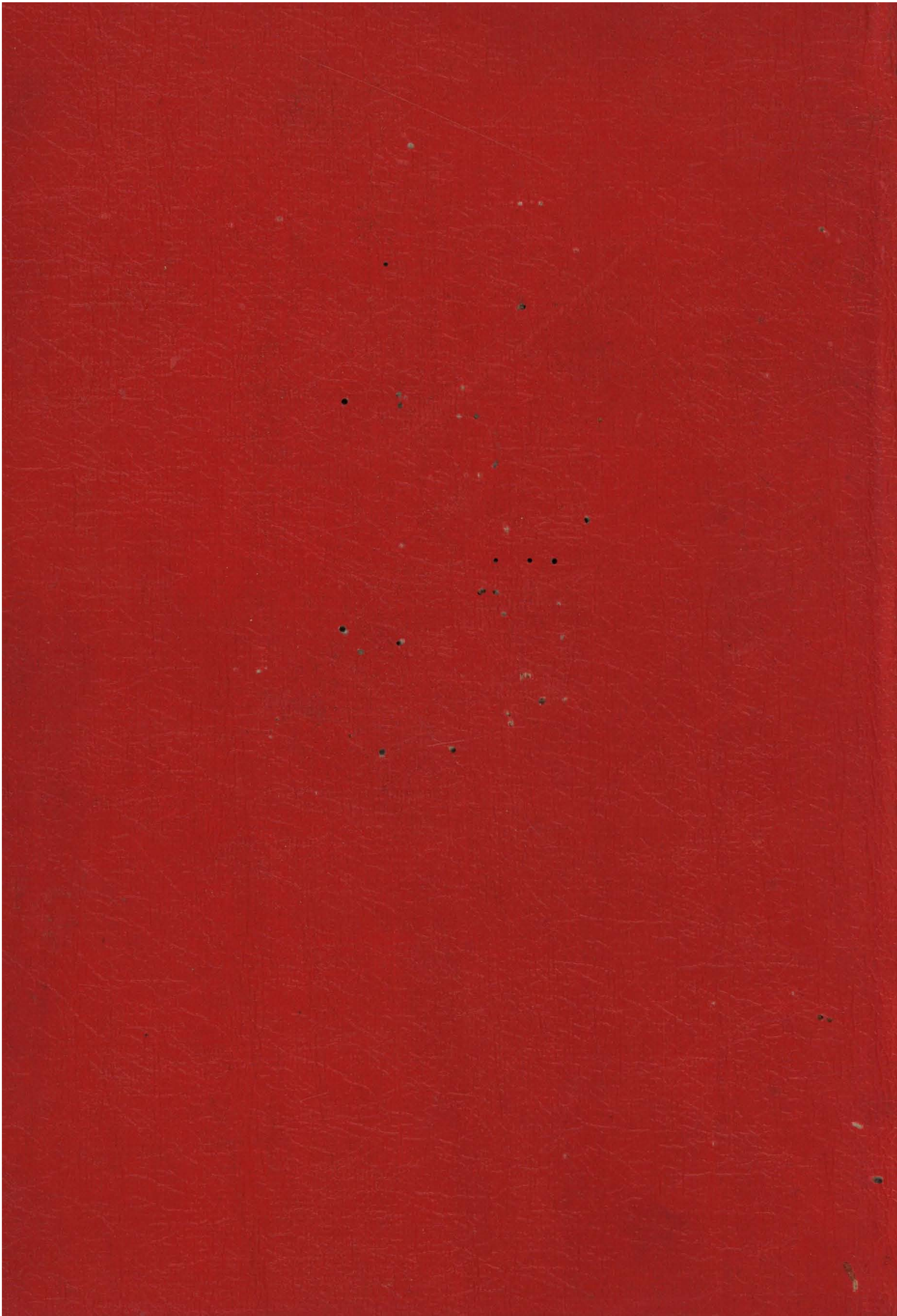

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

A MEETING of the Society was held at 2.15 p.m., on Wednesday, December 6th, at the Chemical Society's Rooms, Burlington House. The President—Professor W. H. Roberts—was in the chair. The following papers were read and discussed:—

"The Examination of Lard," by R. W. Sutton, B.Sc., F.I.C., A. Barraclough, B.Sc., F.I.C., R. Mallinder, B.Sc., F.I.C., and O. Hitchen, B.Sc., F.I.C.; and "The Estimation and Examination of 2-Methyl-1: 4-naphthoquinone," by J. L. Pinder, B.Sc., F.I.C., and J. H. Singer, A.I.C., with introductory remarks on the relationship of 2-methyl-1: 4-naphthoquinone to Vitamin K, by A. L. Bacharach, M.A., F.I.C.

The following candidates have been elected members of the Society:

Ernest Brown, B.Sc. (Lond.), Chemist with experience of analysis of soaps, essential oils and cosmetics.

Alexander Stuart Robertson Inglis, B.Sc. (Edin.), A.I.C., Assistant Chemist in the County Laboratory, Stafford. (*Through the North of England Section.*)

Samuel Gordon Liversedge, F.I.C., Head of the Analytical Department of Messrs. Howards & Sons, Ilford.

Clifford Hanks Robinson, B.A. (Toronto), F.C.I.C., Chief of the Division of Chemistry, Science Service, Canadian Department of Agriculture, Dominion Agricultural Chemist.

Obituary

WILLIAM THOMAS BURGESS

WILLIAM THOMAS BURGESS, a former member of Council and Vice-President of the Society, died at Bedford Park, London, on June 22nd, 1939, at the age of 78.

A native of Brighton, he received a scientific training there, including chemistry, under the late W. Jago, F.I.C. In 1882 he continued his studies at the Royal College of Science, and, in his first year's course, showed his natural aptitude for physics by taking top place in the examination and the Tyndall Prize. In those days Agriculture was given much prominence in the College, and the Associateship could be obtained in that subject. Burgess was selected from among the most

promising chemistry students to act as a demonstrator in a Summer Course in Agriculture, and his deftness at manipulating apparatus and general resourcefulness in experiments was noted by Professor Percy F. Frankland, who proposed that he should be employed as assistant to his father, Sir Edward Frankland, in a private laboratory where the consulting work of both father and son was done. Burgess's acceptance of this post entailed a stoppage of his work at the College. His fellow assistant for a short time was Frederick (later Sir Frederick) Gowland Hopkins, and, for a few years after, James Kear Colwell, now Public Analyst for Finsbury and elsewhere. From 1884 until the death of Sir Edward, in 1899, Burgess was his sole assistant, and, in this capacity, learned all that was known about water supplies and geological strata throughout the country.

On the death of Sir Edward Frankland it was proposed that his official work as "Water Analyst to the Local Government Board" should be transferred to the Government Laboratory. Dr. Thorpe agreed on condition that Burgess would continue the work as a part-time assistant, and a room was placed at his disposal in which to design and lay out the necessary apparatus. Although the greater part of Burgess's time was taken up with this official work, which consisted mainly in regular testing of the water supplied to London by the eight separate companies, his intimate connection with most of the large water undertakings in the country involved reference to him in the conduct of these, and he continued the consultative work for them, first in the laboratory of Dr. Samuel Rideal, and later in his own home. When the Metropolitan Water Board took over, in 1908, the examination of the London water supply, there was not enough official work in the Government Laboratory to keep Burgess employed, and, wishful of devoting his energies entirely to his private work, he left the Laboratory.

His unrivalled knowledge of the water supplies of this country enabled him to continue a sound practice as water consultant until his death. There were few new installations throughout the land where Burgess was not called in for advice either by the authorities or the water engineers.

He devised a simple colour meter for determining the degree of discoloration in water in terms of the Lovibond scale, and he retained his old love for the Frankland combustion method of determining carbon and nitrogen in water, probably later than any other analyst.

He had been a Member of Council, Vice-President and, temporarily, Honorary Treasurer of the Institute of Chemistry, and did much work for the Glass Research Committee during the last war.

Never robust, Burgess kept alive a boyish interest in every new development in chemical and physical apparatus, and his keen appreciation of the value of any novelty, coupled with helpful advice often wrapped up in an anecdote, endeared him to all with whom he came in contact. A. MORE

The Detection and Estimation of Benzedrine

By EDWARD T. ILLING, B.Sc., F.I.C.

BENZEDRINE or β-aminopropylbenzene ($C_9H_{13}N$) is chemically allied to ephedrine and adrenaline. It diminishes gastro-intestinal activity, raises the blood pressure and interferes with sleep, and may cause pronounced psychological changes. When applied to mucous membranes it causes contraction of the blood vessels and shrinkage of the membrane, and is used as an inhalant to free congested nasal passages in coryza. It tends to abolish fatigue, especially in depressed patients. A case of acute aplastic anaemia following self-administration of the drug has been recorded[1]; 190 mg. were taken in the course of 19 days. Severe cardiovascular collapse occurred the day after the last dose. Gradual recovery ensued.

In connection with a case of sudden death due to haemorrhage in the cranium, an endeavour was made to try to prove the absence of benzedrine in the viscera. Very little information about benzedrine could be found, so that it was necessary to make a study of the substance and its general characteristics with a view to working out a satisfactory method of detection and estimation. This paper sets out some of the results of the study.

Benzedrine is a liquid that is slightly soluble in water, more soluble in alcohol, and readily soluble in acids, ether, amyl alcohol, ethyl acetate and chloroform. It is completely volatile at 100° C., and appreciable loss occurs even at low temperatures, as is shown by the following results:

Twenty mg. of benzedrine were dissolved in about 10 ml. of ether, the solvent was evaporated at 70° to 80° C., and the residue was dried in a desiccator; weight, 7 mg. ≡ 65 per cent. loss.

A solution of 7 mg. in ether was evaporated at 38° to 40° C.; residue, 4·2 mg. ≡ 40 per cent. loss.

A solution of 4·2 mg. in ether was evaporated at 33° to 35° C.; residue, 3·3 mg. ≡ 20 per cent. loss.

The acetate also is volatile:

Twenty mg. of acetate were dissolved in 10 ml. of alcohol, the solvent was evaporated below 40° C., and the residue was dried in a desiccator; weight, 12 mg. ≡ 40 per cent. loss.

12 mg. of acetate heated for 15 minutes below 40° C.; residue, 9·5 mg. ≡ 20 per cent. loss.

9·5 mg. of acetate heated for 15 minutes on the water-bath; residue, 4·5 mg. ≡ 50 per cent. loss.

18·8 mg. of acetate heated for 30 minutes in the oven; residue, 5·8 mg.

Heated for a further 1 hour the residue weighed 0·4 mg.

,, ,, ,, ,, 1 ,, ,, ,, ,, nil.

Hence, 19 mg. of acetate were completely volatilised at 98° C. in less than 2 hours.

The hydrochloride, sulphate and tartrate are crystalline salts, are not volatile, and may be heated at 100° C. without decomposition. These salts are soluble in water and alcohol. The sulphate is not so soluble as the chloride and tartrate;

for instance, 100 ml. of alcohol dissolved 60 mg. of sulphate at 17·5° C., whereas more than 50 times this amount of hydrochloride dissolved readily in 100 ml. of alcohol at the same temperature. The salts dissolve readily in 50 per cent. alcohol.

ESTIMATION OF BENZEDRINE IN AQUEOUS SOLUTIONS.—The extraction from solution by means of chloroform depends to some extent upon the degree of alkalinity. Sodium bicarbonate was used for the first trials, but it was found that, although the base could be extracted, a large number of single extractions with chloroform was required. For instance, when 20 mg. of benzedrine sulphate were taken and the alkaline solution was about 50 ml. in volume, a series of extractions with 25, 15, 10 and 10 ml. of chloroform extracted 10·5 mg. of benzedrine sulphate

A second similar series of extractions ,, 7·1 ,, ,, ,, ,,
A third ,, ,, ,, ,, ,, 2·1 ,, ,, ,, ,,

Total extracted 19·7 ,, ,, ,, ,,

When the solution was made alkaline with sodium hydroxide, one series of extractions with chloroform extracted the whole of the benzedrine sulphate. The method adopted is as follows:—The aqueous (generally acid) solution, containing in 50 to 100 ml. the salt of benzedrine, is neutralised approximately with 10 per cent. sodium hydroxide solution, and 1 ml. is added in excess. The solution is extracted with successive quantities (25, 15, 10 and 10 ml.) of chloroform. Each chloroform extract is washed with the same 5 to 10 ml. of water, this wash-water is then shaken with a little chloroform, which is added to the combined extracts, and the whole is washed with 5 ml. of water. The benzedrine is then re-extracted from the chloroform by shaking with four successive portions of 10 ml. of $N/2$ hydrochloric acid. These acid extracts are evaporated to dryness on the water-bath, and finally the residue is dissolved in absolute alcohol, filtered into a tared flask, evaporated to dryness, dried and weighed. In two experiments

(*a*) 20·6 mg. of benzedrine hydrochloride gave 20·6 mg. of the hydrochloride.
(*b*) 20·0 mg. of benzedrine gave 19·5 mg. of benzedrine.

TEST FOR BENZEDRINE.—Benzedrine gives the general reactions for alkaloids, such as those of Meyer and Dragendorff, but there seemed to be no other test available. However, a new test has been found which is quite delicate and has proved useful in confirming the purity of the residues of hydrochloride. Benzedrine gives a purple colour in the modified Mohler's Test already described by me.[2] The conditions are exactly the same as those used in the detection of benzoic acid by this test, and the same precautions have to be taken. The test will detect 0·1 mg. of benzedrine, and the colour given by 1·0 mgm. is a convenient one to match in a volume of 15 ml. The purple colour fades quickly on dilution with water, as does also that given by *m*-diamino-benzoic acid; but it has been found that the colour is quite stable if the solution prepared as follows is used as a diluent:—Twenty ml. of conc. sulphuric acid are added to 40 ml. of water containing 2 g. of potassium nitrate, and the liquid is cooled while 200 ml. of water containing 100 ml. of ammonia (sp.gr. 0·880) are added carefully. Finally, 40 ml. of water containing 0·8 gm. of hydroxylamine hydrochloride are added. This solution

may be used for diluting the final 15 ml. of the test solution to 50 ml. or other convenient volume. From 2 to 3 mg. of benzedrine in a volume of 50 ml. give a colour suitable for matching.

Ephedrine and adrenaline under the same conditions give a deep yellow and a slight yellow colour respectively.

DISTILLATION OF BENZEDRINE.—The benzedrine salt is introduced into a 1-litre round-bottomed flask, fitted as is usual for steam-distillation, and 50 ml. of water, 25 g. of salt and sufficient sodium hydroxide to render the liquid alkaline are added. No condenser is necessary, so that the total condensed distillate is small in volume. The distillate is passed through 10 ml. of *N* hydrochloric acid contained in a conical flask (200 ml.), which is connected by means of a glass tube with the outlet of the distillation flask by rubber tubing; the other end of the glass tube passes through a bung in the neck of the conical flask. From this flask the distillate is passed through a further 10 ml. of *N* hydrochloric acid contained in a distillation flask (100 ml.), and the glass tube leading into this flask is drawn out so as to make a slightly smaller orifice; but this must not be too small, otherwise the pressure required to force the steam through the absorbers would be too great. Sufficient water to form a seal is added to the 10 ml. of hydrochloric acid in each flask, and the steam finally escapes through the side tube of the last flask.

The distillation is continued for about 30 minutes, and the main flask is heated in such a way that a small amount of salt remains undissolved. At the end of the distillation the flame is kept beneath the flask while the steam is stopped and the connection removed, then the outlet of the main flask is disconnected from the conical flask. The combined distillates are evaporated to about 50 ml., transferred to a separator, made alkaline with sodium hydroxide, and extracted as described above.

In two test experiments, in which 20 mg. of benzedrine were taken, 18·7 and 18·9 mg. were recovered.

EXTRACTION FROM VISCERA.—Benzedrine may be separated from viscera by the Stas–Otto process, it being borne in mind that the acetate is volatile, so that if acidification is required at any stage before or during evaporation, tartaric acid should be used. The benzedrine is extracted from the alkaline solution with chloroform. It is suggested that a portion of the chloroform extract should be extracted with hydrochloric acid, as described above, to prove the presence or absence of benzedrine. It is also preferable to render the solution alkaline with sodium bicarbonate when dealing with an unknown specimen and to make further extractions with chloroform.

In one experiment, 3·2 mg. of benzedrine hydrochloride were added to 40 g. of stomach and contents, and 2·8 mg. of hydrochloride were recovered.

Distillation.—The distillation of alkaline mixtures of viscera is practically impossible owing to the excessive frothing. Attempts were made to form the acetate and to distil from an acetic acid solution, but without success. No trace of benzedrine could be detected in the residues.

EXTRACTION FROM URINE.—The following experiment is typical:—The benzedrine (18·9 mg.), as tartrate, was added to 50 ml. of urine, and the mixture was steam-distilled for 1 hour, as previously described. From the distillate 24·5 mg.

of impure hydrochloride (= 19·3 mg. of impure benzedrine) were obtained, and Mohler's test showed that the residue contained 18·0 mg. of pure benzedrine.

BENZEDRINE TABLETS.—Each tablet is stated to contain 5 mg. of β-aminopropyl benzene sulphate. It was found that absolute alcohol did not extract all the benzedrine sulphate (actually only 3·75 mg. per tablet were obtained by extracting 2 tablets), but if the sulphate is converted into the hydrochloride a good extraction is possible.

Thus, 0·892 g. of crushed tablets contained in a 100-ml. conical flask was mixed with a little solid barium chloride and 1 ml. of *N* hydrochloric acid. Twenty-five ml. of absolute alcohol were added, and the mixture was heated on the water-bath for a few minutes. The alcohol was filtered, and the residue was washed with absolute alcohol. All the alcohol was evaporated from the filtrate without allowing the filtrate to evaporate to dryness. The liquid was filtered into a separator, the filter was washed with acidified water, the filtrate was approximately neutralised with 10 per cent. sodium hydroxide solution, and 1 ml. was added in excess. The extractions were made with chloroform, as already described. The amount of hydrochloride obtained was 12·2 mg., which is equivalent to 4·8 mg. of benzedrine sulphate per tablet.

Alcohol of 50 per cent. strength will extract the sulphate completely, and this method is quite satisfactory. The tablets are extracted with 25 ml. of 50 per cent. alcohol and the process described above is carried out, but omitting the treatments with barium chloride and hydrochloric acid. The alcoholic filtrate should be made slightly acid with 2 to 3 ml. of *N*/10 hydrochloric acid. [No more acid should be added during the heating to drive off the alcohol, for if even 1 ml. of 10 per cent. hydrochloric acid is added, no benzedrine will be extracted; moreover, attempts have been made to prepare the solution for extraction by heating the tablets for 1 hour on the water-bath with 25 ml. of water containing 5 ml. of conc. hydrochloric acid, but no trace of benzedrine was obtained in the final residue.]

This process gave 4·9 mg. of benzedrine sulphate per tablet, and Mohler's test showed the residue to be pure.

Distillation.—For this process 1·4162 g. of tablets were taken and the benzedrine was distilled from an alkaline salt solution as already described; 16·8 mg. of benzedrine hydrochloride were obtained, which is equivalent to 4·2 mg. of benzedrine sulphate per tablet. It was found necessary to prolong the distillation to 1 hour, as the benzedrine evidently distils at a slower rate from this solution containing the tablets. In a second experiment, 0·8812 g. of tablets was taken, and the distillation was carried out for 1 hour; 11·4 mg. of hydrochloride were obtained, which is equivalent to 4·55 mg. of benzedrine sulphate per tablet. When 20 mg. of benzedrine were distilled only 18·8 mg. were recovered and, if this is made the basis of a correction, the 4·55 mg. are equivalent to 4·9 mg. of benzedrine sulphate per tablet.

SULPHATE.—One or two tablets, or a portion of crushed and mixed tablets, were heated for 1 hour on the water-bath with 25 ml. of water and 5 ml. of conc. hydrochloric acid. The solution was filtered, the residue was washed thoroughly, and the sulphate was estimated in the filtrate.

The barium sulphate is precipitated slowly and must be allowed to stand some hours, preferably overnight. The precipitation is complete in 3 hours if the tablets are dissolved in 25 ml. of water containing 2 ml. of 10 per cent. sodium hydroxide by heating on the water-bath and then adding 5 ml. of concentrated hydrochloric acid and proceeding as above.

The results of the estimation of benzedrine in the tablets are collected together in the following table:

	Sample taken	Method			
		50 per cent. alcohol mg.	Steam distillation (1 hour) mg.	Determination of sulphate mg.	$BaCl_2$ acid and absolute alcohol mg.
Benzedrine sulphate per tablet	one or more separate tablets	4·9	—	4·5	—
		4·5	—	5·3	—
	20 tablets crushed and portions taken			4·5	
		4·9	4·5	5·3	4·8
		5·2	(corrected figure 4·9)		5·1
		—		5·4	—

In conclusion, I wish to thank Mr. D. R. Wood, F.I.C., for his interest and criticism, and Mr. E. G. Whittle, B.Sc., A.I.C., for his assistance in carrying out some of the determinations.

References

1. I. J. Davies, *Brit. Med. J.*, 1937, II, 615.
2. E. T. Illing, ANALYST, 1932, 57, 225.

THE COUNTY LABORATORIES
TAUNTON, SOMERSET

July 7th, 1939

Examination and Determination of 2-Methyl-1:4-Naphthoquinone

BY J. L. PINDER, B.Sc., F.I.C., AND J. H SINGER, A.I.C.

(*Read at the Meeting, December* 6, 1939)

INTRODUCTION.—The independent discovery, by Dam and by Almquist and their collaborators,[1,2,3,4] of a vitamin necessary for maintenance of normal blood-clotting behaviour in the young chick at first roused interest only among those directly concerned with poultry husbandry. Later investigations, stimulated by Dam's own first clinical trials,[5] pointed to certain important therapeutic uses of the naturally occurring active substance, whose richest sources appear to be certain green leaves, especially those of the chestnut. It is also synthesised by certain bacteria. Intensive research during the last three years has now established

the following facts about the vitamin, which has been called vitamin K ("Koagulationsvitamin"):

(1) It exists naturally in at least two forms; one is the phytyl derivative of 2-methyl-1:4-naphthoquinone and the other is derived from the same quinone and another alcohol, possibly farnesol. The two forms have somewhat different biological activities on young growing chicks.

(2) The blood-clotting time of young chicks deprived of the vitamin rises to many times its normal value, and the animal eventually succumbs to a multiple haemorrhagic condition.

(3) There is no direct evidence for the existence or experimental production of vitamin K deficiency in any animals besides chickens and one or two other species of poultry.

(4) Attempts to use vitamin K preparations on human subjects suffering from obstructive jaundice and certain other conditions associated with prolonged blood-clotting times (specifically with a reduction in plasma prothrombin and an increased "prothrombin time") have given encouraging results. Similar results, though so far only a few, have been claimed from treatment with the synthetic vitamin (phytyl derivative).

(5) 2-Methyl-1:4-naphthoquinone appears to exert on the chick qualitatively and quantitatively the same action as the "natural" forms of vitamin K.

From this it is but a short step to the introduction of the methyl naphthoquinone itself as a therapeutic agent. Evidence is now available of its efficacy.[6] It is probable, therefore, that a method for the detection and estimation of this particular naphthoquinone will be required in the assay and in control of the purity of the substance in bulk and to estimate it in the various preparations in which it may be used; the work described below was undertaken to provide such a method.

Examination and Determination

(*a*) Bulk Supplies.—A common method of preparing 2-methyl-1:4-naphthoquinone is by oxidising methyl-naphthalene with chromic acid, followed by recrystallisation. The quinone itself is decomposed in sunlight, changing from a lemon-yellow colour to a light brown. The end-product is thought to be 2,3,2′,3′-tetrahydro-2,3′-dimethyl-2,3,2′,3′-binaphthalene-1,4,1′,4′-tetrone.[7] A method for assaying the pure substance must permit estimation of the quinone in presence of possible traces of unchanged methyl-naphthalene, and also of traces of the decomposition product. The melting-point (106° C.) is a useful criterion of purity; unchanged methyl-naphthalene has m.p. 32° C., and the m.p. of the tetrone is given as 235° C.[7] The presence of either of these substances would therefore have a considerable effect. Tests must also be applied for excess ash, and for traces of chromium and of solvent. The compound should be stored in brown bottles in the dark, and all tests should be made in the absence of direct sunlight.

The quinone can be estimated by means of its absorption spectrum.

The following chemical assay method was devised after preliminary work had established that under suitable conditions the quinone could be quantitatively

reduced to hydroquinone by means of titanous chloride, the end-point of the reduction being shown by the use of an internal oxidation-reduction indicator.

Determination.—Weigh out accurately about 0·2 g. of the substance, and transfer it to a 200-ml. conical flask. Dissolve it in a mixture of 10 ml. of alcohol (industrial methylated spirit) and 15 ml. of glacial acetic acid. Add 4 g. of anhydrous sodium carbonate and 25 ml. of a 10 per cent. aqueous solution of sodium potassium tartrate (Rochelle salt). Observe these quantities of carbonate and tartrate, as they control the *p*H of the solution and prevent its becoming too acid during the course of the titration. Titrate with an approximately $N/10$ solution of titanous chloride in N hydrochloric acid, using 3 drops of a 0·1 per cent. aqueous solution of potassium indigo-disulphonate as internal oxidation-reduction indicator; a sharp end-point is obtained, the blue dye being changed to the colourless leuco base by the addition of one drop of the titanous chloride solution in excess. Phenosafranine is also a suitable indicator. During the titration pass a steady stream of carbon dioxide through the solution.

The titanous chloride solution is stored under hydrogen or carbon dioxide in the usual way, and is periodically standardised against a ferric iron solution of known strength (1 ml. of $N/10$ titanous chloride $\equiv$ 0·0086 g. $C_{11}H_8O_2$). Suggested limit, not less than 98·5 per cent.

Appearance and Odour.—A lemon-yellow crystalline powder with a faint but characteristic odour. No odour of methyl-naphthalene should be discernible.

Ash.—One g. is ashed in a platinum dish over a Bunsen flame, and finally in the muffle furnace. Suggested limit, not more than 0·15 per cent.

Chromium.—The ash is fused with a small quantity of a mixture of sodium and potassium carbonates and sodium peroxide, the cooled melt is dissolved in water, and the solution is acidified with dilute sulphuric acid and made up to 50 ml. An aliquot part is transferred to a test-tube and diluted with water, and a few drops of diphenyl-carbazide reagent (0·2 per cent. in a mixture of one part of glacial acetic acid and nine parts of alcohol) are added. The violet solution is made up to about 10 ml. and its colour is compared with those produced from known quantities of a standard solution of chromium (0·283 g. of $K_2Cr_2O_7$ in 1 litre: 1 ml. $\equiv$ 0·1 mg. Cr. Suitable dilutions of this stock solution are made as required). Suggested limit, not more than 0·01 per cent.

Loss in Vacuum Desiccator.—One g. is weighed into a small tared glass crystallising dish, which is transferred to a vacuum desiccator The dish is covered with an inverted tin (to exclude light) and dried to constant weight. Suggested limit, not more than 0·2 per cent.

Melting-point.—This should be 104–106° C.

(*b*) Colorimetric Estimation of Small Quantities.—A reliable method for the estimation of small quantities of 2-methyl-1:4-naphthoquinone in tablets and ampoules was also required. A search of the literature revealed several colour tests for quinones; of these, three were investigated.

(1) Raudnitz and Puluj[8] describe a reaction between aldehydes and quinones in acetic acid solution in presence of hydrochloric acid. Many quinones are stated to yield coloured products. The reaction was tried with a variety of aromatic and aliphatic aldehydes and a representative selection

of quinones; in some instances colours were obtained, but further investigation showed that the same colour could be produced by the aldehyde with hydrochloric acid alone. No initial success was forthcoming, and the method was abandoned.

(2) The colour reaction with sodium methylate, proposed by Almquist and Klose[9,10] for natural concentrates of vitamin K, was tried; the results with 2-methyl-1:4-naphthoquinone were inconclusive.

(3) Craven[11] describes colours developing on the addition of ethyl cyanacetate to a solution of a quinone in equal volumes of ammonia (sp.gr. 0·880) and alcohol. This test was tried on a number of quinones; benzoquinone, sodium β-naphthoquinone sulphonate and 2-methyl-1:4-naphthoquinone all yielded strong colours. The mixed reagents alone developed no colour, and the test was therefore investigated at some length.

Preliminary experiments showed that 0·1 mg. of methyl-naphthoquinone in alcoholic solution gave a detectable violet colour in 3 ml. of the ammonia and alcohol mixture with 3 drops of ethyl cyanacetate, whilst 0·5 mg. gave a good colour. The coloured solution on dilution with water underwent little change in tint; it was also fairly stable to dilute alkali. Addition of strong alkali (6 *N* potassium hydroxide solution) produced a stable yellow colour. The original violet colour was destroyed by acid, and was not extracted by ether. The violet colour faded fairly rapidly and was therefore unsuitable for colorimetric estimations, but the yellow colour with alkali gave more promise. Comparison between the colours given by the same quantity of quinone, using in the Hilger (Spekker) absorptiometer the filters giving the maximum readings, showed that the yellow alkali colour was more intense than the original violet. Efforts were then made to find the best conditions for maximum development and stability of the yellow colour, combined with consistency of results.

In the original method 3 ml. of the alcohol and ammonia solution were added to the alcoholic solution of the quinone, followed by 3 drops of ethyl cyanacetate and then 5 to 6 ml. of 6 *N* aqueous potassium hydroxide solution. The boiling-tube was then warmed over a micro burner to incipient boiling of the liquid. The colours produced were not strictly reproducible; this was found to be due to two causes:

(*a*) The initial violet colour, as already indicated, fades fairly rapidly; if the alkali is added shortly after the ethyl cyanacetate, a more intense yellow is obtained than if the alkali is added after the violet has begun to fade.

(*b*) The yellow colour itself is not completely stable, and is more rapidly destroyed on warming than on standing at room temperature. It was found that maximum colour development and reliable duplication of results were obtained by adding the alkali within half a minute of the cyanacetate and by allowing the yellow colour to develop in the cold for about 15 minutes. The solutions are then made up to 50 ml. with water and the absorption intensity is measured.

The effect of the volume of the alcoholic solution of quinone was also investigated, and it was found that, under the conditions outlined above, 0·5 mg. of quinone

gave within experimental error the same reading when dissolved in amounts ranging from 1 to 6 ml. of alcohol.

The following details of the test are therefore proposed:—To 1 to 5 ml. of an alcoholic solution containing 0·4 to 0·8 mg. of the quinone, add 3 ml. of a mixture of equal volumes of alcohol (industrial methylated spirit) and ammonia (sp.gr. 0·880). Add 3 to 5 drops of ethyl cyanacetate and allow the mixture to stand for exactly half a minute. Add 5 ml. of 6 *N* potassium hydroxide solution, mix, and leave for 15 minutes, preferably out of direct sunlight. Dilute to 50 ml. and measure the absorption in the Hilger (Spekker) absorptiometer, using the light blue No. 6 filter. Read off the quantity of quinone from a previously prepared graph.

Application of the Method.—The method may be applied to tablets and pills; these are ground to powder in a mortar, and extracted in the cold with alcohol, the volume being finally adjusted in a graduated flask, and a suitable aliquot part is pipetted out for assay as described above. It may also be applied to solutions in arachis or other oils by shaking a suitable weighed quantity with alcohol, and proceeding with the determination on the resulting emulsion; the alcohol extracts the quinone from the oily solution. Any undissolved oil may be removed after the final dilution with water by shaking with ether, in which the yellow colour is insoluble.

RESULTS

The following table summarises a typical series of results:

Sample	m.p. °C.	Loss in vacuum Per Cent.	Ash Per Cent.	Chromium Per Cent.	Quinone: Volumetric Per Cent.	Quinone: Colorimetric Per Cent.	Spectrographic examination $E_{1cm.}^{1\%}$ 331 $m\mu$	Spectrographic examination $E_{1cm.}^{1\%}$ 250 $m\mu$	Quinone calculated from absorption band at 250 $m\mu$ Per Cent.
A	103–105	0·18	0·18	0·06	97·7	—	143	1110	95
B	104–106	0·19	0·10	0·005	99·1	—	155	1160	99
C	104–106	0·19	0·10	0·005	98·8	—	158	1160	99
D	102–106	—	—	—	93·4	—	145	1090	93
E	125–220	—	—	—	18·3	19	29·5	band obscured	19*

* Calculated from the absorption at 331 $m\mu$.

Special interest attaches to sample E. Part of sample A was transferred to a small glass specimen tube and exposed to the light under laboratory conditions for a period of about six weeks. During this period its colour changed to a light brown. As indicated in the table, it melted over a very wide range. The various assay processes gave results in agreement, indicating that all three methods measure the quinone and are uninfluenced by the presence of the decomposition products.

SUMMARY.—1. A volumetric method for the assay for 2-methyl-1:4-naphthoquinone has been devised.

2. Control tests for likely impurities are described.

3. The spectrographic absorption has been measured.

4. A colorimetric method, capable of application to tablets and ampoules, has been developed for estimating quantities of the order of 0·5 mg.

5. A summary of results obtained by these tests is given.

We have to acknowledge the encouragement and help of Mr. A. F. Lerrigo, B.Sc., F.I.C., during both the execution and the presentation of our experimental work; the co-operation of Miss B. E. Stern, B.Sc., in carrying out all the spectroscopic observations involved; the assistance of Mr. A. L. Bacharach, M.A., F.I.C., in supplying the historical and biochemical data of the introductory paragraphs; and permission to publish this paper given us by the Directors of Glaxo Laboratories Ltd., Greenford.

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Discussion

Mr. L. Eynon asked if 2-methyl-1 : 4-naphthoquinone replaced not only vitamin K_1 but vitamin K_2.

Dr. H. E. Cox said that he found the paper most interesting because it gave a new importance to the naphthoquinones and re-opened the puzzle of the function of substituted naphthoquinones in plant life. He thought that experiments on walnuts, which contain much juglone (5 : hydroxynaphthoquinone), would be worth making. With regard to the titration of methyl naphthoquinone it was interesting to note the effect of the methyl group in reducing the activity of the quinone. He had found that the 2-hydroxy compound was so active a reducing agent that it could not be titrated with titanous chloride and had had to devise a special method for its titration (Analyst, 1938, **63**, 399); when the hydroxyl group was replaced by $-CH_3$ the reducing power was much diminished, though the resulting product was so much more sensitive to light. He hoped the authors would continue their work on other naphthoquinone derivatives.

Mrs. Tritton asked whether 2-methyl-1 : 4-naphthoquinone was as stable as vitamin K. She also asked how the tests were made. Was the substance given by the mouth or by intravenous injection?

Mr. Pinder, replying to Dr. Cox, said that quite a number of batches of 2-methyl-1 : 4-naphthoquinone had been estimated, by means of titanous chloride, with very satisfactory results. He had recently had occasion to try the method on a hydroxyquinone, but the results were unsatisfactory. He had come to the conclusion that the presence of hydroxy groups rendered the method useless.

Mr. BACHARACH thought that methyl-naphthoquinone would replace both vitamin K_1 and vitamin K_2 for all practical purposes. Its stability was about the same as that of vitamin K_1, though it was rather less photo-labile. It was possible that vitamin K_1 might be associated in plant tissues with other naphthoquinone derivatives. This clue to likely sources was, however, not available to Dam and the other earlier investigators—on whose work his (Mr. Bacharach's) table of vitamin K distribution had been based—because the naphthoquinonoid structure of the vitamin had not at that time been made clear. Almost all of the tests on chicks had been carried out by the oral route. In the clinical use of vitamin K_1 or 2-methyl-1 : 4-naphthoquinone, there were three possible routes. First the oral, less practical, because it almost invariably involved simultaneous administration of bile salts; second, intravenous, in diluted aqueous alkalised solution, where this can be prepared; third, intramuscular or subcutaneous, in an oil solution.

The Assay of Mercury

BY J. SANDILANDS, F.I.C.

ALTHOUGH there are numerous methods for the assay of mercury, it is noteworthy that, except in those of Hempel,[1] Personne[2] and a few others, the formation of iodide as a feature of the method has been neglected. This seems strange when one considers the ease with which mercury and iodine combine to form a highly insoluble compound, somewhat refractory to ordinary reagents, and volatile, though not so much so as mercury itself.[3] The readiness with which mercuric iodide forms double salts and complexes may be a reason for this neglect, and, with the exception of the methods in which the element itself is produced in the assay, in nearly every instance the procedure involves the formation of a mercuric salt in some liquid medium from which the sulphide is precipitated and weighed. When the conversion of the material into mercuric salt can be carried out—a tedious process when much organic matter is present—the thiocyanate method,[4] analogous to that of Volhard for halides, is available. This method, however, has distinct limitations.[5] B. Rose[6] found it necessary in his dry assay of mercury to heat the material with quicklime and a finely divided metal, but others have shown that the complete decomposition of all mercury compounds cannot be effected in this way. In consequence of this, such modifications of the method as those of Chism,[7] Holloway (Eschka),[8] Meharg,[9] and H. ter Meulen[10] have been devised.

It should be noted that all wet methods in which prolonged treatment with acid is essential for decomposition are liable to loss, owing to the volatility of the salt from a *dry* part of the reaction vessel. Apparently there is less loss in an acid than in a neutral medium, as is shown in Table I. This gives the results obtained on keeping an aqueous solution of mercuric chloride at a definite temperature for a given time as compared with those obtained with a similar solution in 5 per cent. hydrochloric acid. The solutions were placed in beakers with the same area exposed to the atmosphere. They were heated electrically on a hot plate, and draughts were excluded by having asbestos screens round the

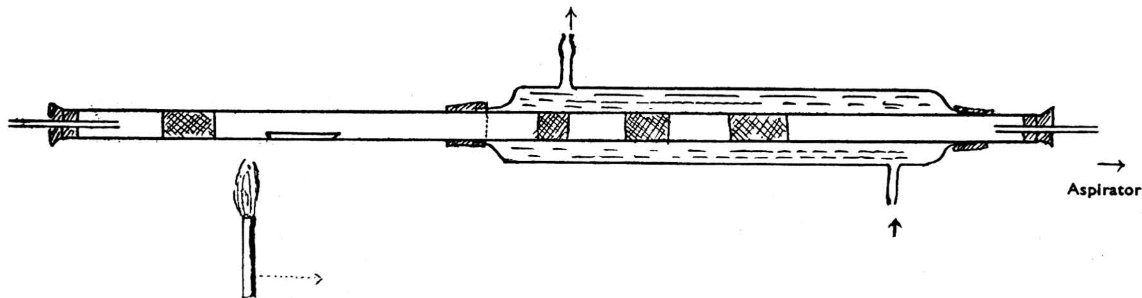
vessels. The temperature selected was 85° C., the duration of each test was 8 hours, and periodically solvent was added to replace any evaporation loss. The results agree closely with those obtained by Sulc,[11] Estere,[12] and Lehmann.[13]

TABLE I

Aqueous Solution of Mercuric Chloride kept at 85° C. for 8 hours.

Pure mercuric chloride taken g.	Mercuric sulphide pptd.	Mercuric chloride equivalent to mercuric sulphide	Difference g.
0·5013	0·3908	0·4681	—0·0332
0·3274	0·2544	0·3046	—0·0228
0·3833	0·2985	0·3578	—0·0256
0·2558	0·1977	0·2367	—0·0191
0·2810	0·2164	0·2593	—0·0262
Five per cent. Hydrochloric Acid Solution of Mercuric Chloride under same conditions			
0·2873	0·2240·	0·2856	—0·0015
0·5094	0·4365	0·5109	+0·0015
0·2654	0·2258	0·2642	—0·0012
0·4048	0·3445	0·4032	—0·0016
0·2775	0·2380	0·2780	+0·0005
0·7058	0·6040	0·7065	+0·0007
0·3928	0·3367	0·3941	+0·0013

The original purpose of this work was to endeavour to get mercuric iodide directly from a compound, but the difficulty of collecting the product in a confined space proved too great an obstacle. Instead, the iodide was dissolved in sodium thiosulphate solution,[14] excess of thiosulphate was removed by addition of a little iodine, and mercuric sulphide was precipitated from the solution in the normal way. The first series of experiments was conducted on a specimen of cinnabar known to contain 57·5 per cent. of mercury. A quantity of ore was weighed into



a combustion boat, mixed with four or five times its weight of iodine, and introduced into a combustion tube, almost half of which formed the inner tube of a water-cooled condenser (Fig. 1). Within the cooled portion, and acting as baffles, were some plugs of asbestos fibre, while a tighter plug was situated at the inlet end of the tube as a safeguard against back-firing. Later, glass beads were substituted for the asbestos plugs. A gentle stream of air was drawn through the apparatus by means of an aspirator, but as the experiments progressed it was found more

advantageous to attach the exit tube to a controlled suction pump. During the tests on cinnabar, the results of which are given in Table II, it was noticed that the condensed product varied in colour.

TABLE II

Ore taken g.	Mercuric sulphide (supposed) obtained g.	Mercury Per Cent.
0·3851	0·3077	68·86
0·2184	0·1630	63·87
0·3966	0·3054	66·39
0·2877	0·2018	60·48
0·4145	0·3115	64·75

To elucidate this irregularity several tests were made without the use of baffles, and some of the solid was obtained. It was washed with carbon disulphide and then with acetone, and the residue was found to contain mercury, iodine and sulphur apparently in combination. The peculiar results thus seem to be due to the formation of thio-iodide; there are a number of such compounds.[15]

Experiments to eliminate the sulphur by using iodine in admixture with potassium chlorate in the combustion boat were usually unsuccessful, owing to the speed of the reaction and the consequent loss of the products. With potassium nitrate, in place of the chlorate, the reaction was also vigorous, while attempts to control the rate of reaction by the addition of a non-reactive diluent, such as sand, were no more hopeful. What did emerge from the tests was that a brighter and more uniformly coloured product was formed.

PROCEDURE WITH ORES.—In order to supply iodine and, at the same time, oxygen for the sulphur, iodic acid was next tried. After some preliminary tests the following method was adopted:—The mixture for the combustion boat consisted of one part of ore to between two and three parts by weight of partly dehydrated iodic acid. By leaving the acid in an oven at 95° C. for some time before use the amount of water formed in the reaction later on was reduced; also, a better mixture of the ingredients was obtained. After the heating, when all signs of reaction had ceased, the excess of iodine was removed, together with the red mercuric iodide, by solution in the minimum amount of sodium thiosulphate solution, and the mercury was precipitated with hydrogen sulphide. The results in Table III were obtained with the ore containing 57·5 per cent. of mercury.

TABLE III

Ore taken g.	Mercuric sulphide from HgI_2 g.	Mercury Per Cent.
0·2101	0·1390	57·03
0·3218	0·2144	57·42
0·3762	0·2579	57·7
0·5466	0·3672	57·9
0·3445	0·2294	57·39
0·3510	0·2342	57·5
0·3813	0·2548	57·61
0·2375	0·1591	57·72
0·4103	0·2767	57·85
0·3758	0·2509	57·57

TABLE IV

	Amount taken g.	Mercuric sulphide obtained g.	Mercury Per Cent.
No. 1. Merthiolate (sodium mercurithiosalicylate).[16]			
	0·2579	0·1470	49·14
	0·2205	0·1272	49·73
	0·2856	0·1629	49·17
	0·3014	0·1728	49·28
	0·2723	0·1564	49·46
	0·3115	0·1780	49·27
	0·2422	0·1384	49·28
	Theoretical figure	..	49·58
	By wet process ..	..	49·36
No. 2. Sublamin (ethylenediamine mercuric sulphate).[17]			
	0·5015	0·2543	43·7
	0·2514	0·1282	43·95
	0·2856	0·1465	43·92
	0·3237	0·1633	43·5
	0·2766	0·1402	43·69
	0·3058	0·1556	43·86
	Theoretical figure ..	..	43·7
	By wet process ..	..	43·66
No. 3. Planochrome (a variety of mercurochrome,* 220; *hydroxymercuridibromofluorescein).*[18]			
	1·1549	0·2850	21·28
	0·8457	0·2095	21·4
	1·1204	0·2767	21·3
	0·8846	0·2181	21·25
	0·7533	0·1873	21·44
	0·7247	0·1780	21·42
No. 4. Merfenil† (a phenyl mercuric nitrate).[19]			
	0·3415	0·2427	61·28
	0·2853	0·2022	61·1
	0·3288	0·2341	61·48
	0·2510	0·1780	61·19
	0·3205	0·2279	61·32
No. 5. Merfenil 002.			
	0·2376	0·1732	62·52
	0·2547	0·1843	62·4
	0·2283	0·1655	62·5
	0·3145	0·2272	62·29
	0·3572	0·2593	62·6
	Figure supplied by makers		62·2
	By wet process (average)		62·34

* This compound is liable to contain water. There appear to be several closely related compounds. A wet assay of the sample gave 21·72 per cent. of mercury.

† The sample was supplied by Messrs. May and Baker, Dagenham; its mercury content was stated to be 61·2 per cent.

As the results were satisfactory and were obtained in much less time than by the wet process, the possibility of applying the method to organic mercurials was next investigated.

Since many of the organic mercury derivatives exhibit varying degrees of resistance to treatment during assay, material was selected to embrace various linkages, including the C-Hg type, which is recognised to be the most resistant.

PROCEDURE WITH MERCURIALS.— A quantity of the substance was weighed and mixed on glazed paper with two to four times its weight of dried (95° C.) iodic acid. The mixture was carefully transferred to the boat, and this was introduced into the tube, the outlet of which was connected with the pump by rubber tubing with a screw clip. Heat was applied very gently at the beginning, and was reduced at the first sign of reaction taking place. A bright red deposit, together with some iodine, was seen in the cooled part of the tube. When all reaction had ceased and no further change appeared to take place on prolonged heating, the tube was allowed to cool, and the cooling water was run off. One end of the reaction tube was closed with a small rubber stopper, and two or three crystals of sodium thiosulphate were introduced at the open end. This was followed by a few ml. of water, after which a second rubber stopper was used to close the tube, and the solution was tilted backwards and forwards to dissolve the iodine and the iodide. In some of the tests a few more crystals of thiosulphate had to be added, and in one experiment a little alcohol was introduced, as the residue seemed to be oily. The *cold* solution was filtered, and the filtrate and washings were treated with a few ml. of iodine (alcoholic) until faint reactivity to starch paper was noted; this was to remove excess of thiosulphate. The liquid was then trebled in volume by adding water acidified with dilute hydrochloric acid and slightly warmed, and hydrogen sulphide was passed in. The mercuric sulphide showed the usual variations in colour, and the black precipitate was finally obtained. This was filtered off in a sintered Jena crucible, washed, purified, dried and weighed. Typical results are given in Table IV.

The method here described for the assay of mercury is more rapid than, and as accurate as, the digestion methods. The substance that gave the greatest difficulty was Planochrome, the assay of which by the wet digestion method took almost eight hours as against two hours ten minutes by the iodic acid process.

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May, 1939

The Potentiometric Titration of Glucose with Alkaline Tartrate Solutions of Copper, including Fehling's Solution

By H. T. S. BRITTON, D.Sc., F.I.C., and LESLIE PHILLIPS, M.Sc., A.I.C.

THE use of alkaline tartrate solutions of copper for the determination of reducing sugars was first suggested by Barreswill in 1844, and the method was subsequently elaborated by Fehling.[1] For the purpose, a solution was recommended containing 69·28 g. of $CuSO_4,5H_2O$, 346 g. of Rochelle salt and 130 g. of sodium hydroxide in 2 litres, *i.e.* a solution containing these substances in the molecular ratio of 1:4·6:10·8, respectively. The choice of such a ratio appears to have been entirely fortuitous, the probable reason being that, in the presence of such an amount of tartrate, cupric oxide is maintained in alkaline solution. It is under these conditions that cupric oxide becomes most readily susceptible to reduction, and will in consequence oxidise glucose and other reducing sugars. Unfortunately, the oxidation of glucose is not governed by any definite stoichiometric reaction; hence the statement of Fehling, that exactly five molecules of copper oxide are necessary to oxidise one molecule of glucose, has been shown by several workers to be erroneous. The precise amount of cupric oxide required depends on experimental conditions, particularly those of concentration and *p*H. The latter factor does not arise with Fehling's solution, but it becomes of importance when sodium carbonate is substituted for sodium hydroxide. Empirical tables relating to titrations at various concentrations have been prepared by Brown, Morris and Millar,[2] Munson and Walker,[3] Pflüger,[4] Peters,[5] and Lane and Eynon.[6]

Various modifications of Fehling's method involving the use of potassium ferrocyanide as external indicator have been advanced. More recently Lane and Eynon have suggested the use of methylene blue as internal indicator. In a reducing medium methylene blue becomes reduced to the colourless form when it has reached a characteristic potential with respect to a platinum electrode immersed in the solution. It will be shown that the choice of methylene blue as an internal indicator was highly successful in view of the potentials indicated at the end-point of the titration of Fehling's solution with a solution of glucose.

In view of the results of some recent work of Britton and Edge[7] on the nature of alkaline solutions of copper oxide and sodium tartrate, it appeared likely that such a titration could be followed potentiometrically. These solutions contained sodium tartrate and sodium hydroxide in varying proportions with respect to copper sulphate. The copper-ion concentrations were exceedingly small, ranging from $3{\cdot}6 \times 10^{-15}$ to $8{\cdot}0 \times 10^{-17}$. Increasing either the relative amounts of tartrate or alkali caused the copper-ion concentrations to be somewhat depressed. Computations of the ionic product, $[Cu^{\cdot\cdot}][OH']^2$, indicated that these complex solutions were only just able to retain cupric hydroxide in solution, the stability of the solutions on boiling becoming less when smaller amounts of alkali tartrate

were present. The ratio of copper : tartrate : alkali in Fehling's solution represents approximately the minimum proportions of tartrate and alkali that give a comparatively stable solution on boiling.

During the progressive addition of glucose to a complex alkaline copper tartrate solution cupric ions are reduced to cuprous ions, thus $Cu^{\cdot\cdot} \rightarrow Cu^{\cdot}$ The cupric-ion concentration will be governed by the precise composition of the alkaline copper tartrate solution and the changes in the ratio of the constituents resulting from the removal of copper in the form of precipitated cuprous oxide. The cuprous-ion concentration will be regulated by the solubility product of cuprous hydroxide and the hydroxyl-ion concentration. Allmand[8] gives 1×10^{-14} for the solubility product of cuprous hydroxide. Hence, in a decinormal solution of sodium hydroxide, which is approximately the alkali-content of the solutions used in the present investigation (see Table I), the cuprous-ion concentration would be about 10^{-13}. The potential set up at platinum electrode during the first part of the titration will be governed by the expression

$$E_{Pt} = \epsilon_{Cu^{\cdot\cdot} \rightarrow Cu^{\cdot}} - \frac{RT}{F} \log_e \frac{[Cu^{\cdot}]}{[Cu^{\cdot\cdot}]}$$

which at 90° C., the temperature at which the potentiometric titrations here described were performed, becomes

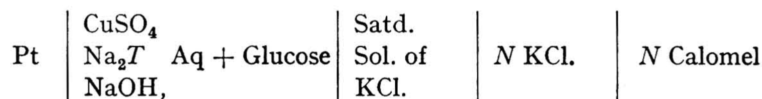
$$E_{Pt} = \epsilon_{Cu^{\cdot\cdot} \rightarrow Cu^{\cdot}} - 0{\cdot}07 \log_{10} \frac{[Cu^{\cdot}]}{[Cu^{\cdot\cdot}]}$$

The end of the titration occurs when the cupric ions are completely removed, and this should be indicated by a rapid diminution in the potential recorded at the platinum electrode. After the end-point has been reached the glucose then added remains in solution, and if it enters into equilibrium with one or more of its oxidation products already formed in the solution, a potential should then be set up at the platinum electrode which will be typical of that particular equilibrium. If such a potential is very different from those prevailing during the first stage of the titration, the end-point will be marked by a steep well-defined inflexion. Preliminary experiments by Dr. H. A. Edge showed this to occur. Regarding the oxidation products of glucose, Nef[8] was able to isolate several products by oxidising glucose with Fehling's solution.

The possibility of estimating glucose potentiometrically has been previously investigated by Daggett, Campbell and Whitman[9] and by Niederl and Müller.[10] The former workers observed that in the titration of a boiling Fehling's solution with glucose the end-point was indicated by the first large permanent deflection of the galvanometer. During the first part of the titration they reduced the small galvanometer deflections to zero by means of an adjustable resistance before each fresh addition of glucose. The latter workers describe a semi-micro potentiometric method in which a very dilute boiling Fehling's solution is titrated with glucose with the use of a platinum electrode, another platinum electrode, immersed in a solution of Fehling's solution of the same concentration, being used as the reference electrode. The inflexion indicating the end-point was not very sharp but was considered sufficiently good for accurate titration. The use of their

particular reference electrode does not permit of a study of the characteristic potentials involved.

EXPERIMENTAL.—The potentiometric titrations were carried out in a Pyrex cylindrical vessel with a capacity of about 250 ml. This was tightly fitted with an ebonite cap with holes for the insertion of one arm of the salt-bridge, a mechanical glass stirrer, two electrodes, a thermometer, a small tube connected with the burette, and with a further two holes—one for the introduction of an inert gas, *viz.* nitrogen, and the other for the removal of steam and air, and (when used) nitrogen. The cell system used for the E.M.F. measurements was



Pt	$CuSO_4$ Na_2T Aq + Glucose NaOH,	Satd. Sol. of KCl.	*N* KCl.	*N* Calomel

Each of the two ends of the salt-bridge, which was filled with a saturated solution of potassium chloride, was plugged with fine asbestos which had previously been immersed in a saturated potassium chloride solution. This reduced diffusion to a minimum. In order to be certain that reproducible results were being obtained two bright platinum electrodes were used and the potential difference between each electrode and the *N* Calomel electrode was measured after each addition of titrant. The electrodes, which consisted of platinum foil, one square centimetre in area, were attached to platinum wires sealed into the ends of glass tubes filled with mercury. The complete cell was placed in a boiling water-bath, and for the titration this was maintained at a temperature of about 90° C., and remained sufficiently constant for the purpose. During titration the burette containing the glucose solution was kept as far away from the water-bath as possible by means of a long drawn-out tube attached to the tip of the burette and leading into the titration vessel.

It was found that continuous mechanical stirring caused rather unsteady potentials to be set up at the platinum electrodes, owing to the enhanced aerial oxidation of the precipitated cuprous oxide, but that steady values were soon reached if vigorous agitation was employed during each addition of glucose and for about half a minute afterwards, and then stopped in order that steady E.M.F.'s between the electrodes and the still solutions might be attained. The E.M.F.'s were measured by means of a potentiometer.

In order to study the titration as usually performed in the presence of air, in only a few experiments was nitrogen passed into the titration vessel; 100 ml. of alkaline copper tartrate solutions were used. They were prepared from copper sulphate, sodium tartrate and sodium hydroxide in the concentrations and ratios indicated in Table I. The solution used for titration No. 2 corresponded with the molar ratio in Fehling's solution, with the exception that sodium tartrate was substituted for Rochelle salt—it having been found that the potassium present in that salt was without effect on the titration.

Typical titration curves are given in Fig. 1 (p. 23), and these refer to solutions similarly numbered in Table I. The potentials set up during the early parts of the titrations were somewhat unsteady, but both electrodes gave the same potentials

after a minute or so in still solutions. The complete reduction of the cupric oxide is marked by a well-defined inflexion extending over more than 200 millivolts, the potentials becoming correspondingly more negative. It is at this stage that cuprous oxide becomes readily oxidised by the air, so much so that the rate of oxidation may become sufficiently large to prevent the potentials from becoming constant, and they tend to fall back to smaller values. This is more marked when using the more dilute solutions of glucose. Any errors thereby introduced can, however, be minimised by adding larger volumes of glucose than are usually employed if the course of inflexion is to be minutely followed. Thus with glucose solutions of 2 g. per litre or less it is advisable to use increments of 0·2 ml. in the vicinity of the end-point. With larger concentrations the potentials are much more stable at the end-point, and additions of 0·1 ml. are permissible. It should be pointed out that when air is completely excluded by using nitrogen these difficulties are avoided.

The potentials indicated after passing the end-point do not appear to alter with increasing amounts of glucose.

Titration No. 10 is interesting in that sodium carbonate was substituted for sodium hydroxide in the complex tartrate solution. The lower *p*H of the solution is reflected in the lower potentials corresponding with the beginning and the end of the titration and also with the smaller range of potential over which the inflexion extends. Another significant feature is that the end-point is attained by addition of less glucose than when caustic alkali is present, for, as Table I shows (No. 10), 6·31 molecules of cupric oxide are required to oxidise one molecule of glucose compared with about 5 molecules when under the influence of free alkali (*cf.* Shaffer and Somogyi[11]).

When once a preliminary titration has been carried out some saving of time may be effected by running into the complex alkaline solution all but about 1 ml. of the glucose solution required to complete the reaction. The course of the inflexion can then be ascertained potentiometrically.

It has also been proved by gravimetric analysis that the point of inflexion corresponds exactly with the complete reduction of the cupric oxide. Although the amount of glucose required to give the inflexion can be read directly from the titration graph, somewhat greater accuracy can be obtained by plotting δ (E.M.F.)/δx against x, where x is the titre of glucose, when the maximum value of δ (E.M.F.)/δx indicates the end-point.

Titrations 1 to 9 illustrate the effects of increasing the relative amounts of (*a*) sodium tartrate, (*b*) sodium hydroxide. On comparing the titres of glucose in titrations 1, 4 and 7, it will be seen that the increasing amount of tartrate had no effect on the amounts of glucose oxidised. On the other hand, the increase in alkali concentration with each of the ratios of copper sulphate to sodium tartrate used, *viz.* 1:4, 1:10, 1:20, slightly increased the titre, or, as the last column shows, reduced slightly the number of molecules of copper oxide which oxidise one molecule of glucose. A further point, which is illustrated by the titration graphs, is that a greater proportion of sodium tartrate renders the potentials given in the early part of the titrations more negative, and this is even more marked with the greater alkali concentrations. This is to be attributed to

the greater stability of the complex copper tartrate solutions under these conditions, resulting in a smaller concentration of cupric ions.

Although alkaline solutions containing 4 to 4·6 mols. of sodium tartrate per mol. of copper sulphate, including Fehling's solution, do not readily decompose on boiling, blank experiments in which the solutions were maintained at 95° C. for the time required for potentiometric titration, *i.e.* about a half-hour, caused a little cupric oxide invariably to be precipitated. This, however, was without effect on the accuracy of the titration of these solutions with glucose. When 10 molecules of sodium tartrate to 1 of copper sulphate are used the complex tartrate solutions remain perfectly clear on boiling.

TABLE I

No.	Concentration of alkaline tartrate solution g.-mols. per litre			Molar ratio $CuSO_4$:Na_2T: NaOH	Glucose g. per litre	Amt. of glucose solution required	Mols. of CuO per 1 mol. of glucose
	$CuSO_4$	Na_2T	NaOH				
1	0·01	0·04	0·04	1 : 4 : 4	2	17·9	5·04
2	0·01	0·046	0·108	1 : 4·6 : 10·8	2	18·1	4·98
3	0·01	0·04	0·20	1 : 4 : 20	2	18·2	4·95
4	0·01	0·10	0·04	1 : 10 : 4	2	17·8	5·06
5	0·01	0·10	0·10	1 : 10 : 10	2	18·1	4·98
6	0·01	0·10	0·20	1 : 10 : 20	2	18·1	4·98
7	0·01	0·20	0·04	1 : 20 : 4	2	18·0	5·04
8	0·01	0·20	0·10	1 : 20 : 10	2	18·1	4·98
9	0·009	0·182	0·182	1 : 20 : 20	2	18·4	4·89
10	0·01	0·10	0·05	1 : 10 : 10	2	14·2	6·31
			(Na_2CO_3)	(Na_2CO_3)			
11	0·01	0·10	0·10	1 : 10 : 10	1	36·8	4·90
12	0·01	0·10	0·10	1 : 10 : 10	3	11·72	5·10
13	0·01	0·10	0·10	1 : 10 : 10	4	8·75	5·14

Titrations 11, 5, 12, 13 (Table I) show the effect of using glucose solutions having 1, 2, 3 and 4 g. respectively per litre. As the last column shows, the amount of copper oxide required to oxidise a molecule of glucose progressively increases as more concentrated glucose solutions are used. Incidentally, these figures are in agreement with those of Lane and Eynon,[6] using methylene blue.

Daggett, Campbell and Whitman[9] used glucose solutions containing about 10 g. per litre. The use of concentrated solutions of this magnitude tends to give sharper inflexions, so much so that the end-point might then be considered as being produced approximately when the amount of glucose added gives the first large, permanent galvanometer deflection, as stated by them.

Potentiometric titrations were also carried out on Fehling's solution, to which methylene blue was added just before enough glucose had been introduced to produce the inflexion, in order to see whether the potential at which it becomes decolorised coincides with that of the true end-point of the reaction. The reduced form of this oxidation-reduction indicator is particularly susceptible to air-oxidation at potentials established at the end-point, as may be seen by the re-appearance of the blue colour on the surface of the liquid. When, however, the solution was kept boiling or, better, when nitrogen replaced the air in the titration vessel, the blue colour was observed to disappear at about —500 millivolts, with

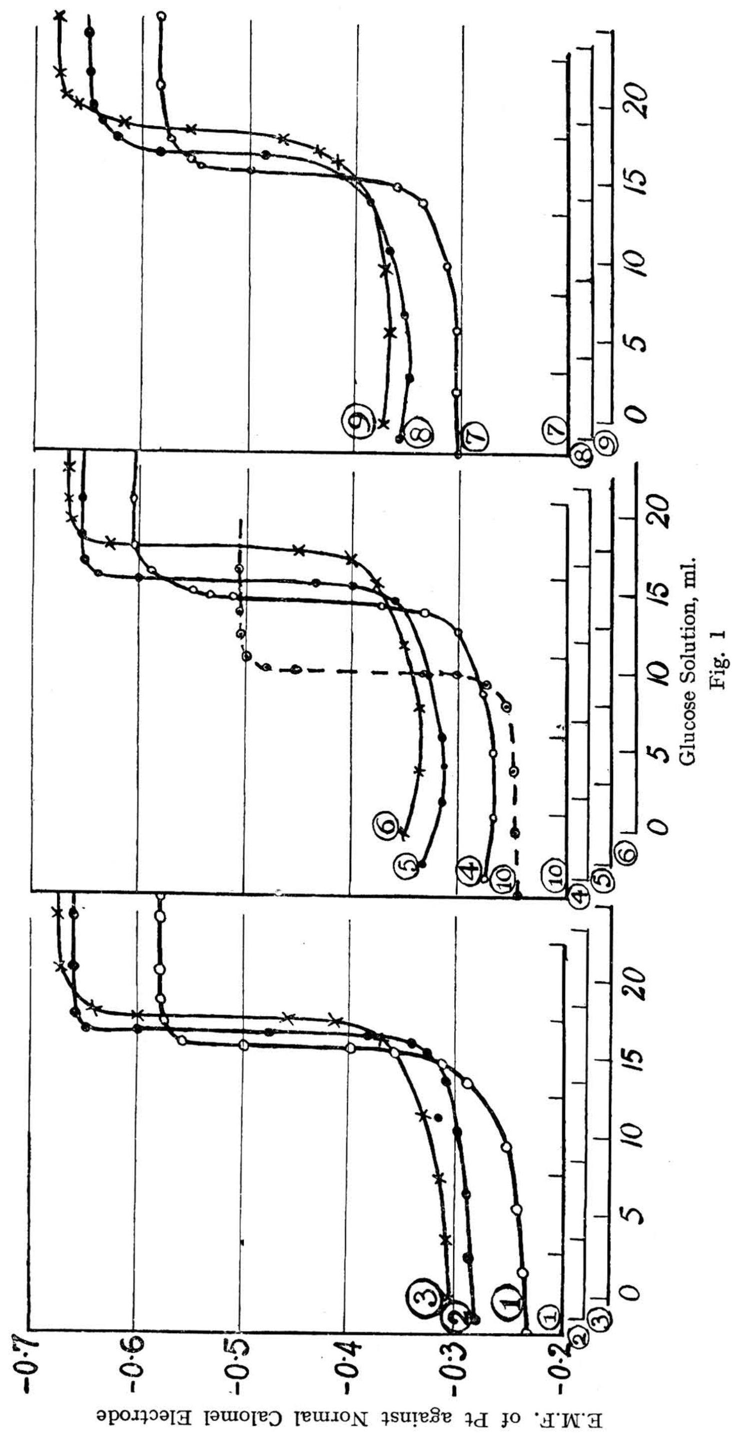


Fig. 1

respect to the *N*-calomel electrode. This is also in accordance with theory, for, according to Clark,[12] the normal reduction potential of methylene blue at *p*H 12·5 (*i.e.* the approximate *p*H of the solution) is —0·15 volt at 30° C. with respect to the normal hydrogen electrode. Temperature has little effect, giving, say, —0·17 volt at 90° C. The range of a "redox" indicator is usually restricted to 0·05 volt above and below this value, and consequently it would be expected that at 90° C. the colour of methylene blue would be completely discharged at —0·22 volt. At 90° C. the potential of the *N*-calomel electrode is 0·265 volt and therefore transition potential referred to this electrode is —0·485 volt.

In conclusion, these potentiometric titrations confirm the accuracy of the methylene blue method of Lane and Eynon for the volumetric determination of glucose. As a method of determination the potentiometric method is quite satisfactory, although it is, of course, not so rapid as that of Lane and Eynon. It is, however, essential to ascertain preferably by separate experiments, or by reference to Lane and Eynon's tables, the precise amount of cupric oxide required to oxidise glucose under comparable conditions with respect to concentration, before any calculations are made as to the amount of glucose in the unknown solution.

The ease with which cuprous oxide becomes re-oxidised by air in the vicinity of the end-point, owing to the extremely low and negative "redox" potentials then prevailing, reveal that inaccuracies must accompany the use of external indicators, *e.g.* potassium ferrocyanide.

It has further been shown that the precise ratio of sodium tartrate to copper sulphate used in the complex alkaline copper tartrate solution is not a matter of great importance, although variations in the oxidising power of the copper oxide present therein is influenced by the alkali content. The use of Rochelle salt in Fehling's solution is unnecessary, sodium tartrate being equally serviceable.

One of us (L. P.) wishes to thank the Senate of this College for a grant from the Andrew Simons Research Fund.

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Washington Singer Laboratories
University College
Exeter

July, 1939

Notes

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

THE DETECTION OF UREA IN URINE STAINS ON CARPETS

In the routine investigation of claims arising from complaints of stains appearing on carpets during use, some difficulty had frequently been experienced in satisfactorily identifying those that were suspected to have been caused by urine.

It was found from many examinations, made in comparison with known urine stains on carpets, that only a few such stains could be identified with certainty by tests depending upon their odour, appearance and deposition, reaction to ultra-violet rays, and the presence of sodium chloride. In the majority of suspected cases of urine staining, especially when the available samples were very small, *i.e.* one or two tufts, it was found that some of the characteristics mentioned above were not sufficiently pronounced to confirm the presence of urine.

Various tests made for urea, depending upon the evolution of ammonia, the formation of biuret, colour reactions, liberation of nitrogen, etc., whether made either directly on the stained wool tufts or on an aqueous extract from them, were entirely unsatisfactory, owing to the wool substance itself interfering with the tests. Since, however, urea is a main constituent of urine, it was considered that if a reliable method for the isolation and detection of urea from urine stains could be found it would provide a very useful test to assist in their identification.

With this in view, several experiments were made on pieces of carpets stained with human and domestic animal urine, and from the results the method here described was devised. It depends upon the extraction of urea from the stained tufts by means of alcohol and acetone and its subsequent identification under the microscope, and has proved very satisfactory in practice.

Three or four of the stained tufts are carefully removed from the carpet, transferred to a test-tube, and extracted successively three times with boiling alcohol (industrial methylated spirits), about 5 ml. being used for each extraction, and the extracts are filtered into a boiling-tube. A few pieces of porous porcelain are added, and the alcohol is carefully evaporated by heating the tube in a water-bath. Any traces of moisture left are removed from the residue by prolonging the heating, since the success of the method depends upon freedom from moisture. The dry residue is then re-extracted three times with successive small quantities (5 ml.) of pure moisture-free acetone, and the combined extracts are filtered into a boiling-tube and concentrated down to 4 or 5 ml. by immersing the tube in a warm water-bath. With the aid of a small pipette, or by careful pouring, this extract is evaporated, drop by drop, on a microscope slide heated over a water-bath, the residue obtained being confined to as small an area as possible.

On cooling, if the stains are caused by urine, there will be a pale yellow transparent ring, either complete or formed by small globules, from which urea separates in characteristic needle-shaped crystals that can be identified under the microscope.

For confirmation, the crystals are converted into the corresponding nitrate, which also has a characteristic crystalline appearance. This is done by carefully warming the crystals with a small drop of conc. nitric acid until they are dissolved. On cooling, urea nitrate crystallises out in either small separate diamond-shaped plates or a series of elongated hexagons, according to the degree of crystallisation.

A little practice is required to acquire the technique, and at first it is advisable to make comparisons with slides prepared under similar conditions from urea.

The method has been found sufficiently delicate to detect urea in the tip of a single urine-stained tuft (*i.e.* of the order of 0·1 mg.), where the quantity of urea is too small to be identified with certainty by chemical tests. If several mg. of urea, however, are isolated, some of it will be available for confirmation by the hypobromite and mercuric nitrate tests.

It was found that the double extractions with alcohol and acetone were necessary to obtain the best results. Whilst alcohol will extract urea readily and conveniently from urine-stained wool, traces of sodium chloride present in the urine, and of sodium sulphate left in the fabric from the dyeing processes, will also be extracted, especially if either the alcohol or wool is moist. These salts will then crystallise out, giving misleading results, especially in the nitrate test, in which sodium chloride is converted into sodium nitrate yielding crystals similar to those of urea nitrate. Subsequent experiments with various solvents showed that dry acetone, whilst not very satisfactory for the direct extraction of the urea from urine-stained wool, is excellent for separating urea, completely free from these salts, from an alcoholic extract of such stains.

The importance of separating urea from sodium chloride should again be emphasised, as many of the stains encountered on carpets, having the typical discoloured appearance of urine stains, are caused by common salt alone.

G. N. Gee

65, Baldwin Road
Kidderminster
November 2nd, 1939

MODIFICATION OF THE STIRRING DEVICE OF THE HORTVET CRYOSCOPE

Elsdon and Stubbs (Analyst, 1934, **54**, 702) devised a mechanical stirrer and blower actuated by an electric motor, and the apparatus, although not described in detail, was stated to give excellent results.

The mechanical blowing and stirring device used in this laboratory was obtained from a British firm. Apart from its expense, it has the drawbacks of noisiness, of being suitable only for laboratories equipped with electric power, and that the high humidity conditions in the tropics are particularly favourable for the swelling of the composition movable parts of this type of blower, with consequent jamming and rupture of the rubber connections.

These drawbacks were overcome by simplification of the stirring and blowing devices. Blowing can be efficiently carried out by means of the ordinary copper water-blast and filter-pump, which is a standard fitting in most laboratories.

For the operation of a vertical-lift type of stirrer the simplest method tried was an adaptation of a vacuum-operated wind-screen wiper, such as is made by the Trico Company. This method has the advantages of being comparatively cheap and noiseless in action, and it can be operated from the suction side of the same filter and blast pump as is used for the supply of compressed air.

The fitting of the commercial motor, which is extremely simple, is briefly as follows:—The actual wiping device is disconnected and discarded, and a short arm is soldered to the motor spindle. The end of the stirrer cord is then attached to the extremity of this arm by means of a simple swivel joint. The vacuum motor is mounted on the back board of the Hortvet apparatus by means of a bracket and is connected with the suction pump by a piece of pressure tubing fitted with an adjustable clip. Complete control of the stirring rate is thus possible, a small knob, easily accessible to the operator, being used for discontinuing stirring after seeding out has taken place. Constructional details and the neatness of the device are indicated in Fig. 1.

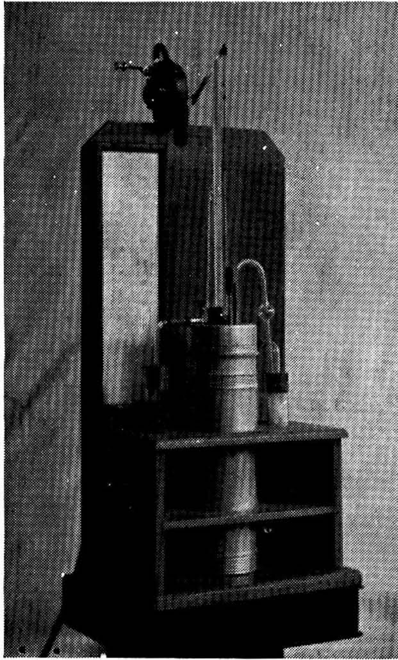

Fig. 1

Modification of the Stirring Device
of the Hortvet Cryoscope

This modified stirring apparatus has been in use for three years and has given every satisfaction. We are indebted to the Director of Agriculture, Fiji, for permission to publish this note.

W. J. BLACKIE
G. F. FLEMONS

CHEMICAL LABORATORY
DEPARTMENT OF AGRICULTURE
SUVA, FIJI
July, 1939

CLEANING JENA GLASS FILTERS

In this laboratory large numbers of Jena fritted glass filters are used in the routine examination of ghee (butter-fat) by the phytosteryl acetate test (*cf.* ANALYST, 1933, **58**, 529). It is found that with continued use the filters work progressively more slowly and, as the obstruction cannot be removed with solvents, they have eventually to be condemned as useless. Some experiments have been made in an attempt to recondition these filters, as new supplies are likely to be unobtainable. It has been found that treatment for a few moments with a small quantity of dilute hydrofluoric acid, followed by immediate washing, clears the obstruction and restores the original filtering speed. The strength of acid used is a 1 in 10 dilution of the strong acid (approximately, 4 per cent. of HF). Presumably the interstices of the filters get clogged by fine particles of glass which have been detached by prolonged use.

HERBERT HAWLEY

GOVERNMENT LABORATORY
KING INSTITUTE
GUINDY, MADRAS

Official Appointments

THE Ministry of Health has issued the following Notification of Amendment (dated 11th November, 1939) of the List of Public Analysts appointed by Local Authorities with the approval of the Minister:—

Authority.	Name of Public Analyst.
MIDDLESEX COUNTY	Miss M. H. PEARSON (Deputy P.A.)
BROMLEY BOROUGH	F. W. F. ARNAUD
BEXLEY BOROUGH	,,
ERITH BOROUGH	,,
ORPINGTON BOROUGH	,,
ESHER URBAN DISTRICT	E. HINKS
,, ,, ,, ...	D. D. MOIR (Deputy P.A.)
SURBITON BOROUGH	E. HINKS
,, ,, ...	D. D. MOIR (Deputy P.A.)
NEWTON-LE-WILLOWS U.D. ..	J. R. STUBBS
LEIGH BOROUGH (LANCS.) ..	,,
RUGBY BOROUGH	W. T. RIGBY
,, ,, ...	F. G. D. CHALMERS (Deputy P.A.)
OLDBURY BOROUGH	H. E. MONK
WORTHING BOROUGH	S. A. WOODHEAD
,, ,, ...	R. F. WRIGHT (Additional P.A.)
ECCLES BOROUGH	G. H. WALKER
MALDEN AND COOMBE BOROUGH..	E. HINKS
ILFORD BOROUGH	B. DYER } (Joint Public Analysts)
,, ,, ...	G. TAYLOR } (Joint Public Analysts)
KEIGHLEY BOROUGH	F. W. M. JAFFÉ
KESTEVEN (LINCS.)	W. W. TAYLOR (Additional P.A.)
LINCS, COUNTY	,,

Notes from the Reports of Public Analysts

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

COUNTY OF ABERDEEN

REPORT OF THE COUNTY ANALYST FOR THE YEAR 1938

OF the 827 samples examined during the year, 229 were purchased formally.

SULPHUR DIOXIDE IN MINCE.—As stated in previous reports, sulphur dioxide is permitted in mince during the months June to September, inclusive, to the extent of 450 parts per million. Two samples examined respectively in April and May and two in October contained from 100 to 166 p.p.m. and were condemned.

COPPER IN TOMATOES AND TOMATO PURÉE.—Eight samples of tomato purée contained from 3·60 to 5·65 p.p.m. of copper. The amounts found in foreign-grown tomatoes (fresh and preserved) were as follows:—Fresh tomatoes (2), 1·05 and 1·00; tomatoes, 0·45; Dutch tomatoes, 0·50 p.p.m.

COMPOSITION OF "SOWANS."—Oat "sids," which will keep for a long period, contain a valuable easily digestible starchy food which when prepared is known throughout Scotland as "Sowans" (cf. *The Scots Kitchen*, by F. M. McNeill, published by Blackie & Son). The solid matter in a sample of sowans gave the following analytical results:—oat fat, 2·78; mineral matter (largely phosphate), 3·43; starch, 55·83; cellulose, 37·96. The starch was in a readily digestible form.

J. F. TOCHER

CITY OF CARDIFF

ANNUAL REPORT OF THE PUBLIC ANALYST FOR 1938

LEAD IN BEER AND CIDER.—Eight samples of beer contained from 0 to 0·45 part of lead per million; two contained 4·0 and 5·4 p.p.m. and were condemned. Up to the present beer and cider containing not more than 0·05 grain of lead per gallon (0·7 p.p.m.) have not been the subject of adverse comment in Cardiff, but as the consumption of 2½ pints of beer containing that proportion would result in the ingestion of 1 mg. of lead, it is obvious that this limit must be revised. The use of tin-washed lead pipes should be discontinued, and it is to be hoped that in the near future no lead pipes will be used in any public house in Cardiff. In the meantime the limit for lead in beverages will be reduced. It seems reasonable to adopt 0·3 p.p.m. as the limit for lead in beer.

So far as is known no cider sold in Cardiff is drawn through pipes. One sample drawn directly from a barrel contained 0·15 p.p.m. of lead.

LEAD, TIN AND ZINC IN CANNED FISH.—The amounts of lead in 12 samples of sardines and one of anchovies were all below the limit of 5 p.p.m., which has been adopted provisionally by Port Medical Officers. Four samples of anchovies and one of sardines taken from a stock condemned by the Chief Sanitary Inspector on account of the "springy" condition of the tins contained amounts of tin ranging from 12 to 18 grains per lb., while the sardines contained 8 grains per lb. These tins contained tomato sauce and all were unlacquered.

Zinc was found in amounts ranging from 17 to 62 p.p.m. in the eight samples examined. It seems probable that zinc occurs naturally in fish in these proportions.

COPPER AND ZINC IN TOMATO PULP.—At a conference of Port Medical Officers convened by the Ministry of Health it was agreed to draw the attention of importers

to the necessity for eliminating as far as possible contamination of tomato products with adventitious copper, and to require, as from January 1st, 1939, that samples taken from consignments arriving in this country should contain not more than 100 parts of copper per million parts of dry matter. It is intended to reduce this provisional limit still further.

Eleven of the 15 samples were contained in cans lacquered on the inside. The four samples in unlacquered cans contained 450, 555, 926 and 1823 parts of tin per million parts of dry solids. The contents of one of the cans that were well lacquered contained only 201 parts of tin per million of dry matter. It is possible that this was introduced during processing, but in any event the amount is very much less than the quantities present in the unlacquered cans. In one can, however, the seam had been soldered, leaving a strip of exposed metal down the inside of the can, and this was completely coated with copper, which was undoubtedly derived from the copper present in the purée.

One of the samples from unlacquered cans also contained zinc to the extent of 114 parts per million, which was equivalent to 722 parts per million parts of dry matter. The rest of the samples gave no indication of the presence of any appreciable amounts of zinc, and this proportion must be regarded as very excessive.

The concentration of these products varied enormously, the total solid matter ranging from 9·91 to 44·18 per cent. In a few instances the approximate amount of dry extract was given either on the label or impressed on the lid of the can, *e.g.* 28–30 per cent., 30–36 per cent.; in others where the labels bore the term "double extract" the dry solids ranged from 33·1 to 40·9 per cent., but with the majority of the samples there was no indication of the concentration of the pulp. It appears desirable, therefore, that makers should declare the amount of dry matter in these preparations or use recognised descriptions such as "single," "double" or "triple" extracts according to their dry tomato contents.

STANLEY DIXON

METROPOLITAN BOROUGH OF STEPNEY

ANNUAL REPORT OF THE BOROUGH ANALYST FOR THE YEAR 1938

OF the 1586 samples submitted under the Food and Drugs (Adulteration) Act, 976 were purchased formally.

QUININE AND CINNAMON TABLETS.—A formal and an informal sample from the same vendor were deficient in quinine sulphate to the extent of 32·4 and 32·8 per cent., respectively. The formula on the bottle showed 12·5 per cent. of quinine sulphate, whereas only 8·4 per cent. was found. The manufacturers stated that their declaration was based on the weight of the uncoated tablets; the uncoated tablet weighed 3 grains and sugar-coated tablets 5 grains. This is misleading, for a tablet might consist of 90 per cent. of coating. To avoid misunderstanding the label should show the actual content of quinine sulphate per tablet. The vendor was cautioned.

D. HENVILLE

Legal Notes

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

BLEACH OINTMENT

A DRUGGIST was summoned at Croydon Police Court on November 8th for the sale of bleach ointment not of the nature, substance and quality demanded.

Mr. W. W. Ruff, prosecuting for the Surrey County Council, said that the amount of available chlorine in ointment prepared according to the formula in the Home Office Air Raid Precautions Handbook, No. 2, was 15 per cent. The amount in the ointment purchased from the defendant's shop was 1·6 per cent.

Mr. G. G. Baker, for the defence, pointed out that the handbook said "can be made," not "should be made." It was, in fact, put forward as a suggestion. No indication had been given to druggists in the district as to the standard the County Council was expecting to be used. In ninety-nine cases out of a hundred a prescription was made up from the British Pharmacopoeia. In this case it was made up from the formula supplied in the A.R.P. Catalogue of a reputable firm of manufacturing chemists.

The defendant said that he had never heard of the A.R.P. handbook, and had never been notified by the local authority of any standard to which he was expected to conform.

Mr. Thomas Tickle, F.I.C. (Public Analyst for the County of Devon), gave evidence that, in his opinion, the chlorine was self-destructive and disappeared, provided that the petroleum jelly had not been bleached (from yellow paraffin) by chlorine already. It made no difference whether there was 1·6 per cent. or 10 per cent. of chlorine, as with white petroleum jelly every particle of chlorine was surrounded by an oily layer.

The Bench dismissed the summons, but refused an application for costs.

At Sutton Police Court on November 8th a druggist was summoned on a similar charge. In this case the ointment supplied by the defendant contained 6·6 per cent. of chlorinated lime and 93·4 per cent. of white petroleum jelly.

The defendant said that he had no knowledge of the Air-Raid Precautions formula. He had consulted the British Pharmacopoeia and the British Pharmaceutical Codex. Neither contained a bleach ointment, but both described liquid preparations containing chlorinated lime. The greatest proportion of available chlorine in any of these formulae was 3 per cent., and he took this as a guide for a bleach ointment.

Mr. Baker, for the defence, said that the primary and essential point was clear. Here was a request for bleach ointment—not a request for bleach ointment for treating mustard gas burns or a special Home Office ointment. There was no indication that the ointment was required for a special purpose. The Bench was asked to say what was the standard formula for bleach ointment. There was no legal standard and the various air-raid precautions issued contradicted themselves.

The Chairman (Mr. Horace Sharp) said that the Bench had had very great difficulty with the case, but they had come to the conclusion that it would not be safe to convict the defendant. They felt, however, that a very useful purpose had been served by the case, for now the general public and druggists would know that something was advised in the air-raid precautions handbook which might be regarded as a standard and which it would be wise to keep to, at least until something more authoritative was found.

Milk and Nutrition

NEW EXPERIMENTS REPORTED TO THE MILK NUTRITION COMMITTEE*

THIS is the last of the reports to be issued by the Milk Nutrition Committee in connection with researches into the nutritive values of raw and pasteurised milk, planned in 1934–35. It presents the results of the complete analysis of the school feeding experiments partly reported in Part II (*cf.* ANALYST, 1938, **63**, 434) and summarises the whole of the experimental work of the Committee, including results published for the first time. Finally, an attempt has been made to assess the practical importance of these researches.

The results of experiments on small animals, mainly rats, and of the chemical tests were described in Part I (*cf.* ANALYST, 1937, **62**, 463), and the results of calf-feeding experiments were reported in Part III (*cf.* ANALYST, 1938, **63**, 893).

CONCLUSIONS DRAWN FROM FEEDING EXPERIMENTS ON CHILDREN.—No constant differences could be detected between the growth-promoting effects of $\frac{2}{3}$ pint of pasteurised and $\frac{2}{3}$ pint of raw milk in height, weight or chest circumference. Initially, a greater proportion of girls than of boys was placed in the higher nutritional categories as assessed clinically by the doctors. The proportions in the teachers' assessments of scholastic ability at the beginning of the test were about equal for both sexes. All the supplements of milk resulted in larger numbers of children being placed in the higher nutritional and scholastic categories by the doctors and teachers than did the supplements of biscuit. The changes attributable to milk were, on the whole, greater for the $\frac{2}{3}$ pint supplements than for $\frac{1}{3}$ pint. No constant differences could be discerned between the effects, on either assessment, of $\frac{2}{3}$ pint of pasteurised and $\frac{2}{3}$ pint of raw milk. The data for the assessments of the condition of complexion, expression, posture, tonsils, teeth and eyes, as well as for "after effects of preceding illness" and "absences since last examination" were found to be ambiguous or inconclusive, or both.

The results of the present investigation, while they add nothing further to our academic knowledge of differences that may exist between raw and pasteurised milk, nevertheless demonstrate that, under purely practical conditions, there is no difference in nutritive value between them.

NUTRITIVE VALUE OF MILK FOR CHILDREN.—The great value of milk for the growth and health of growing children, already established, has been abundantly confirmed by the results of the school feeding test. This experiment, which amounted to a practical trial of the Milk-in-Schools Scheme, has shown that definite improvements in physique, in general appearance and scholastic ability, and to a somewhat less extent in muscular strength, are to be expected from the consumption of $\frac{1}{3}$ pint, or, better still, of $\frac{2}{3}$ pint of pasteurised or raw milk.

THE NUTRITIVE VALUE OF PASTEURISED AND RAW MILK.—Rapidly growing animals, such as the young calf, are particularly sensitive to food deficiencies, and for this reason the calf is the most suitable test animal that can be used to detect small nutritional differences in cow's milk. The fact that no statistically significant differences in growth rate, in general health or in the composition of the blood could be detected in groups of calves fed on raw and pasteurised milk, respectively, during a six-months' feeding trial, is therefore of great importance. It may be argued that there are defects in pasteurised milk relative to raw milk, and that these defects were neutralised by constituents of the other foods—hay and concentrates—that were fed to the calves in the latter part of the experiments.

* Part IV. The Effects of Dietary Supplements of Pasteurised and Raw Milk on the Growth and Health of School Children (Final Report): Summary of all Researches carried out by the Committee and Practical Conclusions. Published by the National Institute for Research in Dairying, Shinfield, Reading. Pp. 69, with 31 Tables. 1939. Price 2s., post free.

The answer is that it is not small defects that are important, but only those that become manifest under strictly practical conditions.

In view of these and other considerations, it is not surprising that the addition, to the house diets of school children, of ⅜ pint of raw or pasteurised milk did not reveal any nutritive differences. As the supplements formed only a part of the total diet of the children, defects in one or the other would have had to be fairly gross to have influenced growth and health. The results showed that there were no differences of practical importance between them.

The matter is different for infants who might be fed exclusively, or almost so, on cow's milk. In the first place, cow's milk contains too little iron for the proper nutrition of the infant, and has therefore to be supplemented with iron in some form (*e.g.* egg yolk, as recommended by the Technical Commission of the League of Nations). Again, commercial pasteurisation, as has been reported in Part I, lowers the vitamin C content of milk, and it is usual to add some fruit juice to milk for infants to counteract this defect. On the basis of existing knowledge, cow's milk, raw or pasteurised, intended for infants, ought to receive such supplements.

The loss in vitamin C following pasteurisation is not *primarily* due to the heat treatment, but to previous exposure of the milk to light. According to present knowledge, if all the vitamin were present in the milk as ascorbic acid at the time of pasteurisation, the heating would not have any effect upon it. Under prevailing conditions, however, exposure of the milk to the shorter light rays causes some of the ascorbic acid to combine with the oxygen dissolved in the milk. The resulting oxidation product—dehydro-ascorbic acid—is easily destroyed by heat and the experimental evidence shows that it is only this fraction that is inactivated by pasteurisation. The presence of minute traces of copper (as derived from copper vessels) will also, without previous exposure of the milk to light, cause the oxidation of ascorbic acid.

The only changes attributable to pasteurisation that have been detected in these investigations are a diminution of 20 per cent. in the vitamin C content (but due primarily to exposure to light) and a slight decrease in the heat-labile fraction of vitamin B (presumably the B_1 fraction).

Department of Scientific and Industrial Research

FUEL RESEARCH

Analysis of Commercial Grades of Coal—Part II*

THIS Report, in continuation of the analyses published in Part I (Survey Paper, No. 37), gives the analyses of a further 277 grades of commercial coal.

The grades were sampled by the B.S.I. methods (B.S.I. Specifications, Nos. 420, 1931, and 502, 1933), and the methods of analysis used were those embodied in *Fuel Research Survey Paper*, No. 44.

Proximate analyses, total sulphur and calorific value determinations were carried out on every grade, and an ultimate analysis on "selected" grades, including both "hards" and "brights," where these occur in the same seam. The moisture-content was determined by heating the coal for 1½ hours at 105°–108° C. in a stream of oxygen-free nitrogen in the "Minimum Free Space" Oven designed at the Leeds Coal Survey Laboratory. The volatile matter in those samples obtained before May, 1937, was determined by the British Standard method B (B.S.S. No. 420, 1931), using six silica crucibles together in an electric muffle furnace at 965° C.,

* Physical and Chemical Survey of the National Coal Resources, No. 48. The Yorkshire, Nottinghamshire and Derbyshire Coalfield, Nottinghamshire and Derbyshire Area. Pp. 166. H.M. Stationery Office, York House, Kingsway, London, W.C.2. 1939. Price 3s. net.

but after that date the determinations were carried out with a platinum crucible in the muffle at 925° C. The total sulphur was determined by the Eschka method, and the forms of sulphur by that of Powell (*U.S. Bur. of Mines, Techn. Paper*, No. 254, 1921; British Standard Methods, No. 687, 1936), while the method developed at the Birmingham Coal Survey Laboratory (*Fuel Research Paper*, No. 44; Alternative Method, p. 43) was adopted for the determination of total carbon dioxide. These determinations, together with the sulphur in the ash, were required for calculating to the "coal substance" the analyses of those grades that had been subjected to an ultimate analysis.

ASH.—Variations in the ash-content are shown to depend *inter alia* on the quantity of impurity present, on the closeness of sizing, and on the method of mining. The ash, as determined by the standard method, differs from the mineral matter in the coal both in amount and nature as the result of changes (described in detail in the Report) that occur during incineration.

The fusion-point of the ash obtained from washed grades and from selected grades of large, hard steam coal may be constant within small limits, but may not be so constant in secondary grades and rough slacks, owing to variations in the amount of adventitious ash.

CHLORINE.—In the examination of seam section in the area under survey it has been found that the amount of chlorine is substantially the same throughout any one section. This regularity of the chlorine-content is sufficient to justify the use of chlorine figures determined in one grade for the calculation of data for other grades in the same seam and colliery. In South Yorkshire, also, it has been found that all grades from a single colliery contain very similar amounts of chlorine (*Fuel Research Survey Papers*, Nos. 20 and 31).

SULPHUR.—For any one seam the highest sulphur-content usually occurs in grades of second quality, which are picked out on account of the visible pyrites they contain. The smaller unwashed grades, with a relatively high ash-content, do not necessarily have a higher sulphur-content than the larger and cleaner grades.

CALORIFIC VALUE.—The calorific value of a grade "as received" will vary according to its moisture and ash contents, but the dry ash-free calorific value of any grade should be constant, within narrow limits, so long as there is no major change in the seam or in the mining conditions.

GRAY-KING CARBONISATION-ASSAY AT 600° C.—The cokes from this assay have been classified into eleven types; photographs and descriptions of these types appear at the end of the report.

New Zealand

ANNUAL REPORT OF THE DEPARTMENT OF AGRICULTURE FOR 1938–1939

THE Report of the Chemistry Section of the Department is by Mr. E. R. Grimmett, M.Sc., the Chief Agricultural Chemist; it describes various investigations carried out in association with the other sections of the Department, such as chemical work on Canterbury pastures in connection with lamb-mortality research and with animal health and nutrition.

COBALT DEFICIENCY IN PASTURES.—More than 550 pasture samples, 135 animal organs, and various soils, limonites, limestones and cobaltised superphosphates were analysed. Low cobalt figures were obtained with a number of pasture samples (below 0·08 p.p.m. of cobalt) in the spring and summer months. A further extension of the cobalt survey of North Island pastures has been made, and special attention has been given to seasonal variation in the cobalt-content of

pastures. Pasture cobalt tends to be at a minimum in late spring and early summer and at a maximum in winter or after prolonged drought.

Analyses of pastures from cobaltised superphosphate top-dressing experiments have shown a high initial uptake of cobalt, but later a rapid fall especially during the period of flush growth. The experiments themselves have been outstandingly successful, and have afforded further evidence of the efficacy and practicability of this method of preventing bush sickness.

Cobalt in New Zealand Soils and Rocks.—Analyses of several New Zealand ultra-basic magnesian rocks gave figures ranging from 67 to 115 p.p.m. of cobalt. Some North Auckland lateritic soils associated with cobalt-deficient pastures have shown figures as high as 10 p.p.m. for total cobalt, but the cobalt present is relatively insoluble, even in strong acid. Owing to the occurrence of cobalt deficiency in the Wairarapa on a paddock built up of a fine limestone wash, analyses of typical limestones were made. Some low figures were obtained, the amounts ranging from 0·2 to 5 p.p.m. of cobalt.

Cobalt in Animal Organs.—The average cobalt-content of the livers of bush-sick sheep is about 0·04 p.p.m. or less, but for healthy animals more than 0·10 p.p.m. A similar difference has been observed for cattle. There is no evidence of copper deficiency associated with bush sickness in the North Island.

FACIAL ECZEMA.—During an extensive outbreak of facial eczema in the Waikato in April large samples of pasture were obtained from fields believed to be still active in causing the disease. Analyses showed that icterogenin (the substance which, it was suspected, might be the active factor causing liver derangement) was not present in detectable amounts. It is possible, however, that the pasture might have passed the active stage when the samples were collected; that an icterogenic substance other than icterogen itself might have been present; or that a toxin may be formed after the pasture has been eaten by the animal. Analytical work in association with records of changes in animal health (if any), changes in botanical composition, and changes in growth rate, succulence, etc., is in progress.

VARIATION IN SOIL NITRATE.—Concurrently with the pasture analyses a series of determinations of soil moisture, nitrate and ammonia has been made in some of the experimental paddocks. A feature that has emerged is the extreme variation in soil nitrate found at intervals of a few hours throughout a single day. This may change from a level as low as 10 p.p.m. in the early afternoon to one of 80 p.p.m. two hours later.

STANDARDS FOR TRACE ELEMENTS IN PASTURES.—In co-operation with the Cawthron Institute and the Dominion Laboratory, work has been begun to obtain quantitative data on the occurrence of a number of elements that are present in many pastures and in animal tissues, but of the possible function of which little is known. Naturally healthy and productive pastures have been selected in several localities, and by the use of movable enclosures clean samples are being obtained several times a year. Determinations of cobalt and of some other elements are being made by chemical methods, and the samples are then handed over to the Dominion Laboratory for spectrographic analysis of the ash.

ARSENIC IN SOILS AND WATERS.—The investigation into the alleged poisoning of live-stock by arsenic occurring naturally in soils and waters has been continued, and a comprehensive report has been submitted to the Lands Department. This includes a map and tables showing the distribution of arsenic in soils, muds and waters; ante- and post-mortem notes and figures for arsenic in the organs of 31 cases of live-stock mortality; the arsenic-content of pasture samples and accounts of experiments on cattle at Reporoa and Wallaceville. Sixty-five samples of muds and soils were collected. The arsenic in muds varied from 0·0068 per cent. to 1·9 per cent. Representative samples of the whole soil to a depth of 9 inches in pasture lands gave results for arsenic ranging from 0·0008 to 0·525 per cent. Thirty-nine samples of water from springs, streams and surface depressions showed

amounts varying from a trace to 2·6 grains of As_2O_3 per gallon. Arsenic was present in the mud deposits in several places, either in combination with iron in impure limonite (the arsenic readily soluble in dilute acid) or as a sulphide (insoluble in dilute acid). In some places amounts of orpiment and realgar up to 5 per cent. were present in orange-yellow siliceous deposits.

The general finding was that arsenic is of widespread occurrence in the spring and drainage waters and the soils of the lower-lying portions of the Reporoa Settlement, but that it is responsible for only a small annual mortality, together with further, but undefined, unthriftiness in live-stock. Clean-growing pasture on arsenical soil normally contains too little arsenic to have any toxic effect, and crops, such as oats, from such soils are also harmless. Farmers are recommended to fence off areas where arsenical springs occur, or where the mud along stream edges or in depressions is strongly arsenical, to provide safe water, and not to graze recently flooded areas while the pastures are still muddy.

Determination of Available Phosphate.—In the soils sent in for examination the usual trouble was phosphate deficiency. Egner's lactate method for estimating available phosphate has been studied and found to show considerable promise. Certain South Island soils showed a very high phosphate-content when extracted with 1 per cent. citric acid solution, but a deficiency by the lactate method. It has not yet been ascertained which is the truer test.

Bromothymol Blue Test for Mastitis.—The staff of the Veterinary Laboratory has been studying the anomalies shown in the bromothymol blue test for mastitis. Frequently the test does not pick out an obviously infected animal. Work done in conjunction with the Dairy Research Institute showed that only about 50 per cent. of the results were correct when checked by the leucocyte assessment in the diagnosis of mastitis. The check results obtained in three other laboratories showed still poorer correlation figures. The factors that made colour testing inaccurate were (*a*) the fact that the milk in many cases of mastitis does not have an alkaline reaction; (*b*) the reading is upset by the quantity of cream, by the interval of time between the taking of the samples and the reading, by the quantity of milk and bromothymol blue taken; (*c*) by the personal factor, many officers being unable to distinguish shades of colour.

Chemical Control of Ragwort.—A farm experiment confirmed the superiority of sodium chlorate as compared with dichromate, thiocyanate, and bisulphite weed-killers. Another experiment is in progress to determine the effect of treatments of individual plants at various times of the year and the toxic dose per plant. Experiments in the Ruakura nursery indicate that injections of chlorate into the soil or applications on the soil surrounding the plants are not as effective as those applied to the foliage in the usual way, that dilution of the spray has little effect, and that the seed from flowering plants sprayed with 2·5 and 5 per cent. sodium chlorate solutions has a germination of 10 to 20 per cent., as compared with 50 to 80 per cent. in unsprayed seed-heads.

Ragwort Feeding to Cattle and Sheep.—A cow that was kept on a diet of 1 lb. of rosette-stage ragwort per day and afterwards given ragwort infusion over a period of three months, has recently calved and appears to be quite normal. Two sheep were kept on ragwort for 2½ years and were then slaughtered and their livers examined. One sheep was normal, but the second showed some increase in fibrous connective tissue. Apparently the amount of toxic substance in rosette-stage ragwort is very small, and insufficient to cause any serious damage to sheep or cattle unless fed in large quantities.

Government of Madras

ANNUAL REPORT OF THE CHEMICAL EXAMINER FOR THE YEAR 1938

THE Chemical Examiner (Mr. S. Rajagopal Naidu, F.I.C.) reports an increase in the work of the department, the total number of cases investigated being 1785, as compared with 1670 cases in 1937.

HUMAN POISONING CASES.—Poison was detected in 241 of the 467 cases investigated. Datura heads the list with 36 cases; then come oleander with 30, opium with 29, and madar juice with 14 cases. Poisoning with organic poisons was nearly twice as frequent as with inorganic poisons. Among the latter, mercury claimed 21 cases, arsenic 20, copper sulphate 12, cyanide 10, and nitrite 9 cases.

Datura Poisoning.—A study of datura cases has shown that the type of criminal who uses datura generally carries with him the powdered seeds, and probably buys sweets at some bazaar to mix with the powder. If only a portion of the powder has been used, the remainder may be expected to be found in his possession. In a case in 1937 the culprit, caught immediately after the offence, had on him a packet of the powdered seeds. In a 1938 case a boy was given sweets by a religious mendicant and soon afterwards became giddy and then unconscious. He was removed to hospital, where, after treatment, he recovered. A mydriatic alkaloid was found in his urine, and particles of datura seed were detected in the stomach washings. Some villagers chased the culprit and found in his possession a packet of vegetable powder.

Oduvan Poisoning.—There were three cases of poisoning by oduvan leaf (*Cleisanthus collinus* Benth.), all of them suicidal. The reactions of oduvan were obtained with the viscera, and particles of leaf with the microscopical structure of oduvan were isolated from each of the stomach contents.

The method employed for studying the poisonous extract from oduvan leaf was the same as that used for the extraction of "nerin" from *Nerium odorum* (*cf.* ANALYST, 1939, **64**, 121). A yellowish-white crystalline substance (m.p. 192–194° C. with decomposition) was obtained. It was freely soluble in alcohol and chloroform, but sparingly soluble in water. It did not reduce Fehling's solution until hydrolysed with emulsin or hydrochloric acid. Its elementary composition was: C, 53·9; H, 6·91; O, 39·19 per cent., corresponding with the tentative formula $C_{22}H_{34}O_{12}$. The name "oduvin" is suggested for this substance. Titration, by Lane and Eynon's method, of the hydrolysed product gave one mol. of an aldohexose calculated as dextrose, from one mol. of the original substance.

In addition to its colour reaction with sulphuric acid, described in the 1937 report (ANALYST, 1939, **64**, 121), oduvin gives a bright vermilion colour with fuming nitric acid or with a mixture of equal parts of conc. sulphuric and nitric acids.

Melia Composita Poisoning.—A man drank the juice of the bark of "malai vembu" (*Melia composita* Willd.) and died in about an hour and a half. No poison was detected in the viscera, but from the vomit a crystalline poisonous substance was extracted resembling the product obtained by extracting the bark with acid ether. Both extracts produced in frogs convulsions similar to those caused by picrotoxin, but whilst picrotoxin gives a red colour when treated with a dilute solution of benzaldehyde and a drop of conc. sulphuric acid (Melzer's test), the two extracts gave a permanganate purple colour. Moreover, the extracts did not give any colour on treatment with potassium nitrate and sulphuric acid and then with excess of potassium hydroxide, whereas picrotoxin gives a brick-red colour in this test. The extract from the bark did not reduce Fehling's solution either before or after hydrolysis.

Poisoning by Manihot utilissima.—The "jungle potato" is used extensively as a food in Malabar and Cochin, and, according to local information, there are two

varieties, one of which is poisonous. There is no appreciable difference between the two, except that the poisonous variety is darker, has redder leaves, and is not so soft when cooked as the non-poisonous variety. According to the local medical officer there have been instances of goats dying after eating the leaves of the poisonous variety, but there is no record of human beings having been poisoned after eating the root when cooked.

A woman and two children were stated to have died after eating uncooked "jungle potato." The bodies, when submitted, were in an advanced state of decomposition. No cyanide or other poison could be detected in the viscera. The root suspected to have caused death was identified as *Manihot utilissima.* When crushed, acidified with phosphoric acid, and distilled it yielded hydrogen cyanide in an amount corresponding with about 14 parts per 100,000 of the root. The poisonous principle thus appears to be a cyanogenetic glycoside. About a pound of the root eaten in the raw state would probably be dangerous to human life.

Bamboo Shoots.—The poisonous crystalline substance previously described (*cf.* ANALYST, 1937, **62**, 742) has been further studied. It melts at 115–116° C., and has a composition corresponding with the formula $C_8H_{11}O_5$. The name "bambusin" is suggested for it. The red colour that it gives with strong sulphuric acid and manganese dioxide can be brought out more distinctly by substituting ceric oxide for manganese dioxide. The substance does not reduce Fehling's solution either before or after hydrolysis.

THE OLEANDERS.—The term oleander is applied to three different plants: (i) red-flowered oleander (*Nerium odorum*), (ii) odallam (*Cerbera odallam*) and (iii) yellow-flowered oleander or yellow oleander (*Cerbera thevetia*).

(i) A note on red-flowered oleander (*Nerium odorum*) and its poisonous principle "nerin" was published in the Annual Report for 1937 (*cf.* ANALYST, 1939, **64**, 121).

(ii) *Odallam* (*Cerbera odallam*) is a tree with fleshy lanceolate leaves, large white flowers and green fibrous fruits enclosing a kernel. The kernel of the fruit is used as poison. The kernel on extraction with petroleum spirit or ordinary ether gave a non-poisonous oil with the following constants:

Constants of Oil from Cerbera odallam Kernels.—Iodine value (Rosenmund and Kuhnhenn), 29·1; saponification value, 178·6; Reichert–Meissl value, 1·21; Polenske value, 1·65; n_D^{28}, 1·4660; unsaponifiable matter, 0·73 per cent. (iodine value, 21·8).

An alcoholic extract of the de-fatted kernels gives, on solution in absolute alcohol and treatment with anhydrous ether, an abundant white precipitate which requires for purification only a few precipitations from absolute alcohol with anhydrous ether and is free from nitrogen.

This extract is freely soluble in alcohol and water, but insoluble in ether, benzene or chloroform. The elementary composition and approximate molecular weight (determined by the cryoscopic method with water as solvent) suggests the molecular formula $C_{20}H_{38}O_{14}$. The name "cerberin" is tentatively suggested for this substance.

"*Cerberin*" reduces Fehling's solution only after hydrolysis with emulsin or with hydrochloric acid. Hydrolysis with emulsin gives colourless products, whereas hydrolysis with hydrochloric acid gives a blue colour and then a dark precipitate. The solution of "cerberin" after hydrolysis with emulsin does not give a blue colour with hydrochloric acid and is no longer poisonous. It appears to be similarly affected by the digestive secretions of animals. The lethal dose for frogs weighing about 10 g. is about 0·025 mg. Paralytic symptoms in dogs were not so pronounced as with "nerin" or "thevetin."

(iii) *Yellow-flowered Oleander* (*Cerbera thevetia*).—The kernel of the fruit is the part most commonly used as poison, though in a few cases the leaves, bark and root have also been used. Cases of accidental poisoning of children through eating the flowers are also known. Two poisonous extracts have been obtained from the

kernels: *Extract A*, from the pressed kernels by extraction with a mixture of ether and chloroform and removal of the traces of oil by petroleum spirit, and *Extract B*, a precipitate obtained by adding anhydrous ether to a solution in absolute alcohol of the alcoholic extract of the residue from the previous extraction. *Extract A* appears to be identical with thevetin described by Dragendorff, but the molecular weight, as determined by the method of Pregl and Rast (in which camphor is used as the solvent), suggests that the formula should be $C_{27}H_{42}O_{12}$ instead of $C_{54}H_{84}O_{24}$ as proposed by Dragendorff. Thevetin is sparingly soluble in water but soluble in ether and readily soluble in chloroform. After hydrolysis with hydrochloric acid or emulsin it reduces Fehling's solution. The sugar residue after hydrolysis gives with thymol and sulphuric acid the reaction given by aldohexoses. About 0·02 mg. is fatal to frogs weighing about 10 g. Ten mg. injected into a dog weighing about three kg. produced defecation, vomiting with retching, frothy salivation, paresis of the hind limbs and terminal spasms. Death occurred in about ninety minutes. Post-mortem examination revealed engorged heart stopped in diastole, intensely congested lungs, congested liver with the gall bladder full, a pale stomach and intestines and somewhat congested kidneys. Thevetin, therefore, appears to be somewhat similar to "nerin" in its poisonous character.

The following colour reactions serve to distinguish thevetin from "nerin":

Reagent	"Nerin"	Thevetin
Conc. sulphuric acid	Immediate crimson.	Yellowish-brown slowly changing to mauve.
Keller's reagent	Immediate crimson in sulphuric acid layer and slow green in acetic acid layer.	Immediate blue in acetic acid layer and slow mauve in sulphuric acid layer.

Extract B was purified by re-dissolving in absolute alcohol and re-precipitating with anhydrous ether a number of times. This extract is soluble in water and alcohol but insoluble in ether, benzene and chloroform. The extract thus purified was free from nitrogen and gave C, 48·6; H, 7·73; O (by diff.), 43·7 per cent. This substance on hydrolysis with emulsin gave an aldohexose, and titration of the sugar by Lane and Eynon's method (the hexose being calculated as dextrose) suggests the tentative molecular formula $C_{20}H_{38}O_{14}$. This substance also agrees with "cerberin," obtained from *Cerbera odallam*, in giving a blue colour with hydrochloric acid and in its toxicity. These facts suggest that *Extract B* of yellow oleander kernels is identical with "cerberin" obtained from *Cerbera odallam.*

Yellow oleander kernels yield (1) thevetin and (2) "cerberin," whereas odallam kernels yield "cerberin" but not thevetin.

EXAMINATION OF HAIR.—A 10 per cent. aqueous solution of potassium cyanide has been found a good clearing agent for the examination of dark specimens of hair. Immersion for about an hour in a solution of one drop of 20 per cent. hydrogen peroxide and three drops of dilute ammonia solution in three ml. of water is also useful for clearing the hair without affecting its structure. For obtaining cross-sections of hair good results are obtained by embedding the sections in a mixture of equal parts of rosin and hard paraffin wax, melted together. The paraffin-rosin sections may be mounted without further treatment in Canada balsam, as the mixture, being transparent, does not interfere with the microscopical study of the hair.

ULTRA-VIOLET LIGHT AND PRINTING INK.—An official minutes book was submitted to ascertain if the sheet of paper bearing pages numbered 11 and 12 was different from the other sheets. On examination under ultra-violet light pages 11 and 12 were found to be, in fact, pages 111 and 112, the extreme left figure of each having been erased. Though the carbon had been removed, the oil of the printing

ink had soaked through the paper and showed under ultra-violet light an unmistakable fluorescence corresponding with the erased figure. There was also evidence of the original paper corresponding with pages 11 and 12 having been torn out and replaced by the present paper, which was similar in other respects, by pasting at the extreme left edge.

British Leather Manufacturers' Research Association

DECONTAMINATION OF LEATHER CONTAMINATED WITH MUSTARD GAS*

TRIALS have been made by the Association in conjunction with the Chemical Defence Research Department, Technical Advisers to the Home Office, and the results are embodied in the present Report.

Decontamination from mustard gas by immersion in boiling water cannot be applied to leather articles because this treatment damages the leather. Although solutions of certain chemicals which destroy blister gas are effective decontaminants of leather, they must be removed by subsequent treatment. Since, however, warm water is equally effective a method involving its use has been developed. At 50° C. (120° F.)—a temperature that can be applied to most types of leather without causing damage—decontamination of the leather from mustard gas is complete within six hours. In the process described the leather articles are first soaked in cold water for one hour and then kept for six hours in water maintained at 50° C. A suitable plant for the purpose is described.

The process effects satisfactory decontamination of leather articles contaminated, even heavily, with drops and smears of mustard gas, but cannot be successfully applied to grossly contaminated articles, which have absorbed large amounts of liquid gas. All types of leather can be satisfactorily treated, except white kid (alum tanned), which becomes shrunken and brittle, and some chamois leathers, which may shrink. Damage may also occur to made-up leather articles that contain components (such as glue or cardboard) which are not water-resistant. The appearance and flexibility of treated articles may be improved by cleaning, and polishing or oiling after drying.

Water dripping from the soaked articles may be contaminated and adequate precautions should be taken to avoid spread of contamination from this source.

Food and Drugs Act, 1938

APPLICATION TO SCOTLAND

THE following provisions of the Act became applicable to Scotland on October 1st, 1939:

Section 8.—Power of the Department of Health for Scotland to make regulations as to the importation, preparation, storage, sale, delivery, etc., of food and for prohibiting or restricting the addition of any substance to, and regulating generally the composition of, any food (other than bread or flour).

Section 30.—Power of the Department of Health for Scotland to make regulations as to the composition of bread and the addition of substances to flour.

Section 31.—Prohibition of adulterants in bakehouses and mills.

Section 33.—Conditions to be observed in dealings in margarine, margarine cheese and milk-blended butter.

* British Leather Manufacturers' Research Association, 1–6, Nelson Square, London, S.E.1.

Part IV.—Provisions as to Importation.

Section 81 (4).—Presumption as to sale for human consumption.

Section 92 (1) to (4).—Supplementary provisions applicable to Food Regulations and Bread and Flour Regulations.

Section 98.—Repeal of two Sections of the Bread Act, 1836.

Section 100 (1).—Definitions of "container," "cream," "preparation" and "substance."

Section 100 (2).—"Milk" refers also to cream and separated milk, but not to dried milk or condensed milk. "Food" includes food "for the manufacture of products for human consumption."

Section 101.—Repeals extending to Scotland—The Bread Act, 1836; The Public Health (Regulations as to Food) Act, 1907, in so far as it empowers regulations to be made regarding food (excluding milk); The Bread Acts (Amendment) Act, 1922; part of The Food and Drugs (Adulteration) Act, 1928, *viz.* Section 6 (except subsections (2) and (7)), Sections 12, 20 and 23, the proviso to subsection (3) of Section 27, and subsections (5) and (6) of Section 28.

Section 103 (1).—Name of the Act and date of operation.

These provisions (other than Part IV) are to be construed as one with the Food and Drugs (Adulteration) Act, 1928, and shall be deemed to be included in Part I thereof, subject to certain modifications detailed in Section 102.

APPLICATION TO NORTHERN IRELAND

The Act does not apply to Northern Ireland except as regards Part IV (Provisions as to Importation), which is slightly modified as detailed in Section 102. Part of The Food and Drugs (Adulteration) Act, 1928, is repealed, *viz.* Sections 12 and 20, the proviso to subsection (3) of Section 27, subsections (5) and (6) of Section 28, and Section 36.

National Institute for Medical Research

INTERNATIONAL STANDARDS FOR PROLACTIN AND FOR THE GONADOTROPHIC SUBSTANCE OF PREGNANT MARES' SERUM

It was stated in a previous issue (Analyst, 1939, **64**, 430) that the Third International Conference on the Standardisation of Hormones, held at Geneva in 1938, had decided that international standards should be established for certain hormones of the anterior lobe of the pituitary gland and analogous substances found in urine and serum, and that international units should be defined in terms of a weight of each such standard. It was further decided that the final preparation of these standards, their dispensing in a form suitable for the use of the laboratory worker, their storage, preservation and subsequent distribution should be undertaken by the National Institute for Medical Research, Hampstead, London.

The first of these new standards, as already announced, *viz.* that for the gonadotrophic substance of human urine of pregnancy—chorionic gonadotrophin—was established in May of last year.

We are now asked to announce that the preparation of two additional international standards has been completed, *viz.* for the gonadotrophic substance of pregnant mares' serum and for the lactogenic (crop-gland stimulating) substance of the anterior lobe of the pituitary gland. The former standard has been prepared from substantial amounts of material generously provided by five manufacturing firms in four different countries, and the latter from material supplied by seven manufacturing firms and two research institutes in five countries. The individual samples for each standard were examined by members of the Conference, and a

suitable mixtures were then made to serve as the respective international standards and finally dispensed in the form of tablets which have been packed in sealed tubes. For each standard each tablet contains approximately 100 international units.

The international standard for the gonadotrophic substance of pregnant mares' serum is dispensed in the form of 25-mg. tablets, each sealed tube containing ten of the tablets, and the international unit has been defined as the specific gonadotrophic activity contained in 0·25 mg. of the standard preparation.

The international standard for prolactin is dispensed in the form of 10-mg. tablets, each sealed tube containing ten of the tablets, and the international unit has been defined as the specific activity contained in 0·1 mg. of the standard preparation.

As with the international standards for other hormones, drugs and vitamins, the above international standards are held, on behalf of the Health Organisation of the League of Nations, at the National Institute for Medical Research, Hampstead, London, N.W.3, and are distributed therefrom to national control centres established in other countries for local distribution to laboratories, institutes and research workers; also to workers in other countries in which the establishment of national control centres has not yet been completed.

With regard to the supply of these new standards to those requiring them in the United Kingdom, applications should be made to the Department of Biological Standards, the National Institute for Medical Research, Hampstead, London, N.W.3.

British Standards Institution

The following British Standard Specification has been issued:*

No. 875—1939. Standard Dimensions for Silica Basins, Crucibles and Capsules.

The series for the basins is based on specified relations of dimensions. Five sizes are provided, *viz.* 20 ml., 50 ml., 75 ml., 100 ml. and 200 ml.

Two types of crucible—tall and squat—are provided, with dimensions based respectively on the following ratios: $\frac{\textit{External diameter top}}{\textit{Overall height}}$ $\frac{1}{1}$ and $\frac{5}{3}$ respectively. Five sizes of tall crucibles, *viz.* 10 ml., 15 ml., 20 ml., 30 ml. and 50 ml., and four sizes of squat crucibles, *viz.* 10 ml., 15 ml., 25 ml. and 40 ml. are provided. Both basins and crucibles are made of transparent or translucent silica.

Capsules with lid for combined moisture and ash determination are made of translucent silica unless otherwise specified. They are provided in four sizes of specified dimensions.

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* Publications Department, 28, Victoria Street, London, S.W.1. Price 2s.; post free 2s. 2d.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Sulphur Content of Foods. M. Masters and R. A. McCance. (*Biochem. J.*, 1939, **33**, 1304–1312.)—Sulphur has been determined in over 300 different varieties of foods. In most instances a hydrogenation method was employed, whilst in the others destructive oxidation of the organic matter with a mixture of nitric and perchloric acids was used, followed by gravimetric estimation of the resulting sulphates (*cf.* Masters, *Biochem. J.*, 1939, **33**, 1313). Drying prior to the analysis of some foods introduced errors into the determination, owing to the presence of volatile or potentially volatile sulphur compounds. Fruits, nuts, cereals, meat and beer lost no sulphur on drying, whilst fish and many vegetables lost small quantities, and onions, horse-radish and mustard and cress lost large amounts. The nitrogen: sulphur ratio was found to be remarkably constant for meat (15·3:1) and for fish (13·8:1), but it varied so widely with material of plant origin as to make any generalisation impossible. For animal tissues, raw or cooked, an estimate of the sulphur-content can usually be made with sufficient accuracy by multiplying the nitrogen-content by the appropriate factor. Some of the most important results are summarised below, the sulphur-contents being expressed in mg. per 100 g.:—

Fruits, raw.—Apples, cherries, damsons, grapes, grapefruit, melons, oranges, peaches, pears, pineapples, plums, rhubarb, 3·7 to 9·1; bananas, blackberries, cranberries, figs, gooseberries, lemons, loganberries, raspberries, strawberries, tomatoes, 10·7 to 18·1; currants (black, red and white), 23·6 to 33·1. *Fruits, dried.*—Prunes, 18·5; currants, raisins, sultanas, dates and figs, 23·0 to 80·8; apricots (probably sulphited), 164·0; peaches (probably sulphited), 240·0. *Tinned fruit.*—Apricots, pineapple, loganberries, peaches, pears, 1·0 to 3·0. *Nuts.*—Chestnuts, cobs, coconuts, almonds, Barcelonas, Brazils, peanuts and walnuts, 104·0 to 377·0. *Vegetables.*—Artichokes (boiled), French beans (boiled), broad beans (boiled), runner beans (raw or boiled), beetroot (boiled), cabbage (boiled), carrots (raw and boiled), cauliflower (boiled), celery (raw and boiled), cucumber, endive, lettuce, onions (boiled), parsnips, potatoes (boiled), turnips (boiled), 5·0 to 30·4; asparagus (boiled), Brussels sprouts (boiled), lentils (boiled), mushrooms (fried), onions (fried), spring onions, peas (boiled), radishes, spinach, 37·5 to 87·8; watercress, 127·0; mustard and cress, 170·0; horseradish, 212·0. *Cereal and starch products.*—Biscuits, 31·8 to 107·0; bread, 54·5 to 114·5; flour, 108 to 123. *Dairy products.*—Butter, 9·1; cheese, 177 to 321; egg yolk, 164·5; egg white, 182·5; milk, 29·2. *Meats.*—Beef, 203 to 341; mutton, 164 to 197; pork, ham, 195 to 233. *Fish.*—Cod, haddock, halibut, hake, herring, kippers, mackerel, plaice, salmon, trout, 169 to 256; shellfish, 265 to 401.

F. A. R.

Gases in the Commercial Handling of Citrus Juices. G. N. Pulley and H. W. von Loesecke. (*Ind. Eng. Chem.*, 1939, **31**, 1275–1278.)—The gases present in Florida grapefruit and orange juices extracted and de-aerated by

various methods have been studied. The apparatus used for determining the gas-content of the juices was a modified Peterson equipment with a Sprengel pump attached. Commercially extracted juices were found to contain (*a*) before de-aeration, and (*b*) after de-aeration: total gas, (*a*) 28·5 to 42·8, (*b*) 4·0 to 18·2; carbon dioxide, (*a*) 14·7 to 28·2, (*b*) 3·0 to 8·1; oxygen, (*a*) 2·46 to 4·17, (*b*) 0·09 to 2·39; nitrogen, (*a*) 9·7 to 11·3, (*b*) 0·9 to 7·8 ml. per litre, at 0° C. and 760 mm. The plate type de-aerators were found to be greatly overloaded in practice, and centrifugal de-aeration was the most effective type in use, the optimum conditions for which required a temperature of the juice of not less than 16° C., with a vacuum of at least 635 mm. The plate type and perforated cylinder type of de-aerator were about equal in efficiency. Oxygen in canned juices rapidly disappears, and the rate of disappearance is more rapid at higher temperatures and may adversely affect the juice, particularly as regards its vitamin C content. D. G. H.

Hydrogen "Swells" in Canned Fruits. T. P. Hoar. (*Tin and its Uses*, 1939, [3], 7.)—Representative samples of pack-rolled tinplate obtained from various sources were made into cans of a common type and packed with various fruits at Campden Research Station. These cans were stored under tropical conditions (95° F.), and as each can became a "swell" it was removed and examined. More than 1100 chemical analyses and nearly 1000 corrosion tests were made, and the separate influence of each variable was worked out by statistical methods. The main conclusions are as follows:—(1) The comparative susceptibility of a can to hydrogen swell may be assessed roughly by a corrosion test of the steel-base with a pure citric acid solution; (2) Steel-base with high copper (up to 0·2 per cent.) and also low phosphorus (less than 0·045 per cent.) gave the slowest rate of hydrogen swell in lacquered-can packs of blackcurrants, gooseberries, loganberries, Pershore plums, raspberries and strawberries; (3) Sulphur-content within the limits investigated (0·02 to 0·09 per cent.) was without influence; (4) The pack of white cherries was not influenced by the copper, phosphorus or sulphur content of the steel-base. The investigation showed that the use of tinplate with a relatively high copper and low phosphorus content will, on the average, roughly double the usual life of such packs.

Natural Ageing of Wines. E. K. Nelson and D. H. Wheeler. (*Ind. Eng. Chem.*, 1939, **31**, 1279–1281.)—The chemical changes that take place during the ageing of wines in vats have been studied. Samples of red and white wines from New York and California were analysed every 3 months during the first year, and at intervals of 6 months during the second year of ageing. Oxidation-reduction ("redox") potential measurements failed to reveal any detectable amount of a reversible redox system at the *p*H of the wines. Sulphuring caused a temporary increase in volatile acids, and, if tannin was used in clarifying, in an increase in colour and tannin. A reduction in sugar-content was noted in the Californian wines, but the sugar did not entirely disappear in 2 years. One sample showed an aldehyde content of 0·005 g. per 100 ml. after 6 months' ageing, the previous sample having only 0·0005 g. and the subsequent one a very slight trace, possibly owing to heating in transit. The aldehyde figures for another sample were very erratic. The wines from California had a considerably smaller percentage

of tartaric acid than those from New York. Except in two samples a steady increase in the amount of volatile esters was observed during ageing. Decreases in extract, alkalinity of the water-soluble ash, total tartaric acid, potassium acid tartrate, colour and tannin occurred during ageing, as the result of oxidation and precipitation of wine lees, which consisted of potassium acid tartrate and oxidised colour and tannin. Wines in hermetically-sealed glass containers, kept at room temperature, showed a greater increase in volatile esters than those stored in vats; this is in keeping with the improvement in quality found to occur in bottled goods during storage. D. G. H.

Rice Bran as a Raw Material of Oil. T. Hidaka. (*J. Soc. Chem. Ind, Japan*, 1939, **42**, 237–239B; *cf.* ANALYST, 1939, **64**, 750.)—The free fatty acids in the oil of rice bran kept in gunny bags increases rapidly on storage at a far quicker rate than in oil from meal treated with castor seed lipase and kept under similar conditions, but if water is added to each and the paste kept, the opposite results are noted. The decomposition power of the castor bean lipase is, in fact, far stronger than that of rice bran lipase. If the rice lipase is destroyed by heating, the increase in acidity in the oil is very greatly diminished. If fresh rice bran from the mill is pressed at once or after destruction of the lipase, a good oil is obtained with only a small percentage of free fatty acids, and this can be purified to produce a good edible oil. The removal of this oil by pressing has a beneficial effect on the oil still remaining in the cake; the increase in free fatty acids in one sample was only from 3·94 to 5·42 per cent. in 4 months. D. G. H.

Chemistry of Fat Spoilage. Analytical Differentiation of the Aldehydes Formed. K. Täufel and K. Klentsch. (*Fette u. Seifen*, 1939, **46**, 64–66.)—The important rôle of aldehydes in the spoilage of fats by ageing is emphasised. They affect the odour, taste and chemical reactions of the fat, and may be produced by oxidation effects resulting from the influence of heat or ultra-violet light (*cf.* Schmalfuss, Werner and Gehrke, *Voratspflege u. Lebens. Forsch.*, 1938, **1**, 98) or by the decomposition of proteins by proteolytic enzymes. Aldehydes of such origin have been classified into epihydrin aldehydes (which give a positive Kreis reaction) and "free" aldehydes, which are detected by Von Fellenberg's fuchsin-sulphurous acid test (ANALYST, 1925, **50**, 245) and determined by means of the quantitative modification of it evolved by Schmalfuss and his co-workers (*loc. cit.*). Since the fuchsin test is a group-reaction (*cf.* Täufel and Müller, *id.*, 1931, **56**, 259), it is necessary in the first instance to be able to distinguish various types of aldehydes, at any rate qualitatively, and in particular, aldehydes of high and low mol. wts. Thus, in Criegee's modification of the test the fuchsin is replaced by Döbner's violet (diamino-fuchsonimonium chloride, a diamino triphenyl methane dyestuff). This is used with sulphur dioxide in the same way as the fuchsin reagent, but the colour produced is dark violet if the aldehydes of low mol. wt. are present (*e.g.* acetaldehyde) and greenish-blue for the higher aldehydes (*e.g.* heptyl or nonyl aldehyde). On the other hand, the method is less sensitive than the fuchsin test (*cf.* Wieland and Scheunig, *Ber.*, 1921, **54**, 2527). These authors

also noted that the compound produced by the reaction between the fuchsin-sulphurous acid reagent and an aldehyde differs in its solubility in organic solvents (*e.g.* amyl alcohol or chloroform) according to the mol. wt. of the aldehyde. Thus, the compounds of the following aldehydes are red and insoluble in chloroform:—formaldehyde, acetaldehyde, propyl aldehyde, acrolein (a violet shade, and forms an emulsion in chloroform), epihydrin aldehyde, butyl aldehyde and valeryl aldehyde (violet shade). Capric aldehyde forms a violet-red compound which is soluble in chloroform to give a violet solution, and the following aldehydes form violet or violet-blue soluble compounds which appear blue-violet in chloroform:—heptyl, nonyl, undecyl, lauryl and palmityl aldehydes. The test is carried out as follows:—The sample (5 g.) is distilled with 20 ml. of saturated salt solution and a little pumice in a 300-ml. conical flask, fitted with a splash-trap and a vertical condenser (length, 20 cm.), which delivers into a 10-ml. graduated cylinder. To 10 ml. of the mixed distillate are added 1 ml. of the fuchsin and sulphurous acid reagent, and the mixture is shaken and allowed to stand for 5 minutes. This serves as a preliminary test. An appropriate smaller portion of the distillate is then used for the final test, and diluted to 10 ml. with water. Two ml. of chloroform and 2 ml. of the fresh reagent are then added (in this order), and the mixture is shaken in a 50-ml. separating funnel. If, on standing, the colour is concentrated mainly in the interface, the funnel should be manipulated so as to bring it into the chloroform layer, but in some instances it is helpful to observe the nature and colour of the emulsion. If the colour in the chloroform layer is very strong, this layer should be removed, and the residual liquid shaken with more chloroform. Since a certain amount of fractionation may occur during distillation, the distillate may contain a larger proportion of the more volatile constituents, and it is therefore desirable to collect the 10 ml. Positive results were obtained with 5 g. of paraffin containing 80γ of butyl aldehyde or 100γ of heptyl aldehyde. Satisfactory results were also obtained with 6 samples of olive oil containing various higher and lower aldehydes, together or singly. J. G.

Analysis of Chaulmoogra Oils. III. *Hydnocarpus wightiana* Oil. H. I. Cole and H. T. Cardoso. (*J. Amer. Chem. Soc.*, 1939, **61**, 2351–2353.)—The characteristics of the oil cold-pressed from selected fresh seeds of *Hydnocarpus wightiana* were:—sp.gr. 25°/25° C., 0·9549; free fatty acids (per cent. oleic acid), 2·7; saponification value, 201; iodine value (Hanus), 98·4; $(\alpha)_D$ + 55·0°; n_D^{25}, 1·4799; unsaponifiable matter, 0·25 per cent. Power and Barrowcliff (*J. Chem. Soc.*, 1905, **87**, 884) reported that the fatty acids of this oil consist mainly of hydnocarpic and chaulmoogric acids and they found evidence of a lower homologue having the formula $C_{14}H_{24}O_2$ but were unable to isolate it. They concluded from the high iodine value (140·7) that acids of the linolic or linolenic series were present. The authors have shown (*J. Amer. Chem. Soc.*, 1938, **60**, 612) that no members of these series are present, but that the high iodine value and high optical activity (+ 50·4) are due to the presence of gorlic acid. By the method of analysis previously described (*loc. cit.*) the percentage composition of the total fatty acids was found to be:—hydnocarpic acid, 48·7; chaulmoogric acid, 27·0; gorlic acid, 12·2; oleic acid, 6·5; palmitic acid, 1·8; lower homologues of chaulmoogric acid (alepric,

aleprylic, aleprestic and aleprolic acids with unidentified acids), 3·4. The fatty acids consisted of 84·2 per cent. of solid acids and 15·7 per cent. of liquid acids. The solid acids were separated from the liquid acids by crystallisation from 80 per cent. ethyl alcohol and the two fractions were converted into their ethyl esters and fractionated. The details of the separation are those described in the first paper of the series (*loc. cit.*), the only change being that the two final crystallisations were made with 80 per cent. acetone to prevent the formation of ethyl esters. Alepric acid ($C_{14}H_{24}O_2$), aleprylic acid ($C_{12}H_{20}O_2$), aleprestic acid ($C_{10}H_{16}O_2$) and aleprolic acid ($C_6H_8O_2$) are newly discovered homologues of chaulmoogric acid (Cole and Cardoso, *J. Amer. Chem. Soc.*, 1939, **61**, 2349). The presence of an optically inactive unsaturated acid with one double bond was deduced from the iodine value, but attempts to isolate it were unsuccessful. A. O. J.

Fermentation Process in Tea Manufacture. IV. Tea Tannin and its Fermentation Products. C. J. Harrison and E. A. H. Roberts. (*Biochem. J.*, 1939, **33**, 1408–1420.)—Freshly-plucked leaves were extracted with boiling water, and the infusion was extracted in succession with benzene, ether and ethyl acetate. The ethyl acetate extract on being concentrated and treated with chloroform, gave a yield of tannin 20 to 25 per cent. of the quantity of total tannins found by analysis of the original leaves. This had all the properties of a true tannin, its solution being precipitated, for instance, by quinine and lead acetate solution, but by gelatin solution only in the presence of acid and salt. The solution gave green to blue colorations when treated with ferric chloride solution. On hydrolysis with acid, no glucose was produced, but some gallic acid could usually be detected. This tannin obtained by hot aqueous extraction was not, however, the true tannin of the leaf. Extraction with cold 5 per cent. oxalic acid solution or 1 per cent. hydrochloric acid gave a tannin with a much higher specific rotation. Moreover, addition of salt precipitated only 25 per cent. of this tannin, whereas all the tannin obtained by hot aqueous extraction was precipitated by salt. *l*-Epicatechin has been isolated from the leaf, and gallocatechin has been detected therein. The galloyl ester of epicatechin appears to be present in some types of leaf only. The native tannin is probably made up of epicatechin and gallocatechin and their simple condensation products. Condensation of the catechins, resulting presumably from combination between the carbinol group of the pyran and the phloroglucinol nucleus, occurs when their solutions are heated or acidified or made alkaline. Under the last-named conditions a pronounced uptake of oxygen can be observed and the tannin gradually loses its tanning properties. From a study of the oxygen uptake, it is concluded that this autoxidation in alkaline solution is a property of the catechol or pyrogallol nucleus. The fermentation of tea tannin, gallic acid, pyrogallol and catechol by means of the peroxidase of the tea leaf was studied, and the rate of oxygen uptake was found to decrease in that order. During fermentation the decrease in reducing power (as measured by titration with potassium permanganate solution) was more rapid than would have been expected from the rate of oxygen uptake, and the discrepancy is attributed to condensation of the tannins to products of low reducing power. During manufacture oxidation of the tannins is 80 to 90 per

cent. complete, and substances with all the properties of condensed tannins are isolated from the extract of "made" tea by precipitation with acid or salt; they have a low reducing power and are precipitated by gelatin. The material precipitated by acid apparently represents the more highly condensed tannins which are responsible for the dark colour of the infusion. Since too great a proportion of this fraction is undesirable, it follows that "if quality of leaf and conditions of manufacture are reasonably constant the acid-soluble tannin-content seems largely to determine the value of the made tea." See also ANALYST, 1939, **64**, 615, 616.

F. A. R.

Determination of the Alkaloids in Preparations of *Berberis vulgaris* and *Berberis agrifolium*. K. Brunner and H. Neugebauer. (*Pharm. Zentr.*, 1939, 80; *J. Pharm. Belg.*, 1939, **21**, 575.)—The method used for the determination depends on the fact that quaternary bases in ammoniacal solution are insoluble in ether, whilst tertiary bases are soluble. The quaternary bases are reduced and extracted and phenolic bases are determined in the solution. Two g. of the root bark of *Berberis*, 30 g. of ether and 2 g. of ammonia are shaken together for 5 minutes and left with occasional shaking for 1 hour, after which 2 g. of calcined sodium sulphate are added, and the mixture is shaken and left for 10 minutes. It is filtered into a separating funnel, and the flask and filter are washed twice with 20 ml. of ether. The tertiary bases dissolve in the ether and are extracted by shaking 3 or 4 times with 10 ml. of water acidified with 0·5 per cent. sulphuric acid. Ammonia is then added, the bases are re-extracted with ether, the extract is dried with sodium sulphate, and evaporated to a few ml., a little water is added, and the rest of the ether is evaporated. The amount present is determined by titration with sodium hydroxide in presence of methyl red (1 ml. 0·1*N* sodium hydroxide solution is equivalent to 0·03041 g. of oxyacanthine). The residue in the flask and that left in the filter are united, the ether is evaporated, 30 ml. of water, 5 ml. of dilute sulphuric acid, 5 ml. of dilute acetic acid and 2 g. of zinc dust are added, and the whole is heated on a water-bath for 2 hours. The supernatant liquid is filtered into a separating funnel, and the residue is heated for 5 minutes with 10 ml. of water and 19 drops of sulphuric acid and filtered. This operation is repeated three times, and the combined filtrates rendered alkaline with ammonia, cooled and extracted with ether. The alkaloids are titrated (dimethyl yellow as indicator) and calculated as berberine. After titration the solution is rendered alkaline by the addition of 1 ml. of sodium hydroxide, and again extracted with 30 ml. of ether. Under these conditions the phenolic bases remain in the aqueous solution. The quaternary bases are titrated, and the difference between the last two titrations gives the phenolic bases.

D. G. H.

Identification of Plasmoquin by a Colour Reaction. A. E. Tchitchibabine and Ch. Hoffman. (*Bull. Soc. Pharmac.*, 1939, **5**; *J. Pharm. Belg.*, 1939, **21**, 755–756.)—The reaction of Schulemann, Schonhofer and Wingler is not specific for plasmoquin, since related substances such as plasmocide give the same colorations. The following reaction is regarded as specific and very sensitive. A feebly acid solution of plasmoquin in sulphuric acid, when treated with iodic acid, develops a violet-blue colour in dilutions up to 1 in 2,000,000. One g. of plasmoquin is

dissolved in 20 ml. of 10 per cent. sulphuric acid and made up to 500 ml. with water. From this, weaker solutions are prepared down to 1 in 2,000,000. Ten ml. of each of these solutions are mixed with 5 ml. of a 10 per cent. solution of iodic acid and allowed to stand. The colour is developed in the cold in 3 to 5 minutes according to the concentration. With plasmocide a less permanent, redder, and less violet colour is formed more slowly. D. G. H.

Contraphthisine. Communication from the Rijks-Institut voor Pharmaco-Therapeutisch Onderzoek. (*Pharm. Weekblad*, 1939, **76**, 1301–1302.)—A substance known as "Contraphthisine" has recently been placed on the market as a cure for tuberculosis. The leaflet accompanying it contains a statement by a Dr. Kalle of the University Clinic, Cologne, verifying its suitability for this purpose; it also states that contraphthisine contains "*Drosera rotundifolia, Thymus serpulli, Vinum absynthium,* Alphyton, *Mel depuratum, Saccharuum album,* Aqua dist." In the work now described it is referred to as a pale yellow liquid, having an odour similar to that of absinthe and a strongly acid reaction; sp.gr., 1·017; dry solids, 4·30; ash-content, 0·640 g. per 100 ml. The presence of the following substances was established:—potassium, aluminium, sulphate, calcium (in small quantities), invert sugar, saccharin, and approximately 3·4 per cent. of alcohol. It was not possible to identify *Drosera, Thymus* and *Absinthium* with certainty, as they have no characteristic constituents apart from plant acids (principally malic and tannic acids) and a little volatile oil. It seems therefore that "Alphyton" is alum, with saccharin to mask its taste. J. G.

New Colour Reaction for the Identification of Cocaine. M. Pesez. (*J. Pharm. Chim.*, 1939, **30**, 200–205.)—A few particles (up to 1 cg.) of the alkaloid are shaken gently in a test-tube with 2 drops of conc. nitric acid and 13 to 15 drops of pure sulphuric acid (sp.gr. 1·84), and the tube is placed in boiling water for 5 to 10 minutes. After cooling, addition of 1 ml. of water produces a canary-yellow colour. The solution is re-cooled, treated with 10 ml. of acetone and cooled again, and 5 ml. of sodium hydroxide solution (about 15 per cent. strength) are added. The tube is then shaken and inverted several times to bring the two liquids in contact. The acetone phase suddenly becomes turbid, while at the junction of the liquids a violet-blue ring is formed. On shaking, the colour of the acetone solution becomes deep azure blue, which slowly changes through violet (3 to 5 minutes) to Bordeaux red (20 to 30 minutes). With 1 mg. of cocaine the blue colour is distinct but fleeting; with a few tenths of 1 mg. a deep rose colour is finally obtained. The colour is specific for cocaine and two other local anaesthetics (delcaine and alypine); hence this reaction should be useful in toxicology. The eucaines give a violet-blue colour, and other local anaesthetics give colours varying from orange-yellow to reddish-brown in the acetone phase. Characteristic colours are also given by some other alkaloids, aromatic hydrocarbons and barbiturates (with phenyl radicals). Benzene gives an intense violet colour, owing to formation of *m*-dinitrobenzene; the reaction can therefore be used for the detection of the nitric ion. Toluene gives a slight pink colour, the xylenes a yellowish-green, and naphthalene a red colour. Normal methyl-ethyl-phenyl-malonylurea (Prominal-Isonal) also colours the acetone solution yellow, but on addition of

soda the colour changes through greenish-yellow and greyish-blue to azure blue, which is stable for a few minutes and then changes slowly through violet to the final Bordeaux red. E. B. D.

Determination of Bismuth, Iodine and Quinine in Quinine Iodobismuthate and its Injectable Preparations. G. N. Thomis and G. Ph. Kopanaris. (*J. Pharm. Chim.*, 1939, **30**, 193–199.)—The complex salt, quinine iodobismuthate, when prepared by the Codex method, has the formula $(BiI_3)_2C_{20}H_{24}N_2O_2.2HI$, but the composition varies with the method of preparation. Methods used for the determination of bismuth, iodine and quinine in the salt itself are unsuitable for its oil suspensions or its special aqueous preparations, which may contain other substances, *e.g.* chlorides, and the following is proposed for these:—(A) *Aqueous preparations.*—These solutions in ampoules usually contain 0·01 g. of bismuth per ml. Ten ml. of the filtered liquid are pipetted into a 150-ml. separating funnel, 2·5 ml. of 15 per cent. sodium hydroxide solution are added, the quinine is extracted by shaking vigorously with (*a*) 50 and (*b*) 30 ml. of chloroform, and the extracts are filtered into a weighed dish through a small, dry, defatted piece of cotton in the delivery tube of the separating funnel (length of tube, approximately 3 cm.). After each operation the sides of the funnel are washed 3 times with 4 ml. of chloroform, without shaking the liquids, and the washings are added to the principal extracts. The solution is evaporated on a water-bath and heating is continued until the initial oily residue becomes white and opaque. (This prolonged heating completely removes ethyl carbamate, if present.) The residue is treated twice with 4 to 5 ml. of ether, which is removed on the water-bath, and dried at 105° C. to constant weight. The gravimetric result may be checked volumetrically by dissolving the residue in a known volume of $N/10$ hydrochloric acid and titrating back, with methyl red as indicator, or, if a micro-burette is available, the method may be shortened by the direct titration of the chloroform extract with alcoholic $N/10$ hydrochloric acid, bromophenol blue being used as indicator (*cf.* Thomis, *J. Pharm. Chim.*, 1936, **24**, 162; Thomis and Iatrides, *ibid.*, 1935, **21**, 585.) Iodine is then determined in the quinine-free solution, to which are added 50 to 60 ml. of chloroform and then, carefully, 5 ml. of nitric acid (sp.gr. 1·4) which has been cooled to about 4° C. The separating funnel is immediately stoppered and shaken vigorously for a few minutes. After clarification the lower layer is run off into a 500-ml. conical flask (the cotton filter having first been removed from the tube), the funnel is washed twice with 5 ml. of chloroform, and the extraction and washing are repeated first with 30 ml. and then with two 5-ml. portions of this solvent. After addition of 1 to 2 g. of pure sodium bicarbonate and 120 to 130 ml. of 95 per cent. alcohol to the combined chloroform extracts, the iodine is titrated with $N/10$ sodium thiosulphate solution. The nitric acid solution, free from quinine and iodine, is decanted or filtered, if necessary, into a 400-ml. beaker, the chloroform is gently boiled off, and the large excess of acid is nearly neutralised with sodium hydroxide solution and, after dilution of the still acid liquid to 200 ml., the bismuth is precipitated with hydrogen sulphide at 20° C. The precipitate is filtered off, washed well with hydrogen sulphide water, alcohol, ether and, finally, re-distilled carbon disulphide

until all free sulphur is removed. It is then dried for an hour at 100° C. and weighed, counterpoised filter papers being used for filtration and weighing. (B) *Solid quinine iodobismuthate.*—Exactly 0·5 g. of the salt is shaken in a 150-ml. separating funnel with 5 ml. of 30 per cent. sodium hydroxide solution until all red particles have disappeared, the salt which finally adheres near the stopcock being detached by water from a washing-bottle. The solution is shaken well for 2 to 3 minutes with 50 ml. of chloroform, 1 to 1·5 ml. of saturated tartaric acid solution is added, and the mixture is again shaken vigorously for 5 minutes and then allowed to stand for 10 minutes. The rest of the determination is as before. (C) *Oily suspensions.*—Ten ml. of the thoroughly mixed suspension are treated with excess of ether, the liquid is decanted through a weighed Schott No. 4 filter with porous glass plate, and the precipitate is washed first by decantation and then on the filter with ether until the washings no longer give an oily residue on evaporation. The precipitate is dried at 100° C. to constant weight and the quinine iodobismuthate in the suspension is calculated. The rest of the analysis is as for (B). *Remarks.*—Analysis of the salt itself showed the presence of an appreciable amount of sodium chloride as impurity. The method is accurate, very simple and rapid (complete analysis requires 3 to 4 hours).
E. B. D.

Chemical Assay of Powdered Ergot. C. Daglish and F. Wokes. (*Quart. J. Pharm.*, 1939, **12**, 451–467.)—The extraction of the total alkaloids of ergot by different methods was investigated. The method of the British Pharmacopoeia, 1932, employing a single half-hour shaking with alkaline ether at room temperature, did not extract the alkaloid completely, but the efficiency was increased to about 80 per cent. when mechanical shaking was employed. It was further increased in high potency ergots by decreasing the drug/menstruum ratio. The method of Hampshire and Page (*Quart. J. Pharm.*, 1936, **9**, 60; *cf.* ANALYST, 1936, **61**, 489), employing a single 5-hour extraction, also failed to give complete extraction, even when the amount of ammonia was increased or when dilute ammonia was used. The average efficiency was 78 per cent., but was higher with ergot of low potency. In both methods the reduced efficiency is due to incomplete extraction, since more alkaloid could be obtained by re-extraction of the marcs. The B.P. method gave slightly higher results than Hampshire and Page's method when both were carried to exhaustion, owing, it is believed, to destruction of some of the alkaloid by heat in the latter method. An improved method of extraction has been devised; it gives an average efficiency of 93 per cent. and yields results not significantly different from those obtained by the B.P. method when both are carried to exhaustion. In this method 5 g. of ergot are shaken mechanically for 30 minutes with a mixture of 49 ml. of acetone and 1 ml. of 10 per cent. ammonia. The acetone extract is filtered, and 40 ml. of the filtrate are mixed with 80 ml. of anaesthetic ether and shaken with four 10 ml.-portions of aqueous 1 per cent. tartaric acid solution. The combined tartaric acid extracts are warmed to remove acetone and ether, cooled, and made up to 50 ml. with tartaric acid solution. One re-extraction of the marc is sufficient to give a reliable figure for the total alkaloids. The authors also show that the experimental errors introduced by the use of a colorimeter are considerably reduced by taking 8 readings instead

of 2, by taking readings against 15-mm. and 20-mm. depths of standard, by comparing solutions differing in colour intensity by not more than 10 per cent., and by eliminating traces of yellow or red colour in the blue colours matched by taking care to remove as much fat as possible from the ergot before beginning the assay.

F. A. R.

Colorimetric Determination of Ergotoxine and Ergometrine. S. A. Schou and J. Bennekou. (*Dansk Tidsskr. Farm.*, 1938, **12**; *J. Pharm. Belg.*, 1939, **21**, 477–478.)—It is shown that the colour reaction given by these alkaloids with *p*-dimethylaminobenzaldehyde and described in the British Pharmacopoeia, 1932, and the modified reaction described by Allport and Cocking (*Pharm. J.*, 1932, **129**, 235; Abst., ANALYST, 1932, **57**, 725) are identical and may be used for quantitative determinations with the Pulfrich photometer. When an aqueous solution of the alkaloids of *p*H 6 is shaken with ether, ergotoxine passes completely into the ethereal layer, and 90 per cent. of the ergometrine remains in the aqueous layer. If the ethereal layer is shaken with a fresh portion of water of *p*H 6, 9/10ths of the ergometrine that it contains is removed, so that an almost complete separation of the alkaloids is effected.

A. O. J.

Biochemical

Effects of Sulphur Dioxide on Vegetation. G. S. Whitby. (*Chem. and Ind.*, 1939, **58**, 991–997.)—The work described originated in complaints of damage, due to sulphur dioxide from the largest non-ferrous smelter (dealing with concentrates of lead and zinc sulphides) in the British Empire, at Trail, British Columbia. The emission of this gas in the peak year (1930) amounted to about 550 tons per day, and, owing to the situation of the works (in a deep valley), the problem became so acute that an International Joint Commission was appointed to investigate it (see also M. Katz *et al.*, "*Effect of Sulphur Dioxide on Vegetation,*" Ottawa; National Research Council, 1939). The main conclusion reached by the Commission was that the damage is trifling so long as the following conditions are observed:—If at any time at a point 7 miles south of the smelter the concentration of sulphur dioxide in the air exceeds 1 p.p.m. for 3 consecutive 20-minute periods, the emission of the gas should be reduced until the concentration at the point in question falls to 0·5 p.p.m. Most of the argument in connection with the Trail complaint centred on the lower concentrations, since it was contended that concentrations of the gas that produced no visible markings on vegetation could, nevertheless, cause injury. Climatic factors, which, as a rule, favour the occurrence of the gas in high concentrations for relatively long periods of time, include low winds, low temperatures, high humidities and, especially, fog or mist. The sulphur dioxide concentrations were recorded continuously by aspirating air through a conductivity cell containing very dilute hydrogen peroxide, which converted the gas into sulphuric acid; the corresponding increase in conductivity was obtained by means of an automatic recording Wheatstone bridge (*cf.* Thomas, *Ind. Eng. Chem., Anal. Ed.*, 1932, **4**, 253). After 20 minutes the air was automatically diverted to a second similar cell for the next 20 minutes, so that the first cell could be renewed. The investigations showed that the sulphur-content of the

surrounding vegetation can be abnormally high at distances from the smelter greater than those at which visible injury to field crops and trees occurs; this is because the sulphur-content is influenced by both the time of exposure and the concentration of the gas, and because in some instances (*e.g.* the needles of coniferous trees) the increase in the sulphur-content is cumulative. Fumigation experiments indicated in fact that, as a rule, the sulphur-content may rise to 4 times the normal value without producing any injury; this probably is because small doses of the gas are assimilated and oxidised to sulphate, whereas heavy doses extending over a short period may result in damage without raising the sulphur-content appreciably (see below). The *p*H and exchangeable-base content of the soil near the smelter (especially in the top 1 inch) were lower than the normal values, but this reduction fell off rapidly at greater distances from the smelter, being almost negligible 8 miles away, although injury to the leaves of plants was then apparent. The sulphate-contents of the water-supplies in the area were not affected, but seemed to depend rather on the nature of the formation through which the water had seeped. Studies of the growth-rates of 10,000 trees (Douglas fir and yellow pine) by comparisons of the widths of the annual rings of trees, inside and outside the area, showed that, whilst the growth rates outside the area could usually be correlated closely with the rainfall, those inside did not respond to this form of stimulation. Experiments with 43 groups of conifers (450 trees) showed that they were far more susceptible to injury at the time of active growth than during periods of dormancy. Transplanted conifers (and especially the larch) were more susceptible than conifers in their natural habitat, and seedlings were less resistant than the older trees. The importance of humidity and of the intensity of the sunlight are illustrated by experiments with barley and alfalfa, which showed that, in general, all factors that favour the opening of stomata and a condition of leaf turgor (corresponding with active photo-synthesis) promoted absorption of sulphur dioxide and, consequently, injury. The moisture-content of the soil also influences leaf turgor, and it was found that wilting plants were more resistant in a dry than in a wet soil. Photo-synthetic activity also varies with the age of the plant, and, consequently, barley was found to be most susceptible when young and alfalfa at middle-age. Measurements of the stomatal openings by three different methods enabled the effects of sulphur dioxide to be traced. Thus, continuous fumigation with 0·40 p.p.m. of gas was without effect for the first 4 days, but subsequently the stomata opened to only 65 per cent. of the normal extent; just prior to this, signs of acute injury were first evident, and after 1 week this was apparent in 27 per cent. of the leaves. Barley is the most sensitive of the cereals, although less sensitive than alfalfa; yet it was shown that continuous exposure of the latter to a low concentration of gas (*e.g.* 0·30 p.p.m.) for a long period (*e.g.* 66·5 hours) injured less than 1 per cent. of the leaves and did not affect the yield. As a rule the chemical composition of the plant was unaffected, although when injury was severe there was some reduction in the contents of sucrose and polysaccharides of alfalfa. The assimilation and respiration of carbon dioxide were measured by passing the air entering and leaving the fumigation cabinets through sintered glass plates in a conductivity cell containing 0·0052 *N* sodium hydroxide solution and a little butyl alcohol (to prevent foaming). If the contents of the cell were

renewed every 2 minutes, the changes in conductivity indicated the carbon dioxide concentration with a sensitiveness of 1 p.p.m. Such measurements were found to be a much more sensitive index of the effects of the gas than the appearance of visible markings, especially where short exposures to high concentrations were concerned (see above); thus, a shock-effect is produced, followed by a recovery without necessarily any visible signs of injury. In general, up to 0·5 p.p.m. was without effect on the photo-synthesis of alfalfa, but when this concentration was exceeded reduced assimilation and increased elimination of carbon dioxide in the day and night, respectively, resulted; this effect corresponds with that produced by a sudden reduction in the intensity of the light. Since recognisable injury to the leaves seldom followed such treatment, it is concluded that sulphur dioxide may inhibit temporarily the chlorophyll mechanism without actually injuring the chlorophyll itself or the protoplasm. J. G.

Method of Ashing Soft Tissues Preliminary to the Determination of Cations. M. V. Buell. (*J. Biol. Chem.*, 1939, **130**, 357–363.)—The two methods most frequently used for ashing tissues are ignition in a muffle furnace and wet digestion with sulphuric acid. There are serious objections to both, and a method has now been developed for digesting the dried tissue, freed from fat, with nitric and perchloric acids at a low temperature. A sample of the moist tissue, weighing 10 to 15 g., is minced and introduced into a tared 140-ml. stout conical flask (Pyrex) fitted with a standard ground-glass joint. The flask is reweighed, 15 ml. of pure acetone are added, and the mixture is thoroughly stirred with a rod. The flask, with the rod, is then placed on a steam-bath until the acetone has evaporated, after which it is transferred to a cold electric oven, and the temperature is raised slowly to 105° C., and maintained at that level for 24 hours. The mixture is ground in the flask with the rod, which is then removed, and the flask is re-weighed, the loss representing the moisture-content of the tissue. The lipids are removed by extracting the tissue successively with 20-, 10- and 10-ml. portions of warm anhydrous ether and three 10-ml. portions of warm low-boiling petroleum spirit. The tissue is dried as before and re-weighed. Ten ml. of water and 10 ml. of conc. nitric acid are added to the dry fat-free residue, the flask is placed on an electric hot plate covered with a sheet of asbestos, and the low heat is turned on. After the volume has been reduced to about 10 ml. and the tissue has dissolved (1 to 2 hours), 3·5 ml. of 70 per cent. perchloric acid are added, and the heating is continued until ashing is complete and the residue is almost dry (20 to 24 hours). As the residue approaches dryness, the sides of the flask are washed down with a fine jet of water, and the solution is evaporated to dryness. While the flask is still warm 12·5 ml. of water are added from a burette, and a ground-glass stopper is inserted. The ash dissolves slowly but completely, and the resulting solution is suitable for analysis without further treatment. Calcium should be precipitated at *p*H 4·2 with the aid of bromocresol green, to avoid contamination with magnesium. Phosphates must be removed prior to the determination of sodium, and phosphates and ammonium prior to the determination of potassium. Both results are achieved by adding to the solution in the digestion flask a small drop of 0·1 per cent. alcoholic phenolphthalein solution and just sufficient solid calcium

hydroxide to make the solution definitely alkaline. The solution is filtered after standing for an hour. Ammonium ion is removed from an aliquot portion by evaporating to dryness on a hot-plate, and the residue may be used for the determination of potassium. Almost quantitative recoveries of calcium, magnesium, sodium and potassium were obtained when known amounts of these ions were added to beef muscle. F. A. R.

Colorimetric Semi-Micro-Determination of Arsenic in Urine. J. V. Harispe. (*J. Pharm. Chim.*, 1939, **30**, 58–70.)—The author's method for the determination of arsenic in urine is a modification of that of Denigès, in which the arsenic sol is stabilised by silicic acid. It can be used to study the elimination of arsenic after the administration of arsenic-containing medicaments. From 1 to 50 ml. of urine (containing 50 to 1000 mg. of arsenic) are treated in an evaporating dish with 5 g. of crystalline magnesium nitrate and evaporated to dryness on a water-bath, and the dish is then heated for 3 to 4 minutes in a muffle furnace at a dull red heat. After cooling, the residue is moistened with water and taken up in 10 ml. of conc. hydrochloric acid. The liquid is again evaporated on the water-bath and the dish is heated in the oven for a few minutes to destroy traces of nitrites. After cooling, 10 ml. of Bougalt's hypophosphorous and hydrochloric reagent (*J. Pharm. Chim.*, 1907, **26**, 13) are added, and the liquid is stirred and treated, drop by drop, with 5 ml. of a solution of 100 ml. of potassium silicate solution (sp.gr. 1·28; SiO_2, 20·3 per cent.; K_2O, 9·47 per cent.) in 500 ml. of water. The resulting colourless liquid is treated with 1 drop of $N/10$ iodine solution and made up to 20 ml. It is of advantage at this stage to centrifuge the liquid for a few seconds in a perfectly dry tube; this is particularly to be recommended if the colour is later to be determined by a photometric method. The clarified liquid is heated in a dry degreased Pyrex test-tube (conveniently 16 $\times$ 180 mm.) in a water-bath for 30 minutes and then allowed to cool in air.

The Standard Colours.—As the presence of mineral salts slightly alters the final colour there is used instead of water in the preparation of the standard colours a solution of 300 g. of magnesium chloride hexahydrate in 180 ml. of water. A quantity of 0·4165 g. of officinal sodium arsenate in the form of transparent non-efflorescent crystals ($\equiv$ 100 mg. of arsenic) is dissolved in the magnesium chloride solution and the volume is made up to 100 ml. with the chloride solution to give the main standard solution. Pyrex test-tubes identical with the first (the diameters must be the same to within 0·5 mm.) are chosen; into eleven of these there are put 0, 0·5, 1, 1·5, 2, 2·5, 3, 3·5, 4, 4·5 and 5 ml. of a 1: 10 dilution of the main solution, and into a further five tubes are put 3, 3·5, 4, 4·5 and 5 ml. of a 1: 5 dilution of the main solution. The volume in each tube is made up to 5 ml. with the magnesium chloride solution, and there are added to each tube 10 ml. of Bougalt's reagent, 5 ml. of the diluted potassium silicate solution, and 1 drop of $N/10$ iodine solution. The tubes are then heated for 30 minutes in a boiling water-bath. To avoid evaporation of the water which is exuded from the upper surface of the gel by syneresis it is advisable to cover the upper surface of the liquid with a few ml. of vaseline oil after cooling; this reduction of evaporation is assisted by using long narrow test-tubes. Such standard colours are

usable for about a year if the tubes are not shaken and if the colour of the liquid in the bottom part of the tube is used for comparison. The quantities of arsenic in the tubes are 0, 0·05, 0·10, 0·15, . . . 0·50, 0·60, . . . 0·90, 1 mg. Directions are also given for making the determination by means of a photo-colorimeter.

E. M. P.

Relation of Cuprous Creatinine to Tests for Sugar in Urine. M. Samson. (*J. Amer. Chem. Soc.*, 1939, **61**, 2389–2392.)—Creatinine, owing to its own reducing power and its property of dissolving cuprous oxide, is the chief source of trouble in determining glucose in urine. Benedict (*J. Biol. Chem.*, 1908, **5**, 484) believed the solution known by his name to be more sensitive to glucose, either in pure solution or in urine, than is Fehling's solution. This is not correct, as some aqueous glucose solutions that yield typical Trommer or Fehling reactions give only an almost imperceptible haze with Benedict's solution. For urinary work, however, Benedict's solution is unexcelled, since it gives bulky turbidities with concentrations of glucose to which other reagents are not sensitive, and clear solutions with urine containing no glucose but responding to the Trommer and Fehling tests. When 0·1 per cent. of glucose is added to the filtrate obtained after treating normal urine with blood charcoal, the mixture gives with Benedict's test the slight haze given by an aqueous solution of glucose of the same concentration. If, however, Benedict's reagent is added to the untreated urine containing this amount of glucose, an opaque turbidity appears, and this indicates that the charcoal removes a promoter from the urine. Lloyd's alkaloidal reagent, a type of fuller's-earth used by Folin and Berglund (*J. Biol. Chem.*, 1922, **51**, 209; Abst., ANALYST, 1922, **47**, 268), has an action similar to that of the charcoal. Of the substances known to be removed from urine by Folin and Berglund's method, creatinine alone was found to be a promoter of the reaction. When the correct amount of creatinine is added to aqueous solutions of glucose they respond to Benedict's test in the same way as urine containing glucose. Experiments on the reduction of Benedict's solution by glucose and creatinine at temperatures below boiling-point showed that at 60° C. a bulky, slightly yellow precipitate and at 50° C. a voluminous white one that agglutinated easily were formed. Analysis of this precipitate proved it to be a compound of cuprous oxide and creatinine containing one atom of copper to one molecule of creatinine, probably the simple addition compound $C_4H_7ON_3.CuOH$. Although susceptible to oxidation, cuprous creatinine is otherwise very stable, the bond being resistant to alkali. The complex is very soluble in alkali hydroxide solutions, but in sodium carbonate solutions it is insoluble and very voluminous. The sensitivity of Benedict's test and the delay in precipitation from solutions containing alkali hydroxide are therefore explained. It is well known that, in addition to creatinine, there are other substances normally present in urine or resulting from pathological states or derived from administered medicaments which interfere with the course of alkali-copper reactions. Reduction by creatinine has a greater interfering effect in those tests that use a larger proportion of urine to reagent (the Trommer and most variations of the Fehling tests) than in Benedict's test, in which the urine comprises less than 10 per cent. of the mixture. Even in this restricted proportion of urine,

creatinine has interfering properties as well as the desirable sensitising effect. Creatinine promotes precipitation only in a range of concentration from 0·03 to 0·15 per cent.—the range in normal urine. This concentration of creatinine sensitises the Benedict test for the range of glucose (0·05 to 0·3 per cent.) that gives doubtful reactions with other tests. A higher creatinine-content, fortunately not common in urine containing 0·1 per cent. of glucose, delays the appearance of the opacity beyond the 3 minutes' heating on the water-bath that is recommended (Folin and McEllroy, *J. Biol. Chem.*, 1918, **33**, 513; Abst., ANALYST, 1918, **43**, 299), and the 0·4 per cent. creatinine level reported by Folin (*Amer. J. Physiol.*, 1905, **13**, 66) for a starch and cream diet prevents the precipitation entirely, as it would in any other copper reduction test. Concentrations of creatinine below the optimum range, such as may occur after copious water intake, cause the urine containing glucose to act in the same manner as an aqueous solution, and the faint red haze obtained with Benedict's solution would be considered negligible in accordance with the author's instructions. From these considerations it may be possible to devise a more nearly infallible test sensitive to any significant concentration of glucose in urine. A. O. J.

Chemical Estimation, Stability, and Form of Aneurin in Urine. D. Melnick and H. Field, Jr. (*J. Biol. Chem.*, 1939, **130**, 97–107.)—The method of estimating aneurin previously described (*cf.* ANALYST, 1939, **64**, 367–369) has been found to give low results when applied to urine, since the presence of large amounts of salts prevents the complete adsorption of the vitamin on the zeolite. Accordingly, aneurin must first be extracted quantitatively from the urine (for which purpose benzyl alcohol is most convenient), and then transferred back to aqueous solution; the estimation is then carried out as previously described. The urine is collected in a bottle containing 10 ml. of toluene and enough 10 per cent. sulphuric acid (20 ml. for a 24-hour specimen) to maintain the *p*H at less than 3. The sample is adjusted to *p*H 5 and concentrated under reduced pressure in a distillation-flask fitted with a tap-funnel containing benzyl alcohol. Anhydrous sodium sulphate (10 g. to a 12-hour sample) is added to the urine to prevent the residue obtained after concentration from becoming gummy, and a few glass beads are added to prevent bumping. When salts begin to separate out, 80 ml. of benzyl alcohol are added, and the concentration is continued until only a few ml. of water remain. The mixture is centrifuged, and the benzyl alcohol layer is removed. The aqueous phase is shaken with more benzyl alcohol (40 ml.) and the mixture again centrifuged. The combined alcoholic extracts are shaken with an equal volume of acidulated water and 4 volumes of ether, and the solvent layer is again extracted with acidulated water. The combined aqueous phases are extracted with ether, and then freed from ether by distillation under reduced pressure. The aqueous extract, now at *p*H 3·5 to 4·5, is subjected to permutit adsorption and elution by the standard procedure. The entire potassium chloride eluate (10 ml.) is poured into a 100-ml. centrifuge bottle, the adsorption tube is washed with an equal volume of 95 per cent. ethyl alcohol containing 50 mg. of phenol, and the washing is added to the eluate. A little thymol blue is added to the mixture, followed by sodium hydroxide solution (40 per cent. solution at

first, then N solution for the final adjustment) dropwise until a faint blue colour appears. Immediately, 20 ml. of the diazotised *p*-aminoacetophenone reagent (Prebluda and McCollum, *cf.* ANALYST, 1939, **64**, 366) are added, and the bottle is stoppered and allowed to stand overnight. The standard solution is prepared in similar fashion with 50γ of aneurin in 0·5 ml. of acidulated water added to 9·5 ml. of a blank potassium chloride eluate. On the following day the coloured vitamin derivative is extracted with xylene, and the colour of the xylene layer from the unknown sample is compared with that from the standard in a micro-colorimeter. The recovery of added aneurin was about 90 per cent., and duplicate experiments gave results that varied by less than $\pm$ 5 per cent. The failure to obtain complete recovery is attributed to the presence of salts not completely removed by the benzyl alcohol treatment. Almost theoretical recovery was obtained by using smaller aliquot portions of urine. The method appears to be specific for aneurin, and numerous drugs were found to give no interfering colour reactions. The vitamin in the urine, preserved in the way recommended, is stable for at least a month. The vitamin is excreted as free aneurin, no phosphorylated material having been detected. F. A. R.

Semi-Micro-Estimation of Amino Acids. H. R. Ing and M. Bergmann. (*J. Biol. Chem.*, 1939, **129**, 603–607.)—An apparatus was designed for the estimation on a semi-micro scale of amino acids in protein hydrolysates by precipitation as sparingly soluble salts. An essential requirement of the method is that the mixture of amino-acids and reagent should be agitated and the amino-acid salt filtered under temperature conditions as constant as possible. A correction for the impurities present in the mother liquor that adheres to the precipitate and filter has to be applied, since the salt cannot be washed. The reaction mixture is placed in a small bottle (in one apparatus of 5 ml., in another of 1·5 ml. capacity), upon the neck of which rests an inverted sintered glass micro-funnel of appropriate size. Both are weighed at the beginning of the test. The whole is enclosed in a suitable centrifuge tube (turned upside down), which is closed by a tightly fitting rubber stopper. Several of these tubes are suspended vertically, stopper downwards, in an ice-bath in a suitable apparatus which rotates backwards and forwards. After being thus agitated for 24 to 48 hours, the centrifuge tubes are removed from the bath, dried and immediately inverted into large centrifuge pots packed with crushed ice and water, the tubes being held vertically by cork collars. In each the small bottle, containing the reaction mixture now rests upside down on the filter, and by centrifuging for 5 to 10 minutes at 2500 to 3000 r.p.m., filtration is rapidly and completely effected, the filtrate collecting in the bottom of the centrifuge tube. The bottle and filter are weighed immediately and re-weighed after being dried in a desiccator to constant weight. The loss of weight on drying enables a correction to be made for the solids contained in the mother liquor adhering to the precipitate and apparatus, provided that the total solids present in the original reaction mixture is known. Thus from the uncorrected weight (m_2) of the precipitate, a weight equal to $x(m_1-m_2)/(v-x)$ must be subtracted, where x is the loss of weight in g. on drying and m_1 is the total solids originally present in the volume v ml. of solution used. The correction

can, of course, be checked by washing the dry amino-acid salt on the filter with an ice-cold saturated solution of the pure amino-acid salt. Examples are given of the estimation of glycine and alanine with sodium dioxypyridate, leucine and arginine with naphthalene-β-sulphonic acid, and proline with ammonium rhodanilate. In no instance did the observed value differ by more than 2 per cent. from the theoretical value. F. A. R.

Distribution of the Fatty Acids in Halibut Intestinal Oil, with a Note on the Presence of Free Fatty Acids in the Intestines of Fish. J. A. Lovern and R. A. Morton. (*Biochem. J.*, 1939, **33**, 1734–1739.)—If the suggestion previously advanced is correct (*cf.* ANALYST, 1939, **64**, 444), that intestinal vitamin A assists in the process of fat absorption in fish, then one would expect to find vitamin A esterified with all the fatty acids concerned, the amounts of the various esters present being in the same proportion as the individual fatty acids in the fat. To examine this suggestion, a large quantity of halibut intestines was extracted with ether, and the resulting oil was first separated by treatment with acetone into soluble "fat" and insoluble "phosphatides." The "fat" contained 73 per cent. of free fatty acids, 6 per cent. of vitamin A and 21·5 per cent. of unsaponifiable matter. Free fatty acids were next (incompletely) removed by treatment with alkali, then the residual oil was freed from cholesterol by cooling the acetone solution, and finally the liquid fraction was subjected to molecular distillation. The acid components of the various distillation fractions were analysed by standard methods, and the composition so derived was compared with the compositions of the fatty acid component of the undistilled neutralised oil, of the free fatty acids and of the phosphatides obtained in the same way. The neutralised oil had substantially the same fatty acid composition as the fatty acids washed out from it, but the phosphatide fatty acids were appreciably different. The composition of the fatty acids of the high-boiling fraction, presumably containing most of the vitamin A esters, was also very similar to that of the free fatty acids. Thus the evidence supports the hypothesis that vitamin A assists in fat absorption processes; it seems unlikely that the phosphatides are directly concerned in the process. Examination of freshly caught fish showed that appreciable quantities of free acid are present in the living gut. F. A. R.

Vitamin A Content of Cheese. A. W. Davies and T. Moore. (*Biochem. J.*, 1939, **33**, 1645–1647.)—A sample of English Cheddar cheese was examined for vitamin A and carotene by the colorimetric method. Vitamin A, as determined by the antimony trichloride test applied to the unsaponifiable fraction, was present in an amount equivalent to 3 I.U. per g., and carotene, determined by the tintometer, in an amount equivalent to 6 I.U. (3·5γ) per g. or 3 I.U. (1·8γ) per g. when the estimation was carried out on the unsaponified fat and on the unsaponifiable matter respectively. Thus the total vitamin A activity of the cheese was 6 to 9 I.U. per g. or 18 to 27 I.U. per g. of fat. This value was confirmed by biological assay, which indicated a potency of about 7·5 I.U. per g.—a value in agreement with the potency of an average milk-fat. Values of the same order were obtained by the colorimetric method with other cheeses made from whole milk, namely,

Camembert, Cheshire, Empire red, Empire white, Gruyère and Stilton, but smaller amounts of vitamin were found in cheeses of lower fat-content such as Dutch Edam and Danish blue. F. A. R.

Seasonal Variations in the Vitamin A Content of Certain Visceral Organs of the Geelbek or Cape Salmon (*Atractoscion acquideus* C. and V.). C. J. Molteno and W. S. Rapson. (*Biochem. J.*, 1939, **33**, 1390–1393.)—The liver oil and the visceral oil (*i.e.* the combined oils from the pyloric caeca and the intestines) of female geelbek were obtained by extracting the minced tissues with ether after desiccation with anhydrous sodium sulphate. Samples were examined each month between November and May of the following year, fish weighing between 12 and 15 lb. being selected. As far as possible, the fish in each month's sample had livers of approximately the same size. The vitamin A content of each oil was measured spectrophotometrically, and the iodine value was also determined. Fish caught in November had small livers with a very low oil content. This oil had a very high vitamin A content, equivalent to nearly 25 per cent. of the weight of the oil, and a low iodine value. As the season advanced and the fish began to feed intensively the liver increased in size and in oil-content, but the concentration and the total amount of vitamin A in the liver oil decreased, and the iodine value increased. Thus the fall in the concentration of vitamin A cannot be accounted for simply by infiltration of fat. About February or March the amount and concentration of vitamin A in the liver oil began to increase, the iodine value fell, and the liver decreased in size. The visceral oil, on the other hand, contained only a small amount of vitamin A in November, and until May this gradually increased to a high value. The diet of the geelbek during the period of intensive feeding is a very fatty one, and the observation that the vitamin A content of the viscera increases during this period is believed to support the suggestion made by Edisbury, Morton, Simpkins and Lovern (*cf.* ANALYST, 1938, **63**, 358) that vitamin A assists in the process of fat assimilation in certain fish. F. A. R.

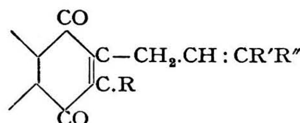
Effect of Soil Treatment on the Vitamin B_1 Content of Wheat and Barley. P. C. Leong. (*Biochem. J.*, 1939, **33**, 1397–1399.)—Five specimens of flour, obtained from wheat grown on plots receiving respectively no manure, dung, a complete mineral manure, ammonium sulphate only, and a complete mineral manure mixed with ammonium sulphate, showed no significant differences in vitamin B_1 content. The values obtained by the bradycardia method were 1·0 to 1·3 I.U. per g. Flour from barley grown on plots similarly treated also showed no significant differences, except that in the 1935 crop a higher vitamin B_1 content (2·0 I.U. per g.) was found in the flour from the plot treated with dung. The other specimens gave values of 0·8 to 1·3 I.U. per g. F. A. R.

Factors Preventing Oxidation of Ascorbic Acid in Blood Serum. E. M. Mystkowski and D. Lasocka. (*Biochem. J.*, 1939, **33**, 1460–1464.)—The atmospheric oxidation of ascorbic acid to dehydroascorbic acid is catalysed by copper, and the reaction is inhibited by substances that form complexes with copper. Serum globulin has now been found to have such an effect, but not to such an

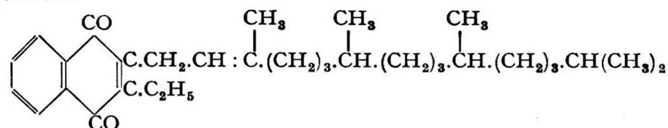
extent as might be anticipated. This is ascribed to the presence, in the globulin, of protein-copper complexes that retain a certain catalytic activity. Casein had a higher inhibitory action than serum globulin, and gelatin a somewhat lower activity than casein. The chloride ion appears to be of particular importance in inhibiting the oxidation of ascorbic acid, and its action is independent of the nature of the cation, and so may be exerted in the tissues and in the serum. The fluoride ion is inactive. (*Cf.* Høygaard and Rasmussen, *Nature*, 1938, **142**, 293.)

F. A. R.

Blue Alkali Salts of α-Phylloquinone (Vitamin K_1) and Similar Compounds. P. Karrer. (*Helv. Chim. Acta*, 1939, **22**, 1146–1149.)—Treatment of solutions of α-phylloquinone (vitamin K_1) or of vitamin K_2 with sodium alcoholate solutions leads to the formation of an intense blue colour, similar to that found by C. Liebermann (*Ber.*, 1898, **31**, 2903; 1899, **32**, 260, 916; F. Michel, *Ber.*, 1900, **33**, 2402; Liebermann and Lanser, *Ber.*, 1901, **34**, 1543). Analogy with these Liebermann compounds, the structural formulae of which are quoted in this paper, lead to the suggestion that vitamin K contains the group



in its molecule; this was confirmed by MacCorquodale, Binkley, Thayer and Doisy (*J. Amer. Chem. Soc.*, 1939, **61**, 1928), who suggested as the probable formula for α-phylloquinone—



E. M. P.

Isolation of α-Spinasterol from Alfalfa. E. Fernholz and M. L. Moore. (*J. Amer. Chem. Soc.*, 1939, **61**, 2467–2468.)—During experiments to isolate vitamin K from alfalfa, small amounts of a crystalline sterol of m.p. 168° C. were obtained. It was identified as a sterol by the formation of a digitonide, and by the Liebermann, Salkowski and Tortelli–Jaffé reactions, and its properties suggested that it might be identical with α-spinasterol first isolated from spinach (Hart and Heyl, *J. Biol. Chem.*, 1932, **95**, 311), and subsequently found in senega root (Simpson, *J. Chem. Soc.*, 1937, 730). There is little doubt that medicagosterol II, isolated by Dam *et al.* (*Helv. Chem. Acta*, 1939, **22**, 313) is α-spinasterol. The substance found in alfalfa yielded an acetate with m.p. 183° C. and a dinitrobenzoate with m.p. 195° C. and, when the difficulty of purifying it is taken into account, its properties were found to agree closely with those found by Dam (*loc. cit.*) and by Larsen and Heyl (*J. Amer. Chem. Soc.*, 1934, **56**, 2663). A study of its catalytic hydrogenation provided further evidence that it is a sterol, one molecule of hydrogen being absorbed in the presence of Adams's catalyst. The resulting compound was still unsaturated, and the m.p. and $(\alpha)_D$ of the dihydrosterol and its acetate agreed well with those reported for the corresponding compounds prepared from α-spinasterol. Titration with perbenzoic acid indicated that

3 atoms of oxygen were consumed by the sterol and 2 by the hydrogenated product. Larsen and Heyl (*loc. cit.*) state that the corresponding amounts of oxygen absorbed by their specimen were 2 atoms and 1 atom respectively. It is not rare to find sterols reacting with more perbenzoic acid than the number of double bonds would indicate, and the results quoted do not necessarily suggest the presence of three double bonds. Alfalfa meal, which is probably the most accessible source of the sterol, yielded 0·8 g. of α-spinasterol from 3·8 kg. (this figure is given as 2·8 kg. of dehydrated alfalfa later in the paper). The meal was percolated with hexane, and from the extract (140 g.) the dark brown, waxy, unsaponifiable fraction (28 g.) was separated and purified by solution in hot acetone. The purified product (20 g.) was dissolved in hexane and washed five times in succession with an equal volume of 95 per cent. methyl alcohol saturated with hexane. This residue (11 g.) was dissolved in alcohol, and the crystalline deposit of sterol forming in two days was recrystallised from a mixture of alcohol and benzene. The final product (0·8 g.) melted at 165° C. and after nine more recrystallisations the m.p. was raised to 168° C. When mixed with a specimen of α-spinasterol (m.p. 169° to 171° C.) the m.p. was 168° to 169° C. Ultimate analysis of the compound agreed with the formula $C_{20}H_{48}O.\frac{1}{2}H_2O$. The α-spinasterol thus obtained was converted into α-spinasteryl-*m*-dinitrobenzoate, α-spinasteryl acetate, and by hydrogenation into α-spinastenyl acetate, which on saponification yielded α-spinastenol. The ultimate analyses of these compounds agreed with their empirical formulae.

A. O. J.

Vitamin P. H. Scarborough. (*Biochem. J.*, 1939, **33**, 1400–1407.)—Vitamin P was defined by Szent-Györgyi as a factor that "brought back the fragile and permeable capillaries to their normal state." The evidence upon which this claim rested was derived from observations made on patients with vascular purpura, thrombocytopenic purpura, infectious diseases, myxoedema and diabetes mellitus, and is regarded by the author as unsatisfactory for deciding whether or not there is a vitamin P. Three substances containing flavanones have now been tested:—crude hesperidin obtained during the desiccation of orange juice, purified hesperidin (m.p. 255° to 256° C.), prepared from it by extraction with pyridine, and "citrin," which appears to be a mixture of eriodictyol glycoside and hesperidin. These substances were given orally, by intramuscular injection or through the rectum, to subjects suffering from multiple vitamin deficiencies. In one individual this was artificially induced by giving a diet containing neither fruit nor vegetables, all food being twice cooked. All the patients showed increased capillary fragility before the flavanone preparations were administered, whether tested by the "positive pressure method," in which fragility is determined in terms of the number of burst capillaries occurring in response to an increased intra-capillary pressure, or by the "negative pressure method," in which fragility is expressed as the amount of suction required to burst a single capillary loop. The administration of ascorbic acid had no effect on the fragility, but all three flavanone preparations increased the capillary resistance to normal. It is claimed that the existence of a factor decreasing capillary fragility is for the first time established with certainty.

F. A. R.

Bacteriological

Preservatives for Preparations Containing Gelatin. L. Gershenfeld and D. Perlstein. (*Amer. J. Pharm.*, 1939, 3, 277–287.)—Two types of gelatin were used in 1 per cent. solutions: (*a*) Pharmagel A, a pork-skin gelatin from an acid-treated precursor, with 75 mg. of tartaric acid added per g. of gelatin to give *p*H 3 to 4, and (*b*) Pharmagel B, a bone gelatin from an alkali-treated precursor, with 0·5 g. of sodium bicarbonate added to give *p*H 7 to 8. The gelatin solutions, with definite concentrations of each preservative, were inoculated respectively with suspensions of *Staphylococcus aureus*, *B. subtilis*, *Proteus vulgaris*, a pink yeast, *Penicillium glaucum* and a suspension of dust from the pharmaceutical laboratory, free access of air being allowed to each. Controls were run and macroscopic readings and sub-cultures for growth were made at monthly intervals over the 4-month test period. The following preservatives were effective for the acid type gelatin:—sodium benzoate in 0·1 per cent. concentration; thymol (0·1); chlorobutanol (0·5); sodium salicylate (0·1); cresol (0·4); parachlorometaxylenol (0·1); oxyquinoline sulphate (0·1); alcohol (8·0); ethyl hydroxybenzoate (0·15); propyl hydroxybenzoate (0·15); butyl hydroxy benzoate (0·15). For the basic type gelatin the following were effective:—thymol in 0·1 per cent. concentration; chlorothymol (0·1); chlorobutanol (0·5); β-naphthol (0·2); phenol (0·5); cresol (0·4); parachlorometaxylenol (0·1); parachlorometacresol (0·25); alcohol (8·0); ethyl hydroxybenzoate (0·15). D. G. H.

Estimation of Foreign Organisms in Yeast. K. E. Jensen. (*Internat. Congress Agric. Industries*, July, 1939; *J. Inst. Brewing*, 1939, 45, 500).—Methods are described for the quantitative estimation of small amounts of foreign organisms in pressed yeast. *Oidium lactis*.—The main solution contains 1 per cent. of sodium acetate, 1 per cent. of ammonium chloride and 1 per cent. of agar. To each litre of this are added 10 ml. of 10 per cent. potassium dihydrogen phosphate solution, 10 ml. of 1 per cent. calcium chloride solution, 30 ml. of 1 per cent. magnesium sulphate solution, 1 ml. of 1 per cent. sodium chloride solution and 1 ml. of 1 per cent. ferric chloride solution. Five g. of yeast are diluted to 100 ml. with sterile water, and tests are made with 1 ml., 0·1 ml. and 0·001 ml. of the suspension on the medium in Petri dishes. These are incubated for 4 days at 30° C. and the colonies are then observed. It is claimed that one oidium cell in 500 million yeast cells can be detected. *Mycoderma*.—The main solution described above is used, but the agar is omitted, and 0·5 per cent. of glucose and 0·3 per cent. of "Difco" yeast extract are added. Five ml. of this liquid medium are put into test-tubes, 2·5 cm. in diameter, the measured quantities of the yeast suspension are added, and the tubes are incubated at 37° C. and observed once or twice a day. Those in which no surface film has formed are shaken to promote its formation.

Diagnosis of *Streptococcus mastitis* by Cultural Methods. S. J. Edwards. (*J. Comp. Pathology and Therapeutics*, 1938, 51, 250–263.)—Methods have been devised to facilitate diagnosis of chronic *Streptococcus mastitis* in quarter

and composite milk samples. The results of an investigation of the relative streptococci in whole milk, sediment, and gravity cream from the same milk show that on the average cream contains 3·2 times as many streptococci as the milk, and the sediment from 10 ml. 16·2 times as many as 1 ml. of the milk. The number of streptococci in milk is generally very large in mastitis cases, ranging in the tests recorded from 8000 to 375,000 per ml., and it is suggested that 0·01 ml. of the cream is a satisfactory test inoculum; this is conveniently delivered by means of a standard loop wire constructed so as to present two loops 2·3 mm. in diameter. Direct incubation of milk samples does not compare favourably with cultural examination in blood agar for the detection of *Str. agalactiae*, and does not give reliable results, since non-specific growths are frequently encountered. Crystal violet—aesculin—blood agar is recommended, the loopful of milk being first mixed with a small quantity of sterile normal saline and then well mixed with 10 ml. of melted blood agar before being poured into the plate. For the selective cultivation of *Str. agalactiae* a glucose broth medium containing crystal violet (1 in 1,000,000) and sodium azide (1 in 10,000) and adjusted to *p*H 6·8 was successfully employed; this medium inhibits the growth of *B. coli*, and the presence of *Str. agalactiae* is indicated by the formation (after incubation) of a large flocculent deposit with complete clearing of the supernatant liquid. Its presence may be confirmed by sub-cultivation on blood agar. The addition of sodium azide to milk in a concentration 1 in 20,000 was found to prevent the multiplication of *B. coli* and other organisms during storage and not to affect the number of *Str. agalactiae*. Instead of samples being left in the ice-chest overnight to obtain gravity cream, by the use of azide they can be kept at room temperature.

D. R. W.

Toxicological

Toxicology of Manganese. A. C. Lemos. (*J. Pharm. Chim.*, 1939, **30,** 206–212.)—Experiments on rabbits have shown that soluble manganese salts, when injected or absorbed in large doses, are highly toxic, but manganese dioxide, whether ingested or inhaled, has no immediate toxic effect; the animals treated with it, when killed or dying accidentally after various periods up to 5 months, frequently showed injuries to liver and kidneys. The study of the distribution of the metal in the organism has shown that the impregnation of the principal organs, particularly the brain, increases with the time, but that the amounts in the blood and muscle remain practically constant. Thus, the manganese-contents of various organs, after 2 and 5 months respectively, were (in mg. per 100 g.): heart, 0·27 and 2·5; kidneys, 1·00 and 1·90; lungs, 0·07 and 1·7; brain, 1·07 and 5·8; skin and coat, 1·49 and 3·9. Manganese-contents of the muscles were 0·54, 0·47 and 0·45 for 2, 3 and 5 months, and of blood, 0·25 and 0·26 for 3 and 5 months. For a control animal, the respective results for heart, liver, kidneys, lungs, and skin and coat were not greater than 0·03; brain, 0·67; muscles, 0·01; blood, traces. The manganese-content of small organs (surrenals, marrow, testicles and spleen) was frequently high; this, with the localisation in the nerve centres, may explain the accidents notified among workers exposed to the prolonged absorption of considerable quantities of manganese products. E. B. D.

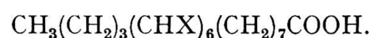
Organic

Wet Combustion Method for Determination of Carbon and of Oxygen Consumed. B. E. Christiansen and J. F. Facer. (*J. Amer. Chem. Soc.*, 1939, **61**, 3001–3005.)—A modified Pettenkofer method was employed (Lunge and Ambler, "*Technical Gas Analysis*," 1934, p. 221). The organic compound (10 to 20 mg.) together with potassium iodate is heated at 190° C. in a reaction vessel with 3 ml. of conc. sulphuric acid for 20 to 40 minutes. The evolved carbon dioxide is absorbed in standard baryta solution, the unused excess of which is neutralised by titration with acid with the use of thymol blue indicator. Oxygen consumed is obtained from a determination of the potassium iodate remaining unused in the reaction vessel, by the usual method of titration with thiosulphate in presence of potassium iodide. S. G. C.

Chromatographic Separation of Palmitic and Stearic Acids from a Mixture of Oleic, Palmitic, and Stearic Acids. C. Manunta. (*Helv. Chim. Acta*, 1939, **22**, 1156–1160.)—When a mixture of saturated and unsaturated fatty acids is adsorbed on magnesium sulphate or, better, on Franconite, the unsaturated acids are found at the top of the adsorption tube, followed by the saturated acids in order of increasing chain length. The following experimental procedure was adopted. A petroleum spirit solution of oleic, palmitic and stearic acids (1 g. of each) was adsorbed in three tubes (3 cm. wide and 60 cm. long) containing magnesium sulphate, $MgSO_4 \cdot \frac{1}{2}H_2O$, previously washed with 100 ml. of petroleum spirit for each tube. Each tube was then washed slowly with 300 to 400 ml. of petroleum spirit, and its contents were divided into six 10-cm. zones, and the corresponding zones of the three tubes were united and washed out with ether. The ethereal solutions were evaporated, and the residues were dissolved in absolute alcohol. After evaporation to 2, 3, or 4 ml. (according to the quantity of material) the alcoholic solutions were chilled and allowed to crystallise, the crystals being filtered off in the cold, washed with a little absolute alcohol and kept in a desiccator. Six fractions consisting of mixtures of palmitic and stearic acids were obtained. Fractions showing similar melting points were united, dissolved in petroleum spirit and again adsorbed on two magnesium sulphate columns. The resulting fractions were again sorted into two lots and once more separated on two columns, 1·5 cm. wide and 36 cm. long. They were thus each separated into three zones. The upper zone of the first consisted of almost pure palmitic acid and the lowest zone of the second lot of fairly pure stearic acid. Similar results were obtained by adsorption on Franconite, the solvent consisting of a mixture of petroleum spirit with 20 per cent. of ether. E. M. P.

Reaction of Wijs' Solution with Tung Oil. S. W. Wan and D. B. Hu. (*J. Amer. Chem. Soc.*, 1939, **61**, 2277–2283.)—In estimating the degree of unsaturation of tung oil with Wijs' solution the presence of elaeostearic glyceride causes much uncertainty because the iodine value thus obtained varies with the experimental conditions (Ho *et al.*, *Ind. Eng. Chem.*, *Anal. Ed.*, 1936, **7**, 96). Various methods for the determination of the percentage of elaeostearic acid in tung oil have been tried but have not given concordant results (Toms, ANALYST, 1928,

53, 69; Croxford, *ibid.*, 1929, **54**, 445; Bolton and Williams, *ibid.*, 1930, **55**, 360; Kaufmann and Baltes, *Fette u. Seif.*, 1936, **43**, 93). The properties of elaeostearic acid causing analytical difficulties have been explained by Böeseken *et al.* (*Rec. Trav. Chim.*, 1927, **46**, 158, 619; Abst., ANALYST, 1928, **53**, 54) by assigning to it the constitution $CH_3(CH_2)_3CH{=}CHCH{=}CHCH{=}CH(CH_2)_7COOH$ which according to Thiele's theory of partial valency will absorb halogen (X) in two stages represented by $CH_3(CH_2)_3(CHX)_2CH{=}CH(CHX)_2(CH_2)_7COOH$ and



$$CH_3(CH_2)_3(CHX)_6(CH_2)_7COOH.$$

With Wijs' solution, X_2 being a molecule of iodine monochloride, the first stage occurs much more rapidly and produces a chloro-compound (Böeseken *et al.*, *loc. cit.*). On the other hand, compounds with non-conjugate double bonds take up the theoretical amount of iodine chloride in a short time, forming iodochloro-compounds. In order to apply the above-mentioned property of elaeostearic acid to the determination of its glyceride in tung oil the kinetics of the slower second stage reaction were investigated. The α-elaeostearic acid used was prepared by the method of Thomas and Thomson (*J. Amer. Chem. Soc.*, 1934, **56**, 898) and the Wijs' solution was modified to contain exactly equivalent amounts of iodine and chlorine. The iodine values were determined in the usual way with certain refinements in the standardisation and measurement of reagents and thermostatic control of the temperature conditions. The experiments were made with (*a*) pure α-elaeostearic acid and a reaction time of 5 minutes, (*b*) pure α-elaeostearic acid with a reaction time of 2 minutes, (*c*) pure elaeostearic acid with a reaction time of 10 minutes, (*d*) pure oleic acid with a reaction time of 10 minutes, (*e*) a known mixture of α-elaeostearic acid and oleic acid with a reaction time of 5 minutes, (*f*) tung oil with a reaction period of 5 minutes. Similarly experiments were made in which definite volumes of the reaction mixture were withdrawn at definite intervals and the course of the halogen absorption was studied. The reactions investigated were (*g*) the reaction between α-elaeostearic acid and the Wijs' solution in conventional proportions, (*h*) the same reaction with an equivalent amount of the Wijs' solution sufficient for the first-stage reaction, (*i*) the same reaction with an excess of the Wijs' solution, and (*j*) comparison of the halogen absorbed by α-elaeostearic acid and by tung oil. With the aid of the results of these experiments a study of the kinetics of the two-stage reaction was made. The values of the velocity constants calculated from the experimental results do not support the assumption that Böeseken's second-stage reaction is either bimolecular or trimolecular. The mechanism suggested by Böeseken is therefore incorrect, and the reaction is most probably a combination of two simultaneous bimolecular reactions—one between the acid and the iodine liberated in the first stage, and the other between the acid and iodine monochloride. The compounds thus formed are $CH_3(CH_2)_3CHClCHClCHICHICHClCHCl(CH_2)_7COOH$ and $CH_3(CH_2)_3CHClCHClCHICHClCHClCHCl(CH_2)_7COOH$ respectively, the product of the first stage being a tetra-chloro compound. The difference in halogen absorption between α-elaeostearic acid and oleic acid has been applied to the analysis of a mixture of these acids and to the determination of elaeostearic glyceride in tung oil, and further work is being undertaken for improving the

latter method. The increase of the iodine value of the oil with increase of temperature or time of contact with the reagent is explained. The absorption of chlorine from iodine monochloride in the first stage explains the requirement of a minimum excess of Wijs' solution. The slight decrease of iodine value with increased amount of oil when the same amount of reagent is used may be accounted for by the fact that a relatively larger amount of α-elaeostearic glyceride absorbs a correspondingly larger amount of iodine monochloride in the first stage, and leaves a smaller excess of halogen which decreases the rate of absorption in the second stage. A. O. J.

Determination of the Eugenol-content of Essential Oils. P. A. Rowaan and J. A. Insinger. (*Chem. Weekblad*, 1939, **36**, 642–643.)—The effects of extraction with solutions of potassium and sodium hydroxide of various strengths and at temperatures ranging from 20° C. to 100° C. have been compared, and the following procedure is recommended:—Ten ml. of the dried and/or filtered oil are pipetted into a 100-ml. flask, the neck of which is graduated from 0 to 10 ml. in 0·1-ml. divisions. To this are added 80 ml. of 1·0 *N* potassium hydroxide solution (A.R. quality), and the flask is then shaken well at intervals of 5 minutes for 30 minutes. Sufficient of the potassium hydroxide solution is then added to bring the level of the unabsorbed oil up into the neck of the flask, where its volume may be read to within 0·05 ml.; the reduction in volume corresponds with the eugenol originally present. Satisfactory results were obtained with oils of clove, pimento, cinnamon leaf and New Guinea lawang oil (an oil from the bast of a plant of the *Cinnamomum* species); bay oil, however, gave unreliable results, owing to the presence of other phenolic substances (*e.g.* chavicol). If an emulsion is formed during the shaking operation, the oil should first be shaken with a little tartaric acid solution, which is subsequently removed, and the residual oil is dried with anhydrous sodium sulphate and then filtered. J. G.

Boric Acid Colour Reaction of Flavone Derivatives. C. W. Wilson. (*J. Amer. Chem. Soc.*, 1939, **61**, 2303–2306.)—Lemon juice evaporated with boric acid produces a brilliant yellow colour and the substance causing the colour reaction has been identified as a constituent of Szent-Györgyi's citrin (Armentano *et al.*, *Deutsch. Med. Wochschr.*, 1936, **62**, 1326). The reaction will detect 0·004 mg. of citrin or 0·002 mg. of quercitrin in 0·5 ml. of solution. An attempt to determine the specificity of the reaction was made. Several representative flavones and flavanones were purified by recrystallisation from alcohol, and hydroxychalcones were obtained by the action of alkali on naringin and hesperidin. The reagent used for the determination of the reactivity of these compounds with boric acid was made by mixing equal parts of a saturated solution of boric acid in absolute acetone and a 10 per cent. solution of anhydrous citric acid in absolute acetone. The separate solutions are stable, but the mixture is stable only for one day. To determine the reactivity, the flavone derivative (0·5 mg.) was dissolved in about 1 ml. of dry acetone and the solution was divided into two parts. To one part about 2 ml. of the boric acid and citric acid mixture were added, and the other part was diluted to the same volume with a mixture of equal parts of acetone and the citric acid and acetone solution. After a few minutes the colours of the two

tubes were compared, a stronger colour in the tube containing boric acid being regarded as a positive reaction. By comparing the reactions of the aglycones with the reactions of two glycosides of quercetin, *viz.* isoquercitrin and quercimeritrin it was established that neither the presence or absence nor the position of the sugar group influenced the colour reaction. Of all the flavone derivatives tested, the flavanones gave no colour reaction with boric acid, whereas all the flavones except fisetin gave a reaction. When the lack of reaction of fisetin is compared with the reactivity of quercetin, the importance of the hydroxyl (or other auxochromic) group in the 5-position is evident. The reactivity of kaempferol compared with the failure of naringenin leads to speculation whether the hydroxyl group in the 3-position or the double bond between the 2- and 3-carbon atoms is responsible for the difference, and the opening of the pyran ring to form the reactive chalcone establishes the importance of the double bond and indicates that the pyran ring is unnecessary for colour formation. The configuration of the chalcones suggests that of curcumin, which reacts with boric acid to give a pink colour. If this colour formation is comparable with the flavone reaction, the benzene ring near the ketone (or quinone) group is unnecessary, provided that an auxochromic group is attached to the second carbon atom from the C = O group. The indicated configuration for reactivity is

$$R - \underset{(v)}{\overset{\overset{a}{|}}{C}} - \underset{(w)}{C} - \underset{(x)}{\overset{\overset{O}{\|}}{C}} - \underset{(y)}{C} = \underset{(z)}{C} - R'$$

in which a is an auxochromic group which may probably be $= O$, OH, OCH_3, etc., and in which R, C(v) and C(w) may form a benzene ring, and C(x), C(y) and C(z) may form part of a pyran ring. In all the compounds examined, R' has been a benzene ring containing either methoxyl or hydroxyl groups. When the pyran ring of fisetin is opened by treatment with alkali, the formation of the hydroxyl group in the ortho position on one of the benzene rings fulfils the requirement of the position of the auxochromic group, but the double bond between carbon atoms on the other side of the ketonic group is missing. A compound of this type may exist in an enolic form (Buswell, Rodebush and Roy, *J. Amer. Chem. Soc.*, 1937, **59**, 1767) having a double bond in the required position. Another possible explanation is that in the alkali treatment of fisetin other secondary compounds formed are responsible for the colour reaction. The following substances which may occur in nature with the flavones did not give the colour reaction:—tannic acid, coumarin, coumarinic acid, dextrose and sucrose and the products of their treatment with alkali, phloroglucinol, quinhydrone and salicylic acid. A. O. J.

Pectic Substances of Plants. VI. Relation between Jelly Strength, Viscosity and Composition of Various Pectins. E. W. Bennison and F. W. Norris. (*Biochem. J.*, 1939, **33**, 1443–1451.)—The jelly strengths of various pectins were measured by a modified form of the pectinometer described by Buston and Nanji (*Biochem. J.*, 1932, **26**, 2090) and viscosities by an Ostwald viscometer. An attempt was made to correlate these two properties with the chemical composition of the preparations. Little relation could be found, although, in general, a high urone content is associated with a satisfactory jelly strength.

Methoxyl-content is not a criterion of jelly strength, as has sometimes been maintained, for first, esters of pectolic and pectic acids prepared by methylation failed to give jellies, and secondly, the methoxyl-contents of prepared pectins bore no relation to their power of forming jellies. The method of preparation is an important factor, and autoclave extraction in presence or absence of sucrose inhibits jelly formation. The jelly strength and viscosity of the same pectin are closely related, and both are reduced when the pectin is heated. Although the ability of pectins to form jellies depends to some extent on their composition, it is primarily determined by molecular size, as indicated by viscosity. Any treatment tending to disaggregate the polygalacturonide chain tends to cause loss of jelly strength (*cf.* ANALYST, 1939, **64**, 000). F. A. R.

Inorganic

Determination of Aluminium Oxide in Metallic Aluminium by Means of Cupric Ammonium Chloride. T. Nakamura and S. Yamazaki. (*J. Soc. Chem. Ind., Japan*, 1939, **42**, 296–297B.)—A 10-g. sample of aluminium is allowed to react with a solution of 240 g. of cupric ammonium chloride, added little by little to prevent violent interaction. The liquid is finally heated, and the residue of aluminium oxide is filtered off and washed with 1 per cent. hydrochloric acid to remove basic copper compounds as far as possible. The remainder is ignited and fused with potassium bisulphate, and the aluminium in the melt is determined by the usual procedure, the weight being calculated as aluminium oxide. The amounts of aluminium oxide found by this method in virgin ingots ranged from 0·01 to 0·06 per cent. The authors satisfied themselves (1) that anhydrous alumina is practically unattacked in the process; (2) that the aluminium in association with elements such as silicon is almost completely dissolved. S. G. C.

Reaction of Gallium with Perchloric Acid. L. S. Foster (*J. Amer. Chem. Soc.*, 1939, **61**, 3122–3124.)—Metallic gallium, which dissolves very slowly in the common mineral acids, has been found to dissolve vigorously in hot perchloric acid (72 per cent. strength); 5 g. of gallium (form not stated) dissolved in 60 ml. of the perchloric acid in 1 hour. The gallium perchlorate produced is soluble in the hot acid liquid, but separates almost completely on cooling, in the form of coarse white crystals of the hexa-aquo salt. A mixture of two-thirds conc. sulphuric acid and one-third perchloric acid was more rapid in solvent action than perchloric acid alone. S. G. C.

Detection and Determination of Germanium with Hydrogen Selenide. V. J. Kouznecov. (*J. Ob. Chem.*, 1939, **9**, 1049–1054.)—Hydrogen selenide reacts with quadrivalent germanium in mineral acid solution to form a yellow-orange precipitate; this reaction is specific. When the concentration of germanium is as low as 1 in 10^{-5} a yellow turbidity is produced almost instantaneously, but as a red precipitate of selenium is formed after a few minutes, the test is doubtful for such low concentrations. Owing to this ease of oxidation and to the poisonous nature of hydrogen selenide, an organic derivative which it forms with formaldehyde is used instead of the selenide itself. This compound is produced in a colourless

solution when hydrogen selenide is passed into a solution of formaldehyde which is just acid. It is fairly stable in air, and concentrated solutions become turbid only after standing many hours (sometimes days) in an open test-tube, after which a yellow precipitate is slowly deposited on the sides (the red precipitate of selenium is not formed). In dilute solution the formaldehyde compound causes turbidity after an hour. Its reactions with metallic ions are the same as those of hydrogen selenide solution. With germanium a curdy yellow precipitate, somewhat less reddish and more stable to light than germanium selenide, is formed. It is insoluble in conc. acids (even in hot sulphuric acid) but readily soluble in alkalis. The reaction is sensitive for 0·2 p.p.m. of germanium, and silicic, hydrofluosilicic, hydrocyanic and other acids do not interfere with it. The test is made by adding 2 or 3 drops of the solution prepared as described below to 5 ml. of the solution examined, which contains approximately 1 ml. of conc. hydrochloric acid (the degree of acidity is important). *Preparation of reagent.*—Twenty g. of selenium and 14 g. of aluminium powder are ignited in a closed crucible, with addition of 20 g. of ignited sodium chloride to moderate the reaction. From 5 g. of the impure aluminium selenide thus formed, hydrogen selenide is liberated, in a small flask, by the action of water from a dropping funnel; finally, addition of dilute hydrochloric acid liberates hydrogen (from the excess of aluminium) which sweeps out the remaining hydrogen selenide. The gas is passed at room temperature, with thorough shaking, into a mixture of 10 ml. of commercial formalin (40 per cent.) and 50 ml. of water acidified with 1 to 2 drops of conc. hydrochloric acid. If the resulting solution is slightly coloured by traces of heavy metals present in the formalin it should be filtered. The product should be kept at a low temperature in a closed vessel. It becomes turbid after 2 or 3 days. By certain modifications, described in the original, germanium can be detected in presence of 100 times its amount of arsenic, tin, selenium (as selenite) and other elements. E. B. D.

Reduction of Molybdate in the Silver Reductor. C. F. Hiskey, V. F. Springer and V. W. Meloche. (*J. Amer. Chem. Soc.*, 1939, **61**, 3125–3127.)—In confirmation of the work of Birnbaum and Walden (*id.*, 1938, **60**, 64) it was found that when molybdate in 2 *N* hydrochloric acid solution is passed through the silver reductor and the reduced solution is exposed to the air the molybdenum is present entirely in the quinquevalent condition. S. G. C.

Microchemical

Quantitative Micro-Determinations with the Use of Ordinary Analytical Balances. A. A. Benedetti-Pichler and R. A. Paulson. (*Mikrochem.*, 1939, **27**, 339–347.)—Ordinary student-type analytical balances are used for the work, but with 5-mg. substituted for 10-mg. riders. The precision of each balance is determined for a load of 10 g. on each pan, and the sensitivity is adjusted to 5 or 6 divisions of the pointer-scale per mg.; one balance of rather poor precision was among those tested. The balances can be used, in conjunction with the Emich filter-stick technique, for the determination of different inorganic ions, *e.g.*,

sodium in sodium oxalate. The sample (6–10 mg.) is weighed into a platinum crucible, converted into sulphate and weighed as such. Percentages of sodium varying from 34·15–34·46, as compared with calculated value 34·38, were obtained, the average deviation from the mean being ±0·08 per cent. With the poor balance the same deviation from the mean (5 determinations) was obtained. The balances have also been used for the determination of aluminium in alum by the 8-hydroxyquinoline method. With samples weighing 6 to 8 mg. the average deviation from the mean (a_s) was ± 0·04 per cent. of Al_2O_3 and the relative average deviation of a single determination from the mean (a_s') was 0·37 per cent.; with samples of 16 to 18 mg., a_s = ± 0·03 per cent. of Al_2O_3 and a_s' = ± 0·28 per cent. In determinations of calcium in Iceland spar by the oxalate method on samples of 16 to 18 mg., a_s = ± 0·20 per cent. of CaO and a_s' = ± 0·36 per cent. In the determination of magnesium by the ammonium phosphate method a_s = ± 0·07 per cent. of MgO and a_s' = ± 0·41 per cent. Micro-methods can therefore be put into general practice without expenditure on a micro-balance. J. W. M.

Salts of Complex Cations Applied to the Microscopical Detection of Anions. W. A. Hynes and L. K. Yanowski. (*Mikrochem.*, 1939, **27**, 336–338.)—*Mono-aquopentamminocobaltic chloride* (*roseocobaltic chloride*).—The reagent is added as a solid of the size of a pinhead, at one side of the test drop containing about 1 per cent. of the ion to be tested. Characteristic crystals are obtained with the following substances:—oxalates, sulphosalicylic acid, dithionates, ferri- and ferrocyanides, silicofluorides and phosphomolybdates. Of these, only the first four reaction products are recommended for identification of the corresponding anions. No reactions are obtained with benzoic, boric, citric, formic, maleic, malonic, oxalic, salicylic, succinic, sulphanilic or uric acid, or with the alkali metal salts of the following anions:—acetate, azide, bicarbonate, bisulphate, bisulphite, bitartrate, borotartrate, bromate, bromide, carbonate, chlorate, citrate, dichromate, fluoride, formate, hypophosphite, iodide, metaborate, nitrate, nitrite, nitroprusside, orthoarsenate, orthoarsenite, secondary orthophosphate, perchlorate, permanganate, pyrophosphate, selenate, selenite, sulphate, tartrate, tellurite or thiocyanate. Eight photomicrographs are given. J. W. M.

Chemical Microscopy of Some Toxicological Alkaloids. W. F. Whitmore and C. A. Wood. (*Mikrochem.*, 1939, **27**, 249–334.)—The behaviour of each of twenty alkaloids with different types of reagents was investigated. The alkaloids include the following:—*local anaesthetics* : β-eucaine, cocaine, procaine, stovaine; *cinchona alkaloids*: cinchonine, cinchonidine, quinine, quinidine; *opium alkaloids* : codeine, dionine, heroine, morphine, narcotine, papaverine; *nux vomica alkaloids* : brucine, strychnine; *purine bases* : caffeine, theobromine; *miscellaneous* : atropine, nicotine. Optical properties of the crystalline products, with the exception of refractive indices, were ascertained with the aid of a polarising microscope. The reagents included the following:—I *Halogen reagents* (1) *Bromine in sodium bromide solution.*—This is a general precipitant for all alkaloids; it forms crystalline precipitates with six alkaloids, the best with theobromine, caffeine and atropine. (2) *Iodine trichloride* gives precipitates of little value for identification purposes. II *Oxygen acids and salts, including organic salts.*—(3) (*a*) *Perchloric acid* gives

distinctive derivatives with strychnine, brucine, morphine and cocaine; (*b*) *ammonium hypochlorite* forms crystals also with novocaine, cinchonine and quinidine. (4) *Sodium perborate* yields the free bases. (5) *Ammonium vanadate* forms crystals with strychnine but they are not very characteristic. (6) *Potassium anthraquinone sulphonate.*—The best crystals are obtained with quinidine. (7) *Potassium xanthate.*—Only brucine and strychnine give crystalline xanthates. (8) *Cupferron*, crystals not well developed with β-eucaine. (9) *Potassium acid phthalate* forms crystals with strychnine. (10) *Potassium bromide* gives crystals with β-eucaine, codeine and stovaine. III *Salts leading to complex or double salt formation.*—(11) (*a*) *Stannous chloride* gives crystals with strychnine, brucine, β-eucaine and cocaine; (*b*) *Stannous bromide* behaves like (*a*) but forms better crystals with cocaine. (12) *Titanous chloride* gives characteristic crystals with strychnine, brucine and papaverine. (13) *Bismuth trichloride* forms crystals with strychnine and brucine. (14) *Potassium antimonyl tartrate.*—Strychnine gives isotropic crystals. (15) *Arsenic trichloride* does not form crystalline precipitates. (16) *Mercurochrome* forms non-crystalline precipitates. IV *Anions of complex salts or acids.* (17) *Sodium silver iodide* gives a characteristic crystalline iodo-argentate precipitate with cocaine. (18) (*a*) *Potassium auro-iodide* is a general precipitant; caffeine and nicotine form crystalline products; (*b*) *Hydrogen auro-iodide* ($HAuI_2$) behaves like (*a*) except that a crystalline precipitate is obtained with theobromine. (19) (*a*) *Sodium auro-bromide* ($NaAuBr_4$) and (*b*) *Hydrogen auro-bromide* ($HAuBr_4$) give more sensitive tests than the chloro compound. (20) *Iridium chloride* ($IrCl_4$) behaves like (24) but the crystals are more soluble. (21) and (22) *Rhodium chloride* ($RhCl_3$) and *Ruthenium chloride* give crystals with strychnine and brucine, and ruthenium chloride also produces, with cocaine, a crystalline precipitate with oblique extinction. (23) *Sodium osmium chloride* ($NaOsCl_6$) behaves like (20), forming yellow precipitates. (24) (*a*), (*b*) and (*c*) *Hydrogen platinichloride* (H_2PtCl_6) in 20 per cent. HBr, and in 5 per cent. HBr and *Sodium platinibromide* (Na_2PtBr_6). These reagents are more sensitive than the generally used chloroplatinic acid. Good crystals are obtained with dionine and with cocaine, for which the test is highly selective; cinchonine crystals show oblique extinction. (25) *Potassium platinic iodide* (K_2PtI_6) is a sensitive reagent giving with all the alkaloids precipitates of which the cinchonine compound is the most characteristic. (26) *Caesium cadmium iodide* (Cs_2CdI_4) is similar to (27) (*a*), *cadmium iodide* (CdI_2), which forms characteristic crystals showing oblique extinction with codeine, parallel extinction with morphine; (*b*) *Cadmium bromide* is similar to (*a*), but the crystals with novocaine are characteristic; (*c*) *Cadmium chloride* gives soluble crystals with heroine. (28) (*a*) *Potassium mercury bromide* (K_2HgBr_4) and (*b*) *Potassium hydrogen bromide* (K_2HgBr_4), when acidified, give insoluble salts with all the alkaloids; (*a*) gives crystals with morphine, nicotine, codeine and brucine, (*b*) also with strychnine, dionine and β-eucaine. (29) *Potassium mercury thiocyanate* ($K_2Hg(SCN)_4$) is useful as a general precipitant. (30) *Potassium thallic iodide* gives only amorphous precipitates. (31) (*a*) *Hydrogen stannic chloride* (H_2SnCl_6) is a very useful reagent, yielding crystalline derivatives with those alkaloids that produce crystals with stannous chloride; these compounds have distinctive optical properties; (*b*) H_2SnCl_6 + KBr forms crystals similar

to those obtained with (*a*) but smaller. (32) *Hydrogen antimony chloride* ($HSbCl_4$) is a good reagent for strychnine. (33) *Manganese sulphate + ammonium thiocyanate.* (34) *Cobalt nitrate + ammonium thiocyanate.* (35) *Chromium sulphate + ammonium thiocyanate.* (37) *Cadmium nitrate + ammonium thiocyanate.* Complex thiocyanates were obtained with nicotine and brucine. (38) (*a*) *Potassium palladium bromide* and (*b*) *Palladium chloride* in HBr give precipitates suitable for identification under the microscope with β-eucaine, brucine, strychnine and caffeine; the crystals, however, are not particularly distinctive. (39) *Sodium stannic iodide* (Na_2SnI_4).—The most distinctive crystals are obtained with cinchonidine. There are 132 drawings and photomicrographs of crystals. J. W. M.

Micro-Estimation of Threonine. R. J. Block and D. Bolling. (*J. Biol. Chem.*, 1939, **130**, 365–374.)—A practicable method of estimating small amounts of threonine (α-amino-β-hydroxybutyric acid) has been worked out, in which the amino-acid is quantitatively oxidised to acetaldehyde by means of lead tetra-acetate solution, and the acetaldehyde is then condensed with *p*-hydroxydiphenyl, with the formation of an intense red-violet colour (Eegriwe, *Z. anal. Chem.*, 1933, **95**, 323). The intensity of the colour is proportional to the amount of acetaldehyde and thus to the amount of threonine. The apparatus consists of 6 tubes connected together in series by means of rubber tubing. Tube 1 is a Pyrex test-tube (20 × 2·5 cm.) fitted with a rubber stopper carrying a long and a short tube, and containing 20 ml. of conc. sulphuric acid, which serves to clean and dry the air that is bubbled through it. Tube 2 is a blank tube exactly like tube 1, and serves as a trap for the sulphuric acid spray. Tube 3 is the reaction tube, and consists of a 20 × 3 cm. Pyrex test-tube with a standard ground-glass joint, into which fits a head containing an air inlet tube of 4 mm. inside diameter, extending to within 2·5 cm. from the bottom, and an air outlet. The tube is immersed in a water-bath maintained at 30° C. It contains the reaction mixture, consisting of 25 ml. of glacial acetic acid (purified by refluxing for 7 hours with 0·5 to 1·0 per cent. of potassium dichromate and then distilling), less than 10 mg. of amino-acids, and at least 1 g. of lead tetra-acetate (recrystallised from boiling glacial acetic acid). Tube 4 is similar to tube 1 and contains a 2-inch column of sodium hydroxide pellets to remove the last traces of acid; it is kept at room temperature. Tube 6 is the absorption tube and is of the same construction as tube 3. It contains 10 ml. of conc. sulphuric acid (analytical grade that has been allowed to stand over a small amount of lead shavings), 5 drops of water and 100 mg. of *p*-hydroxydiphenyl (purified by recrystallisation several times from acetone) in suspension; this tube is kept at 0° C. Air is drawn through the system at a constant rate for 1 hour. No pink or blue colour should be found in tube 6 when a blank estimation is carried out with the reagents only; an appreciable blank is usually due to impurities in the acetic acid. At the end of the reaction tube 6 is kept at 0° C. until it is desired to remove the excess of *p*-hydroxydiphenyl, when this tube is immediately transferred to a boiling water-bath for exactly 2 minutes (stop-watch). The tube is immediately cooled to 0° C. and subsequently allowed to attain room temperature. The colour, which varies from red to violet, depending on the amount of acetaldehyde, on the time, and on the temperature, is stable for 24 hours. The solution

absorbs strongly in the green part of the spectrum and has an absorption maximum at 560$m\mu$. The colour can be measured in an Evelyn photoelectric colorimeter with filter 560$m\mu$; a Duboscq visual colorimeter with the same filter, or a Pulfrich step-photometer with filter S57. A specimen of commercial synthetic *dl*-threonine is employed as standard, or a calibration curve may be constructed. All the other amino-acids tested failed to give colours, with the exception of *dl*-serine and *dl*-alanine, 10 mg. of which formed as much acetaldehyde as 0·03 mg. and 0·5 mg. of threonine respectively. A method of overcoming the interference of alanine is being investigated. All the carbohydrates tested yielded no detectable amounts of acetaldehyde on oxidation with lead tetra-acetate. Recoveries of 97 $\pm$ 3 per cent. of threonine added to protein hydrolysates were obtained. The following amounts of threonine were found in different kinds of protein:—casein, 3·5 per cent.; gelatin, 0·5 per cent.; normal human serum protein, 5·9 per cent. F. A. R.

Physical Methods, Apparatus, etc.

Determination of Water in Ether. R. Gaspart and G. Serrure. (*Bull. Soc. Chim. Belg.*, 1939, **48**, 283–292.)—Gaspart's earlier work on the determination of water in acetone by means of measurements of absorption-spectra in the far infra-red (*cf.* ANALYST, 1939, 459) has been extended to solutions and suspensions in ether. Ether was purified by fractional distillation in presence of sodium metal in the dark (*cf.* Timmermans and Martin, *J. Chim. Phys.*, 1928, **25**, 434) in a Pyrex glass apparatus, through which was passed a current of pure dry nitrogen. An apparatus for the accurate preparation of solutions of water of different concentrations in ether, and for the transference of these to the cell of the spectroscope without appreciable contamination from external sources, is described and illustrated. The method makes possible the preparation of solutions of known concentration, with an accuracy ranging from 0·5 to 14 per cent. according to the concentration. The cell was cooled in a mixture of solid carbon dioxide and alcohol during these operations, and at once hermetically sealed; measurements were carried out with a thickness of liquid of 1 mm. and at 20° C., over the range 3700 to 3400 cm.$^{-1}$ (*cf.* ANALYST, *loc. cit.*) on solutions containing 0 to 50 parts of water per 100,000 by volume. The data are tabulated, and curves are plotted showing the relationship in each solution between the wave-length and the percentage transmission as obtained from the ratio of the energy-equivalent of the radiation after traversing the solution in question, to that obtained after passage through a comparison cell containing carbon tetrachloride. It is considered that the error of measurement, which arises mainly from reflection at the air-cell and cell-liquid interfaces, is of the order of 0·5 per cent. The curves show that for low concentrations of water a band (due to water-ether addition compounds) is obtained at 3598 cm.$^{-1}$ This band increases in magnitude as the concentration increases, until at 6·6 p.p. 100,000 a second band (at 3525 cm.$^{-1}$) becomes apparent. With acetone (*loc. cit.*) this second band was masked by the band (at 3420 cm.$^{-1}$) characteristic of the —C:O linkage, the first band (due to the acetone-water addition compound) being at 3618 cm.$^{-1}$ Curves showing the ratio of the above percentage transmission of each solution to that of pure ether as a function of the

wave-lengths, demonstrate these two bands clearly and show how their magnitude (and especially that of the first) is dependent on the concentration of the water. The absolute and molecular coefficients of extinction for the band 3598 $cm.^{-1}$ are plotted against the concentration. The molecular coefficients are less than the corresponding values for acetone (*loc. cit.*), and since they are only constant for concentrations exceeding $1:21 \times 10^{-6}$, it is therefore necessary to construct a standardisation curve; the sensitiveness of the method then corresponds with a concentration of the order of $1:2 \times 10^{-6}$. Similar data and curves are given for colloidal solutions of gold in ether containing various quantities of water. J. G.

Reviews

INTRODUCTION TO PRACTICAL ORGANIC CHEMISTRY. By F. G. MANN and B. C. SAUNDERS. Pp. ix + 191. London, New York and Toronto: Longmans, Green & Co., Ltd. 1939. Price 4s. 6d. net.

This book is a "severely abridged version" of the authors' larger work—*Practical Organic Chemistry*. It is intended as an introduction to the practical side of this branch of chemistry. It differs from the authors' advanced book in that the more difficult estimations and compounds, and also the sections on vacuum distillation and enzyme action are omitted.

Part I is devoted to methods of manipulation. In it careful and detailed, if somewhat lengthy accounts, are given of most of the fundamental processes used for the purification of organic compounds. The criteria for ascertaining the success of these operations are also fully discussed. In the course of this discussion many interesting (and too often forgotten) facts are mentioned. Hints contributary to success and to the avoidance of accidents are given freely.

Part II is concerned with preparations; in all, about forty-five are described, the aliphatic and aromatic series being about equally represented. In many instances alternative methods are given. The chief reactions and points of theoretical interest connected with each compound are also set forth clearly. The section on sugars is especially good; all the common types seem to be represented.

The next section is taken up with the reactions and methods of identification of organic compounds. The scheme, which is on the orthodox lines, is satisfactory and comprehensive, covering most of the common classes of compounds. In testing for elements Middleton's zinc and sodium carbonate method is recommended. Timely warning is given of the dangers attached to the sodium method when used with polynitro compounds and with carbon tetrachloride or chloroform. The special reactions for the identification of individual compounds seem to have been selected with great care.

The final chapter deals with various quantitative analyses. Among those described are the determination of molecular weights and methods for the determination of nitrogen (Kjeldahl), aniline, urea and acetyl groups.

The book closes with an appendix containing, *inter alia*, notes on the preparation of reagents, on first aid and on fire. Logarithms, antilogarithms and a good index are provided.

An examination of the book will show that a very satisfactory course of introductory practical organic chemistry is given. Students who have worked faithfully through it will have gained a practical knowledge that will be invaluable, not only as a basis for further organic laboratory work, but also for a clear understanding and appreciation of the theoretical side of the subject.

HAROLD TOMS

AN INTRODUCTION TO CRYSTAL CHEMISTRY. By R. C. EVANS, M.A. Pp. xi + 388. Cambridge University Press, 1939. Price 18s.

The story of the development of crystal chemistry (and physics) is a remarkable instance of the dependence of a branch of science on the assistance furnished by the inventor and designer of instruments. True, certain regularities were apparent to the seeing eye, and were duly noticed, but the story of modern crystallography begins with the invention of the goniometer by Carangeot in 1780, and was enormously advanced by the invention of the reflecting goniometer by Wollaston in 1809.

Much was accomplished in crystal chemistry—in which the chief interest lies "in the interpretation of the observed crystal structures and in their correlation with physical and chemical properties"—during the nineteenth century; but the major discovery of the diffraction of X-rays by a crystal acting as a grating, has resulted in most astonishing advances in our knowledge of the intimate structure of solids, advances which it is becoming increasingly necessary to present in such a form that the chemist of to-day may be able to appreciate their significance and importance.

It is too commonly assumed that this appreciation demands an equipment surpassing that possessed by the chemist who has not had the advantage of an early and systematic training in the modern outlook and technique. This is, perhaps, true if attention is concentrated on what may be termed the topographical aspect of the subject, but Dr. Evans has demonstrated that it is possible to begin at the beginning and to give his readers a comprehensive picture of modern views on the crystal lattice, and of the applications of these views to such topics as metallic elements and alloy systems, homopolar compounds, and ionic and molecular compounds, without making heavy demands on their mathematical knowledge.

Dr. Evans's book may be unreservedly commended as an admirably clear account of a fascinating and important subject.

ALLAN FERGUSON

FAT, TOTAL SOLIDS AND MOISTURE. By R. D. MASON, M.Sc., A.I.C. Pp. 101. London: A. Harvey. 1939. Price 7s. 6d. net.

The standardisation of control tests is essential in modern industry, and by this is meant the use of standard apparatus as well as technique. In connection with the determination of total solids by drying *in vacuo* and the determination of fat by the well-known Rose–Gottlieb method, this standardisation is carried to a high level by the ingenious Mojonnier tester, now well known to most chemists. This very excellence, however, seems to have stultified any effort to overcome certain defects that it undoubtedly possesses. An English machine has now been made which has taken advantage of the criticisms levelled at the

original one, and this book gives in a concise, and therefore extremely useful, form the details for testing by its means a large range of food products for fat and total solids. The value of the book is enhanced by an appendix giving tabulated data for all the products dealt with. It is strongly recommended to those who can afford the unit. E. B. ANDERSON

ESSENTIALS OF PHYSIOLOGICAL CHEMISTRY. By ARTHUR K. ANDERSON. Pp. 323 + ix. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. 1939. Price 13s. 6d.

When the first edition of this book was reviewed just over three years ago (*cf.* ANALYST, 1936, **61**, 443), it was suggested that a new edition would be needed "in the not very distant future," but the extent of the revision necessary could hardly have been foreseen at that time. Indeed, a comparison of the two editions gives one some idea of the vast amount of research work that has been carried out during this period, and emphasises the necessity of revising adequately, or perhaps one should say re-writing, textbooks of physiological chemistry, every few years. One hopes that the obvious success of *"Essentials of Physiological Chemistry"* will encourage Professor Anderson to revise just as thoroughly each future edition that may be required.

The volume under review contains thirty more pages than its predecessor, including an entirely new chapter on "The Composition of Tissues." This is an especially interesting section of the book, as it deals mainly with substances of high molecular weight, the composition of which can at present only be guessed at, and one visualises this chapter being expanded into several as the years go by and our knowledge of these substances increases.

Of the other chapters, there are four or five that have especially increased in size. These are the chapters on "Carbohydrates," with nine additional pages; on "Lipids," with seven extra pages; on "Fat Metabolism" and "Carbohydrate Metabolism," the latter, in particular, having been largely rewritten to conform with modern theory. As one might expect, the chapters on "Enzymes," "Endocrine Organs" and "Vitamins" have also had to be enlarged, the last chapter showing the biggest proportional increase of them all. New subjects dealt with in these chapters include the oxidases, co-enzymes, sex hormones, the hormones of the pituitary gland, the vitamin B complex and vitamin E.

One striking exception to the general tendency to devote more space to everything is provided by the account of the chemistry of muscle contraction, which is reduced from two pages to two paragraphs; one can only conclude from this that the author has grown less dogmatic on this subject as the years have gone by!

There are one or two errors to which attention should be directed; thus, "leucine" is spelt "lucine" on page 192, and Na_2CO_3 is surely intended for $NaHCO_3$ in the formula on p. 234, but alas, the besetting sin of the last edition, an apparent difficulty in presenting accurate structural formulae, is again in evidence in this edition. Thus the formula for vitamin B_1 is given on p. 287 without the bridge methylene group, and the formula given on p. 267 for corticosterone does not include the angle-methyl groups, and, in addition, an oxygen atom is shown in

ring C instead of a hydroxyl group. The formula of α-tocopherol on p. 301 would have been less ambiguous if the double bonds of the benzene ring had been indicated.

Whilst it is unfortunate that the accuracy of a textbook of this nature should be impaired by such minor errors as these, it should be clearly understood that the book is a reliable guide, well written and well produced, to that borderland of science that lies between biology and chemistry. One could, of course, if one were so minded, quarrel with several of Professor Anderson's more controversial statements, but no author could be expected to enter upon a full discussion of every question that is open to doubt in a textbook of this kind, and one can only hope that future editions will bear the same impress of wide reading and careful assimilation as does this edition. F. A. ROBINSON

VITAMIN E. A Symposium held under the Auspices of the Food Group (Nutrition Panel) of the Society of Chemical Industry. April, 1939. Pp. viii + 88. Cambridge: W. Heffer & Sons, Ltd. Price 5s.

Every time a new war breaks out the names of unheard-of towns become familiar; in the same way each time another vitamin is isolated another obscure reagent claims a place in one's memory. On this occasion it is allophanic acid, since it is in the form of crystalline allophanates that vitamin E is isolated. The vitamin itself is now called tocopherol. The classically educated mind may puzzle for a moment to decide from whence these names are derived.

The volume under review, as its title implies, is made up of a number of addresses delivered at a symposium last spring, and it presents in a vivid manner the state of knowledge of vitamin E at that time. The first section of the book, dealing with the chemistry of the vitamin, begins with a clear-cut review by Professor A. R. Todd of the isolation of vitamin E, the elucidation of its structure, and finally its synthesis. The narration of elegant work by the organic chemist leading to the final success of a synthetic production of a natural substance is always fascinating, and every chemist will read this survey with pleasure. Other articles follow, dealing with synthetic analogues of the vitamin and methods for its chemical estimation. It appears that a promising method for the determination of vitamin E is based on its power to reduce ferric chloride.

The satisfaction induced in the reader by the tale of achievement recounted in the first part of the book begins to evaporate as soon as he enters the two sections that follow. The physiological functions of vitamin E and its usefulness in human and veterinary therapeutics are beset with doubts and contradictions. These doubts weigh increasingly on the reader as, having followed a review by Professor J. C. Drummond of the confusing physiological situation, he is plunged into the thick of the trouble in articles dealing with the difficulties of producing standard conditions of vitamin E depletion in rats, and hence the difficulty of measuring the vitamin. Other contributions are concerned with such points as the relation of vitamin E to the endocrines and the distribution of vitamin E in rat tissues.

Vitamin E has been used therapeutically in both veterinary and human medicine. An excellent review by P. Vogt-Möller covers the literature in both fields. Then comes a paper by E. Shute expounding his theory that vitamin E

and oestrogens are in antagonistic equilibrium in the blood. By this time the scientific reader begins to appreciate that he has left the realms of exact experimentation and entered the field of clinical research where the doctor who is reluctant to make negative controls of any of his patients is forced to draw his conclusions without them. The present practical position is summed up thus by Professor F. J. Browne, who was chairman of the clinical session of the symposium: while one worker claims that habitual abortion can be cured with vitamin E, another states that it cannot but that the vitamin will prevent threatened abortion. He is contradicted by a third. Where seven women out of eighteen cases of habitual abortion, however, *were* reserved as negative controls and were *not* given vitamin E, all gave birth normally. Thus at present the published findings of the clinical use of vitamin E cancel themselves out.

The bibliography at the end of the book is one of its most valuable parts and will recommend it to all those working on the subject. For the more general reader the volume provides at once a clear summary of the present state of knowledge of vitamin E written by many of the leading workers in the field; at the same time the presentation as a series of papers gives a vivid impression of the discoveries of the future in the making. Finally, while the Public Analyst values the establishment of a chemical method for the assay of vitamin E preparations, he awaits, with perhaps even more interest, the decision of the biologist as to whether the human species is allied with the rat, which requires vitamin E for fertility, or claims relationship to the goat, which multiplies happily without it.

MAGNUS PYKE

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FLUORESCENCE ANALYSIS. By J. A. RADLEY and J. GRANT. 3rd Ed. Pp. xvi + 424. London: Chapman & Hall. 1939. Price 22s. 6d. net.

MICRO-DIFFUSION ANALYSIS AND VOLUMETRIC ERROR. By E. J. CONWAY. Pp. xiii + 306. London: Crosby Lockwood & Son, Ltd. 1939. Price 25s. net.

METALLURGICAL ANALYSIS AND ASSAYING, By J. S. REMINGTON and F. L. JAMESON. Pp. 101. London: The Technical Press, Ltd. Price 5s. net.

TEXT-BOOK OF QUANTITATIVE CHEMICAL ANALYSIS. Y. H. YOE. Pp. ix + 219. New York: Wiley. London: Chapman & Hall. 1939. Price 12s. 6d. net.

BRITISH CHEMICALS AND THEIR MANUFACTURERS. Association of British Chemical Manufacturers. Pp. 393. 1939.

REPORT OF THE DEPARTMENT OF HEALTH OF MONTREAL (CANADA) FOR THE YEAR 1938.