or solution of the resin in a solvent of low viscosity and repeated filtration with the aid of Filter-Cel. Where mixed resins are suspected, fractionation must be attempted by extraction or pptn. methods and the fractions examined separately. The separated resin is fused with sodium and the tests for the elements are applied as in qualitative organic analysis. The acid value and the saponification value are determined by the usual methods. For determining the acetyl value a mixture of pyridine and acetic anhydride is used as acetylating agent. According to the elements present and the ranges of the sap. value and acetyl val. the resins are placed in 8 main groups. The following tests are then systematically applied. Note the degree of solubility of *ca.* 1 g of resin in *ca.* 10 ml of a number of organic solvents. Note the colour obtained in the Liebermann–Storch reaction and the odour when the resin is fused with alkali carbonate. Test for nitrates with diphenylamine. To detect carboxylic esters, treat the resin with 6% fresh alc. potassium hydroxide and a drop of sat. alc. hydroxylamine hydrochloride. Boil and add 1 drop of aq. 1% ferric chloride soln. and just enough 10% hydrochloric acid to dissolve the ferric hydroxide and, cautiously, a few drops in excess. A violet colour indicates carboxylic esters. Fuse the resin, heat strongly and note the odour and presence or absence of distillate. Test for phthalates by heating the resin with phenol and conc. sulphuric acid to produce phenolphthalein, and reverse the test to detect phenolic resins, using phthalic anhydride as the reagent. Phenolic resins may also be detected by the red colour formed when the resin is boiled with Millon's reagent. To detect cumarone-indene resins dissolve *ca.* 0·5 g of resin in 10 ml of chloroform, add 1 ml of glacial acetic acid and 1 ml of 10% bromine soln. in chloroform and leave overnight. A red colour (observed in comparison with a blank test) indicates cumarone resins. Shake 1 ml of the coloured layer with 1 to 2 ml of 0·1 *N* sodium thiosulphate. The colour of the blank will change to a light yellow; a red colour in the test sample is evidence of the presence of cumarone resins of high or medium mol. wt. To detect formaldehyde resins, heat a small piece of resin with 2 ml of 72% sulphuric acid and a few crystals of chromotropic acid in a test-tube for 10 min. in a water-bath at 60°–70° C. A bright violet colour indicates formaldehyde, but a blank test should be made simultaneously to allow for chance contamination with traces of formaldehyde from laboratory air. The xanthoproteic reaction with nitric acid (a yellow colour deepened to orange by ammonia) indicates a phenyl group and identifies certain proteins containing it. It is also shown by some oils and phenolic resins. To detect acetates, treat a small piece of resin on a spot plate with 5% aq. lanthanum nitrate soln. and 1 drop of 0·1 *N* iodine followed by a drop of conc. ammonia. A brown or blue colour indicates acetates or propionates. Aldehydes in acetals are detected by means of a 0·01% aq. soln. of azobenzene phenylhydrazine sulphonic acid. Heat a small piece of resin with 1 ml of reagent and 0·4 ml of sulphuric acid on a steam-bath for 3 min. To the cooled mixture add a few drops of pure methanol, a layer of chloroform and 0·5 ml of conc. hydrochloric acid and shake well. In presence of aldehydes a red to purple colour appears in the chloroform layer. A tabular scheme is given for each main group whereby individual types of resins may be recognised by the results of these tests. A. O. J.

Enzymic Splitting of Starch and the Evaluation of Desizing Materials. H. Rath, D. Keppler and I. Roesling (*Melliand Textilber.*, 1944, **25**, 18–21, 58–64)—The chemistry, mechanism and analytical control of the process are discussed, with special reference to the relative merits of

pancreatic, bacterial and malt amylase preparations. The rate of disappearance of the starch-iodine colour falls off in the above order, and is a rough measure of the dextrinisation rate. Malt amylase produces the slowest drop in the viscosity of starch paste. Since commercial preparations are not pure, the above effects may be masked or modified, *e.g.*, by inhibition of activity due to the presence of adsorbed substrate, certain metal ions (*e.g.*, copper, zinc, mercury, cobalt, nickel) and residues on the fabric from washing, wetting and bleaching agents. The following modification of the iodine method is preferred to determinations of the fall in viscosity or the increase in copper-reducing power of a starch soln., for evaluating desizing materials. Make 200 g of arrowroot starch into a paste with 750 ml of water, on a water-bath for 30 min., cool the paste and dilute it to 1 litre. Pipette out 100 ml, heat to 53–54° C., and add 10 ml of a soln. of a standard desizing agent (2 g in 500 ml of hard water); note the time. After each subsequent 2 min., pipette out 1 ml, and transfer it to a test-tube containing 15 ml of hard water and 2 ml of a mixture of equal vols. of 0·01 *N* iodine and 0·5% hydrochloric acid. At once seal the tubes, and, when the colours are no longer changing, select as standards 10 tubes covering the colour range blue-violet-red-yellow. Repeat this procedure, using the same starch soln., with a soln. of the sample to be tested, the concn. of which is adjusted until a similar set of 10 tubes is obtained for the same time range. The sample and standard may then be compared directly. Alternatively, in order to test the rate of action as distinct from the total activity, compare the curves for the sample and standard obtained by plotting the colour-stage against the time required to attain that stage. When it is desired to make the test on the actual sized fabric, immerse 5 g of fabric in 200 ml of a dil. standard soln. of the desizing agent, remove small portions at intervals, wash them successively in hot and cold water, immerse in dil. iodine soln. for 30 sec., wash with cold water, squeeze and dry. Note the colours produced after various times, and compare with the effects produced by a standard desizing preparation. The results are less accurate than when a starch paste is used. J. G.

Characteristic Reaction of "Antique Purple" on Fibres. L. A. Driessen (*Melliand Textilber.*, 1944, **25**, 66)—Heat the fibre at 60–65° C. for 20 min. with 5 ml of 1·0 *N* ammonia (or 1·0 *N* sodium hydroxide with cotton fibres) and 0·25 g of sodium hydrosulphite. This reduces the dyestuff to the yellow leuco-compound. Place the fibre, while it is still moist, between 2 glass plates, cover one-half of it, and expose the other to sunlight or to ultra-violet light for 15–20 min. The reaction is complete when the exposed part has developed a strong fluorescence. Wash and dry the fibre and expose it to air. If "antique purple" (6:6′-dibromo indigo) or the corresponding chloro- or iodo-compound is present, the exposed and unexposed parts gradually change to blue and violet, respectively. J. G.

Inorganic

Routine Turbidimetric Determination of Tin in Soap. G. M. Compeau and E. W. Blank (*Oil and Soap*, 1944, **21**, 275)—Methods for determining tin in soaps to which tin salts have been added to inhibit the development of rancidity are subject to error. Ashing in presence of magnesium nitrate prevents losses due to volatilisation and oxidation (*cf.* Halliday, *Oil and Soap*, 1937, **14**, 103; Cassil, *J. Assoc. Agric. Chem.*, 1937, **20**, 173), while it is convenient to determine the metal in the ash turbidimetrically as stannic sulphide. *Method*—Add 10 ml of a saturated alcoholic soln. of magnesium nitrate hexahydrate to 10 ± 0·1 g of the sample contained in a porcelain dish, 85 mm in diam., containing a short stout glass rod, and warm on a hot plate over an asbestos sheet with constant stirring until homogeneous. Evaporate the mixture to dryness, breaking with the rod any bubbles which form, and gradually raise the temperature until the mass is thoroughly dry. With some samples it may be necessary to use a Bunsen flame to ensure complete carbonisation. Heat the dish in a muffle furnace below 600° C. for 2 hr., meanwhile disintegrating and mixing the ash, which should be dark gray in colour. Allow to cool, cautiously add 25 ml of 1 : 1 nitric acid and warm to dissolve the soluble material; filter into a 125-ml conical flask marked at 100 ml, wash the carbon residue twice with hot water, add to the filtrate 40% sodium hydroxide soln. until magnesium hydroxide begins to ppt., then add 2 ml of conc. hydrochloric acid and 6 drops of strong bromine water. Dilute to 100 ml with water, pass in hydrogen sulphide for 10 min. and leave for about 15 min. Prepare standards in a similar manner from a solution of pure tin in hydrochloric acid treated with strong bromine water to a permanent yellow and containing 1 mg of Sn per ml; these should contain 4 g of magnesium nitrate hexahydrate and 5 ml of 1 : 1 nitric acid. A blank determination should be conducted on the reagents. A table is reproduced which indicates that recovery of added tin from soaps is satisfactory. It is pointed out that the mass must be thoroughly charred before insertion in the muffle, since it will otherwise take fire, with consequent loss of tin. The method will determine tin present in soap in concns. between 0·01 and 0·25%. J. A.

Microchemical

Improved Phenolphthalin Technique for the Micro-determination of Cyanide. W. A. Robbie (*Arch. Biochem.*, 1944, **5**, 49–58)—The method depends upon the oxidation of phenolphthalin to phenolphthalein by cyanide and copper. Mix 0·5 ml of a 1% soln. of phenolphthalin in absolute ethanol with 99·5 ml of 0·01% $CuSO_4 5H_2O$ soln. This reagent is stable for a day or more at room temp. and will keep in a refrigerator for months without change. Put 1 ml of 0·05% potassium hydroxide soln. in the micro cell of a photoelectric colorimeter, add 2 ml of the cyanide soln. to be tested and then 1 ml of the reagent. Mix the solns. by inverting the tube several times, and measure the colour immediately in the photoelectric colorimeter, using a No. 548 narrow range filter. Calculate the amount of cyanide in the unknown from a standard curve prepared in the same manner from cyanide solns. of known concn. Solns. which are 0·00015 *M* or less give a colour that is within the optimal range for photoelectric determination. Stronger solns. should be diluted before test. When the soln. to be tested is buffered, it may be necessary to vary the amount of potassium hydroxide to overcome the buffer effect. With sea water an excess of potassium hydroxide must be avoided, as otherwise insoluble hydroxide ppts. may form. With the aid of this method as little as

0·5 μg of cyanide (or 1 part in 4 millions can be determined with an error of not more than 3%. Only 2 ml of soln. are necessary. F. A. R.

Physical Methods, Apparatus, etc.

Photometric Determination of Phosphorus in Limestone. J. A. Brabson, J. H. Karchmer and M. S. Katz (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 553–554)—The method is based on the phosphovanado-molybdate colour reaction and is suitable for amounts of phosphorus ranging from 0·002 to 0·4% of P_2O_5. Calcium and iron, in the quantities encountered in limestone, cause no interference and organic matter is destroyed by initial calcination. Any simple commercial filter photometer can be used. *Reagents*—Ammonium vanadate soln.: dissolve 2·35 g in *ca.* 400 ml of hot water, add 14 ml of 72% perchloric acid, cool, and dilute to 1 litre. Ammonium molybdate soln.: Dissolve 100 g of molybdic acid (85%) in 300 ml of water and 80 ml of ammonium hydroxide, filter, boil the filtrate for 20 min., cool and dilute to 1 litre. Standard phosphorus soln.: 0·4263 g of diammonium hydrogen phosphate per litre. Check the phosphorus content gravimetrically (1 ml = 0·1 mg of P). Calibration curve: Transfer aliquot portions of the standard phosphorus soln. to 100-ml volumetric flasks containing 17 ml of 72% perchloric acid. Add 10 ml of ammonium vanadate soln., dilute to 75 ml, and cool to about 25° C. Add 7·5 ml of ammonium molybdate soln. while swirling the contents of the flask, dilute to 100 ml, mix well and leave for 30 min. Measure the absorption on a photometer, using a blue filter. *Method*—Ignite a suitable weight of limestone in porcelain for 30 min. at 900° C. (for large amounts of organic matter ignite first for 15 min. at 500° C.). Add 20 ml of water to the ignited residue and dissolve in the following proportions of perchloric acid—0·5 g in 18 ml, 1·0 g in 19 ml, 2·0 g in 20 ml, 5·0 g in 25 ml. Evaporate to fuming, cover the beaker and continue fuming for a further 5 min. Cool slightly and add 10 ml of ammonium vanadate soln. Rinse down the sides of the beaker with a little water, cool to room temp. and filter into a 100-ml flask. Wash the beaker and paper 3 times, cool the filtrate to 25° C., add, with constant agitation, 7·5 ml of ammonium molybdate soln. and dilute to the mark. Leave for 30 min. and measure the absorption as before. Results by this method on Bureau of Standards samples gave good agreement with certificated values. C. F. P.

Photometric Determination of Silica in Condensed Steam in Presence of Phosphates. F. G. Straub and H. A. Grabowski (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 574–575)—The method is based on absorption measurements of the molybdenum blue colour developed by reduction of the yellow silicomolybdate complex, and is particularly suitable for amounts of silica ranging from 0·02 to 2·0 p.p.m. The reducing agent is 1-amino-2-naphthol-4-sulphonic acid; oxalic acid is added to prevent interference by phosphates. Only colourless solns. free from organic matter have been tested. *Reducing agent*: Dissolve 30 g of sodium bisulphite and 1 g of sodium sulphite in 200 ml of water and add 0·5 g of 1-amino-2-naphthol-4-sulphonic acid; warm gently to complete solution. *Method*—Mix 1 ml of hydrochloric acid (1+1) and 2 ml of ammonium molybdate soln. (10%) in rapid succession with 50 ml of the sample. After 5 min. add and mix 1·5 ml of oxalic acid soln. (10%) and 2 ml of the reducing reagent. After 1 min. measure the absorption on a suitable instrument at $\lambda = 700\ m\mu$. Prepare a calibration curve, using a standard silicate soln. The times given must be followed closely. C. F. P.

Polarographic Use of Organic Reagents. Magnesium with 8-Hydroxyquinoline. K. G. Stone and N. H. Furman (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 596–598)—A soln. of 8-hydroxyquinoline in an ammonia-ammonium chloride buffer of *p*H 10 gives a polarograph wave of which the height is proportional to the oxine concn., while the half-wave potential (1.39 V vs S.C.E.) is independent of the concn. Thus if a standard oxine soln. is treated with a magnesium soln. the residual oxine concn. may be determined polarographically and a measure of the added magnesium obtained. The method, as described, gives a straight-line calibration curve for amounts of magnesium from 5 to 200 μg in 25 ml. The magnesium ppt. need not be filtered out before recording the polarogram; the error due to the solubility of the ppt. is less than 1 μg in 25 ml. *Reagents*—Oxine soln., 0·5% in 5% ethyl alcohol. This soln. decomposes slowly and regular blanks are necessary. Buffer soln.: 0·25 *M* in total ammonia and approx. 0·036 *M* in ammonium chloride, adjusted to *p*H 10. *Method*—Take 5 ml of oxine soln., 10 ml of buffer soln. and a suitable amount of the unknown magnesium soln. (or standard magnesium soln. when calibrating). Dilute to 25 ml, shake frequently for 1–2 hr., bubble pure nitrogen through the soln. for 15 min. and record the polarogram. Other common cations, except aluminium, titanium and calcium, which give a ppt. with oxine under these conditions, may be removed by prior electrolysis in a Melaven cell. Ammonium tartrate (25–50 mg) will prevent interference from 150 μg of aluminium. Titanium must be removed as hydroxide. As much as 0·5 mg of calcium in 25 ml of soln. does not interfere. The method has been found satisfactory for the examination of tapwater and plant materials, *e.g.*, tobacco. L. A. D.

Purification of Solvents for Absorption Spectroscopy. M. M. Graff, R. T. O'Connor and E. L. Skau (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 556–557)—The ultra-violet transparency of natural or synthetic hydrocarbon solvents is improved by passing the liquid down a 120 cm × 4 cm column packed with silica gel (a proprietary American product is mentioned). Silica gel is preferred to other adsorbents because the column allows the solvent to flow through rapidly, but decolorising carbon and activated charcoal are also effective. The gel can be re-activated for most purposes by washing with water and heating for 24 hr. at 325° C. in a stream of air. The column filling is covered by a glass-wool plug and the top is not allowed to run dry until all the solvent has been added. About 1 to 1·5 litres of cyclohexane, 2 litres of *n*-heptane or 4 litres of *iso*octane can be treated with 400 g of silica gel. L. A. D.

Study of Instruments for the Measurement of Opacity of Paper. Institute of Paper Chemistry (*Paper Trade J.*, 1944, **119**, 26th Oct., 27–28, 30)—This paper deals principally with the calibration of the Bausch and Lomb Opacimeter, but a useful method for the preparation of a standard white is described. Cut pure magnesium ribbon

into *ca.* 8-in. strips, hold a strip between pliers in the flame of a Bunsen burner, and hold a flat-surfaced block of magnesium carbonate (ordinary grade) at about 4 in. above the tip of the flame in the rising stream of magnesium oxide smoke. The deposition should be carried out under a hood, and the operator should wear goggles with dark glasses. The strips are burned until a black dot, which should previously have been made on the surface of the block (near to, but not in, the area to be used in the opacimeter), is no longer visible. The final surface should not be covered with glass or compressed, as this introduces an error. The standard alters in reflectance on standing, and when finished with temporarily should be scraped, and resurfaced before use again. J. G.

Review

PHARMACOLOGY. By J. H. GADDUM, Sc.D., M.R.C.S., L.R.C.P. 2nd Ed. Pp. xvi + 460. London: Oxford University Press. 1944. Price 21s.

That the second edition of this work has followed so soon after the first (1940) is substantial evidence of its value. The original plan has been retained, but the incorporation of additional material has extended the book by 53 pages. If the comprehensive title should encourage anyone to hope that pharmacology is only a small department of learning, the expectation will soon be corrected by the preliminary bibliography of other works. In this section it is truly stated that: "The pharmacopoeia cannot always be kept up to date because it is not published often enough," but, rather strangely, there is no allusion to the Addenda.

Without attempting to define his subject, Professor Gaddum has adopted a physiological and biochemical approach, thus clearly demarcating his discourse from the hybrid study of practical therapeutics and pharmacy known in this country as "materia medica." In the United States it would seem that this division is no longer maintained, for it cannot be without significance that Torald Sollmann, in the 6th edition (1943) of his great *Manual of Pharmacology*, alludes to materia medica as an obsolescent title which includes the study of the actions and uses of drugs now generally called pharmacology. Classification is always a problem when writing a treatise concerning drugs, the question being whether the basis of discussion is to be physiological or chemical. In practice the two principles have to share the burden, and Professor Gaddum's modern outlook is reflected by the titles of the first, second, third and fourth chapters which are on Diet: Inorganic Salts and Fats; Vitamins; Hormones of Known Structure; and Hormones of Unknown Structure, respectively. The subject is then carried along for twelve more chapters on strictly physiological lines by extremely interesting and well-illustrated expositions on the Central Nervous System: Stimulants; Narcotics; The Brain; Body Temperature; Sensory Nerves; Motor Nerve Endings; Muscles; The Alimentary Canal; Circulation; Blood; Kidney; Respiration. A return is then made to a predominantly chemical division, the account being continued under the headings: Proteins; Toxic Elements; Drugs which Destroy Life; Chemotherapy: Worms and Protozoa; Chemotherapy: Bacteria. The last and twenty-second chapter on General Pharmacology is largely concerned with the principles of biological assay and contains an engaging account of the application of statistical methods for the assessment of results. Finally, the concluding Key to the Interpretation of Chemical Names will be useful to students and also serve to emphasise the importance of chemistry in medical research.

In the preface to this second edition the author states that references to important papers have sometimes been passed over in favour of more recent but less important publications on the same subject. One ventures to ask if this is really the correct policy to adopt in writing a book intended for students: should not the reader's attention be directed to the discoverers and initiators? Surely they are the people to whom recognition is due. Thus, in connection with the line test for the assay of vitamin D, the originators of the method (McCollum, Simmonds, Shipley and Park, *J. Biol. Chem.*, 1922; **51**, 41) are not mentioned, while the single reference which is given—admittedly to an excellent paper—might convey a false impression as to the date of its inception. Again, ergotoxine was first isolated from ergot by Barger and Carr (*Chem. News*, 1906, **94**, 89), the slightly later work alluded to being concerned with the conversion of ergotoxine into ergotinine. Still, this question of references is matter for debate, and in this instance the answer would seem to be that so good a text warrants more extensive documentation.

Although this volume is so richly packed with information, it is difficult to detect anything approaching to an error. Perhaps Table V, giving the approximate alcohol content of

beverages, is rather optimistic for these times, and indeed the figures would seem to be reminiscent of those dimly distant days before the last war. No doubt there will be something to say about trichloroethylene as a volatile anaesthetic in the next edition. It is stated that chloral hydrate is reduced in the body to trichloroethyl alcohol, which then combines with the glycuronic acid in the liver to form urochloralic acid. This, the generally accepted view, was originally proposed by von Mering (*Z. physiol. Chem.*, 1882, **6**. 480), but it might have been worth while to mention the work of Lehmann and Knoefel (*J. Pharmacol. Exptl. Therap.*, 1938, **63**, 453), who found but little urochloralic acid in the urine after the administration of chloral hydrate, whereas much more was found when trichloroethyl alcohol had been taken. One doubts if it is altogether correct to assert that pilocarpine is not much used, for it is known to be a less irritating miotic than physostigmine and is therefore often preferred in ophthalmological practice.

The author's direct and humorous style is an outstanding feature, and is valuable as an aid to memorising the facts. For example, a laboratory assistant saved himself trouble by making up isotonic solutions with tap-water and, when he took a holiday, Dr. Sidney Ringer made them himself, using distilled water, but the excised hearts refused to beat properly, whence it was discovered that calcium is essential for maintaining the activity of isolated tissues. The caption to Fig. 12, showing the effect of an oestrogen on a cock is characteristic: "Portraits of the same animal before . . . and after the implantation of a tablet of 65 mg of oestrone. His comb has shrunk and his outlook on life has evidently altered." The discussion of the pharmacology of ethyl alcohol affords several diversions, including an observation of its effect on sex which "was neatly summarised by the porter in *Macbeth*: 'It provokes the desire, but it takes away the performance.'" N. L. ALLPORT

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

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

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

Notes on the writing of papers for THE ANALYST

Manuscript.—Papers and Notes should preferably be typewritten.

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

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

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

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

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

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

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

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

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

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

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

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