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Obituary

JOCELYN FIELD THORPE

JOCELYN FIELD THORPE was born in Clapham, London, on December 1st, 1872, the sixth son of William George Thorpe, F.S.A., of the Middle Temple. He received his early education at Worthing College, and in 1888 entered King's College, London, as an engineering student under Professor Wilson. The two years spent in the engineering laboratories, and particularly the experience derived from the shops at Alexander Wilson's Vauxhall Works during each long vacation, appealed to Thorpe's practical sense and laid a foundation of experience in engineering science which he found of great service in his future career as a chemist. His connection with King's College was appropriately recognised when he was elected a Fellow of the College in 1924.

It was a somewhat revolutionary step in those days to choose chemistry as a profession, but this bold course was decided upon by Thorpe's father on the advice of Sir Edward Thorpe, in whose department, at the Royal College of Science, South Kensington, he then entered as a student in 1890. Here he remained for two years, taking only the first year course of elementary chemistry and physics and the third year of advanced chemistry, chiefly organic chemistry, in which he found his vocation. In 1892, again on the advice of Sir Edward Thorpe, he entered the research school of Victor Meyer at Heidelberg, at that time the leading school in organic chemistry; he worked there for two years under the direction of Carl Auwers, and obtained the degree of Ph.D. in 1895.

On his return to England, Thorpe went to Owens College, Manchester, where he remained for ten years. At first he was a Research Fellow and obtained the degree of D.Sc.; later he became a member of the teaching staff. He carried out much important work at this period, a good deal of it in connection with the synthesis of degradation products of the terpenes, which was attracting considerable attention at that time. Towards the end of this period in Manchester, Thorpe was elected a Fellow of the Royal Society. In 1902 he married Lilian, the only daughter of the late William Briggs, J.P., of Hale, Cheshire.

In 1909 Thorpe was awarded the Sorby Research Fellowship of the Royal Society, and spent four years at the University of Sheffield, working mainly on various problems of aliphatic chemistry, such as the isomerism of the glutaconic acids and the reactions of the imino-compounds, including the reaction which bears his name.

In 1914 he was appointed Professor of Organic Chemistry, University of London (Imperial College), a post he occupied until his retirement in 1938. As soon as he was appointed he set out to expand the then limited facilities for research; this expansion was necessarily interrupted by the war, but he came back to the task in 1919 and founded a school of research second to none in the

country. His enthusiasm and encouragement stimulated the activity of a large band of pupils and attracted research students not only from home and the Dominions but also from foreign countries.

Thorpe's ability and shrewd common sense were well known, and many public bodies were glad to avail themselves of his services. Thus he was member of the Chemical Defence Committee, War Office, and in 1917 received the honour of C.B.E.; he served on the Advisory Council, Department of Scientific and Industrial Research, 1916–22. He was President of the Indian Chemical Services Committee, 1919–20, member of the Safety in Mines Research Board, 1924–35, and Chairman of the Explosives in Mines Committee, Department of Mines; and member of the Dyestuffs Development Committee, Board of Trade, 1925–34.

Thorpe gave many years of devoted service to the Chemical Society, of which he was in turn Treasurer, Vice-President and President during the important period of 1928–31.

He was also a Member of Council of the Royal Society 1923–25, and President of the Institute of Chemistry 1933–36. He was awarded the Longstaff Medal of the Chemical Society in 1921, and the Davy Medal of the Royal Society in 1922. In 1933 he was elected an Honorary Member of this Society. In 1939 a Knighthood was conferred upon him in recognition of his valuable public services.

Thorpe owed much of his success, both as a teacher and as a man of affairs, to his personal qualities, his joviality and charm of manner. He had an incorrigible faith in the goodness of human nature and refused to see anything but the best in the people with whom he came in contact. His kindly interest in his old students will also be widely remembered. He was an admirable host, in which capacity he was ably supported by Lady Thorpe, and a large circle of friends will long cherish happy memories of their kind and gracious hospitality.

On his retirement, as Professor Emeritus, from the Imperial College, Thorpe found great happiness in his delightful home at Cooden Beach; here he carried on his literary work and the duties connected with numerous committees of which he was an active member. He had a great love for, and a wide knowledge of, old English china, of which he made a valuable and unique collection; he also took a keen interest in his garden, especially in the growing of roses. The end came peacefully, but with tragic suddenness, on June 10th, 1940, a few hours after he had devoted the usual daily attention to his rose garden.

M. A. WHITELEY
G. A. R. KON

Spectrophotometric Assay of Vitamin A, with Special Reference to Margarine

BY J. R. EDISBURY, B.Sc., PH.D.

(*Read at the Meeting, May* 1, 1940)

THE recent Regulation of the Minister of Food, requiring all margarine sold in the British Isles to be vitaminised, will have directed attention to the problems involved in the assaying of vitamin concentrates sold for this purpose and of the vitaminised margarines offered to the public.

As with all physiologically active materials, the biological approach to the problem of assaying is an indispensable first step and the ultimate court of appeal in cases of doubt, but in ordinary practice it is open to objection on account of the time required for an assay, the highly specialised technique involved and the relatively large experimental error inseparable from biological methods. Those concerned with the assaying of vitamin A preparations are fortunate in having available chemical and physical means for securing the desired information, and

in being able to link up such observations with the biological effect of the vitamin. None of these methods is free from difficulty, and where the degree of dilution is high—as in margarines and butters—special skill and experience are needed both in carrying out the tests and in interpreting the results.

The physical assay of vitamin-A concentrates may be undertaken by any, or all, of the following well recognised means:—(1) Blue value in various guises (Carr-Price,[1] Moore Blue Units,[2] Dilution Strength,[3] Dann and Evelyn L-620$m\mu$ value[4]). (2) Vitameter[5] and simple photoelectric devices (*e.g.* McFarlan *et al.*[6]). (3) Spectrophotometric technique.

The first of these depends on measurement of the intensity of blue colour developed on adding a solution of antimony trichloride in chloroform to a solution of the concentrate, also in chloroform. The second depends essentially on measuring the intensity of light absorption in the ultra-violet at a wavelength of 325$m\mu$, the vitamin A absorption maximum. The spectrophotometric method, essentially a combination and refinement of the first two methods, is of special value in that the tests are complementary and provide additional evidence of normality or abnormality—a matter of importance in the interpretation of results. From a spectroscopic point of view, the "blue value" determination involves measurement of the intensity of light absorption at 620$m\mu$, and to support this observation, measurements at 580$m\mu$ are also made. Margarines in particular respond better to spectrophotometric treatment than to other methods, although with careful attention to detail (including "calibration" of the observer) an abridged form of the "dilution test" described by Andersen and Nightingale[3] is specially useful in routine checking.

It is proposed here to describe briefly the application of spectrophotometric methods to the assay of vitamin A in margarines and the "concentrates" used in their vitaminisation.

Apparatus.—For a comprehensive assay, two instruments are required, *viz.* for the colour test, a visual spectrometer with photometer; for the ultra-violet examination, either—and preferably—a medium quartz spectrograph and photometer, taking 10 in. × 4 in. plates, or a vitameter. Provided that the limitations inherent in measurements confined to 325–330$m\mu$ are recognised, and that the instrument is adapted for photography[7] and calibrated spectrometrically, the Hilger vitameter affords an inexpensive alternative to the ultra-violet spectrophotometer, and is very nearly as accurate in use (probably ±3 per cent.).

Solvents and Reagent.—Special attention should be given to the choice of solvent for dissolving the material under ultra-violet examination, and to the preparation of antimony trichloride reagent. The solvents normally in use for ultra-violet work are cyclohexane, ethyl and *iso*propyl alcohols, etc., and between these solvents the choice usually depends almost entirely on convenience. Chloroform or ether should be avoided.[8,9] For general purposes cyclohexane may be safely recommended; *iso*propyl alcohol has very occasionally been found to give reproducible results a few per cent. higher than cyclohexane. For measuring the absorption at 620$m\mu$, which is characteristic for normal vitamin A when treated in solution with antimony trichloride, it is essential to use cyclohexane or chloroform as solvent—the alcohols obviously cannot be used.

We have found that special care is necessary in the preparation of the antimony trichloride reagent, to ensure removal of any trace of ferric chloride. The standard 20–25 per cent. solution of antimony trichloride in chloroform should be purified by adding 0·1 per cent. of water, shaking thoroughly and leaving in a refrigerator overnight before decanting the clear reagent; this should be kept in a colourless glass bottle of good quality, painted on the outside to exclude light. Coloured glass bottles give up sufficient iron to spoil the reagent.

Examination of Concentrates.—The general procedure is varied according to the character of the material. For concentrates, in which the potency may be

of the order of 1000–200,000 I.U. or more of vitamin A per gram, the recommended procedure is as follows:

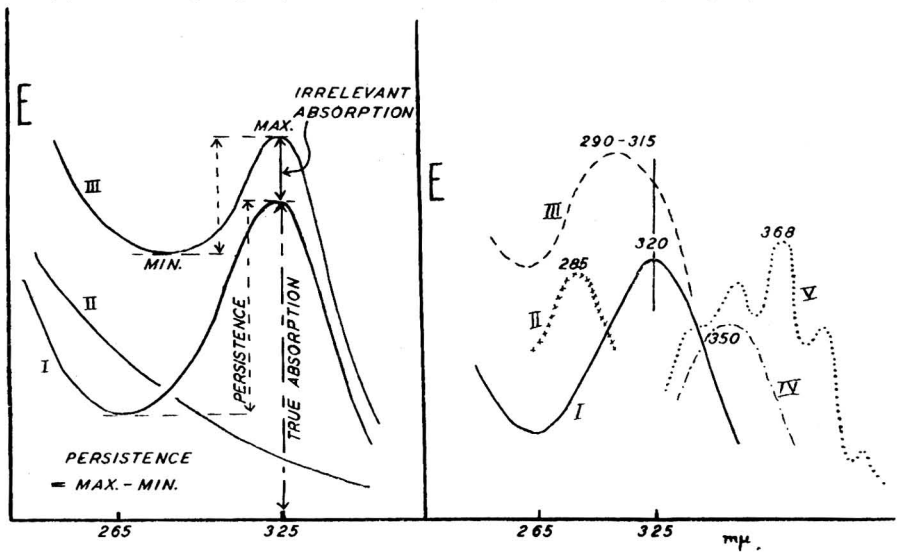
Prepare a solution in pure cyclohexane and dilute as required; 1 per cent. (w/v) is suitable for a concentrate of 5000 I.U. per g., and concentrations in inverse proportions for materials of other potencies.

1. For *ultra-violet assay*, photograph the absorption spectrum of duplicate portions in two stratum thicknesses* that differ by about 10 per cent. (say 3 and 3·3 mm.) and determine the $E^{1\%}_{1\,cm.}$ 325$m\mu$ value. This represents the total light absorption at 325$m\mu$ due to the combined effects of the vitamin A and the diluent oil, if any (Fig. 1). The absorption of the refined and

Fig. 1

IRRELEVANT ULTRA-VIOLET ABSORPTION

(*a*) *Removed by saponification.* (*b*) *Not removed by saponification.*



I. Vitamin A.
II. Glycerides, etc.
III. Observed summation curve. Note diminished persistence.

I. Vitamin A.
II. Unknown component of whale-liver oil (A_3?).
III. Typical commercial whale-liver oil = summation of I and II.
IV. Vitamin A_2.
V. Cyclised vitamin A (crude).

deodorised oil often used varies from sample to sample over a range of about 0·07 to 0·2 at this wavelength, and must be deducted in order to obtain the true (net) vitamin contribution. If, as is most likely, a sample of diluent oil is not available for direct test† a standard deduction of 0·1 from the gross or total $E^{1\%}_{1\,cm.}$ 325$m\mu$ value will thus bring the result to within 0·1 of the true figure. Such correction is not required for values exceeding $E^{1\%}_{1\,cm.}$ 325$m\mu$ = 10. With further dilution (by 5, in the example cited) the solution will be suitable for vitameter readings.

In the routine testing of a normal concentrate, more than this is not strictly required. The colour reaction forms a useful check, however, particularly when the result is doubtful, and in the examination of an unknown material it provides a convenient first approach from which appropriate conditions for ultra-violet assay can be calculated, or further treatment planned.

* Alternatively, one portion can be diluted slightly—say 10 per cent.—using an N.P.L. cylinder, and the same thickness employed again.

† If available, a 1–1·5 mm. thickness of the diluent oil is convenient, the concentration being taken as 92 per cent. for purposes of calculation.

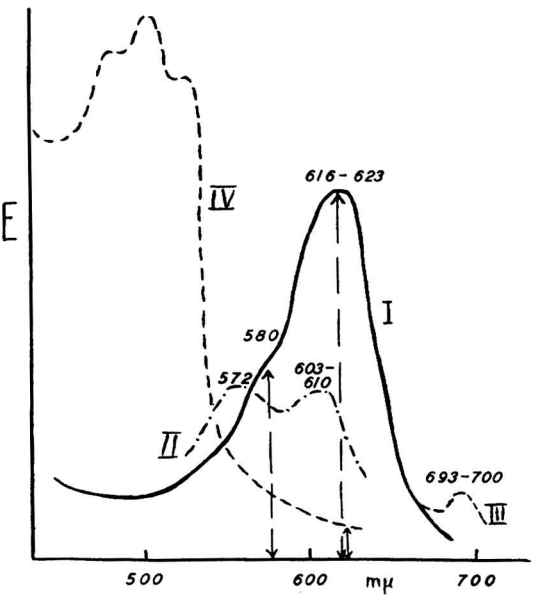
2. *Colour Test.*—The same cyclohexane solution can be employed. The presence of up to 15 per cent of cyclohexane has not been found to have any discernible effect on the antimony trichloride reaction, and the saving of time by the use of one solution is considerable.

The Carr-Price blue value is estimated in the standard manner, but one point requires emphasis: the solution must be diluted (N.P.L. cylinder) until under the accepted conditions of 0·2 ml. of solution and 2 ml. of reagent in a 1-cm. cell the colour *matches 5 Blue* on the Lovibond scale (plus the usual corrections, say + 1·5Y — 0·3N). A low "match" gives high results, and *vice versa.*

In the spectrophotometric colour test, the cyclohexane solution (1 per cent. for 5000 I.U. per g.) is again used, but otherwise the technique devised by Morton[8] is followed in essentials. Intensity readings are taken at the absorption maximum, 616–623$m\mu$, and also at 580$m\mu$, where

Fig. 2

ABSORPTION SPECTRUM OF ANTIMONY TRICHLORIDE COLOUR REACTION



I. Normal vitamin A. Note 2 : 1 ratio between intensities at 620 and 580$m\mu$.

II. Severely inhibited vitamin A as in cod-liver oil. The ratio is altered and the wavelength-maxima displaced. On saponification, normality is restored. II and I are typical of the same weight of vitamin A before and after saponification of a weak oil.

III. Vitamin A_2.

IV. Complex formed between antimony trichloride and colouring matter of a typical margarine. This gives a relatively stable red colour, the 620$m\mu$ absorption of which can be determined after the vitamin A band has faded.

in normal circumstances the intensity is about half that at 620$m\mu$ (Fig. 2). Any marked deviation from a wavelength maximum of 620$m\mu$ (say to 603–606$m\mu$, as seen in the direct testing of cod-liver and similar oils) or from a bright blue colour, calls for further investigation. Reliable estimates of vitamin content cannot as a rule be based on uncorroborated data obtained under these conditions.

With a 2-cm. cell, matching at between 1·8 and 2·2 on the colour density (log I_0/I) scale is recommended. As in the Carr-Price test, unreliable results are otherwise likely to be obtained. Cloudiness of the solution can usually be cleared by addition of a drop of acetic anhydride.

Even with all precautions, the absorption intensity or colour density at 620$m\mu$ is often lower than might be anticipated from the ultra-violet results, probably owing largely, but not exclusively, to inhibition[11] (mechanical as well as chemical) of colour development by components of the diluent oil. Some typical results are summarised in Table I.

TABLE I

TYPICAL ULTRA-VIOLET AND COLOUR-TEST DATA

	$E^{1\%}_{1\,cm.}$				
		620$m\mu$		Blue value	
	325$m\mu$ obsd.	obsd.	calcd. from obsd. u.v.	obsd.	calcd. from obsd. u.v.
Normal rich fish oils or concentrates..	50	155	155	2500	2500
	32	100	100	1600	1600
Whale-liver concentrate	60	130–180	180	2800–3000	3000
Whale and fish-liver concentrates in vegetable oil:					
(i) direct	3·6	9·4	10·9	165	175
(ii) *via* unsap.	3·2–3·5	9·5–10	10–10·9	—	—
Cod-liver oils	1·1	1·5*	(3·4)	20	(55)
	0·5	0·6*	(1·5)	8	(25)
Same cod-liver oils *via* unsap. ..	0·80	2·4	2·48	40	40
	0·32	0·95	1·0	15	16
Two abnormal liver oils	14	28	43·5	650	700
	56	107	174	1700	2800
Margarine:					
(i) on fat itself	>0·1	<0·01	>0·3	—	>5
(ii) *via* unsap.	>0·03	0·03	>0·1	*ca.* 0·5	>1·5

"Pure" vitamin A ($C_{20}H_{29}OH$)—approximate data from which vitamin A content can be calculated:

$E^{1\%}_{1\,cm.}$ 325$m\mu$, 2000; $E^{1\%}_{1\,cm.}$ 620$m\mu$, 6200; $E^{1\%}_{1\,cm.}$ 580$m\mu$, 3100; L-620$m\mu$ Value,[4] 4800; Blue Value, 100,000; Moore Blue Units per g., 5½ million; Dilution Strength[3], 7-10 million.

* Maximum 603–606$m\mu$.

To "unmask" the vitamin A in the original mixture, saponification is the best method. The following technique, based on S.P.A. procedure,[12] is rapid and reliable, and equally applicable to margarine concentrates and to fish oils of the cod-liver oil type, with the reservation that unaccountable losses of 10–15 per cent. may occasionally occur, despite all precautions:

Dissolve 0·5 to 2·0 g., according to expected potency, in 5 to 10 ml. of cyclohexane, and test as above, thus obtaining useful preliminary and confirmatory information. Boil duplicate 1-ml. portions with 0·2 to 0·4 ml. of 60 per cent. potassium hydroxide solution and 5 ml. of pure alcohol for 5 to 10 minutes (without porous pot, etc.), dilute with 10 ml. of water, extract twice with 25 to 30 ml. of *freshly distilled*[8] ether, wash combined extracts with 10 ml. of water, 10 ml. of dilute potassium hydroxide solution,† 3 × 10 ml. of water, all at 30° C. Remove ether. Add a few drops of pure alcohol to the wet residue, and blow until dry two or three times with nitrogen or carbon dioxide at 100° C. Avoid strong sunlight and maintain anaerobic conditions at all stages; the saponification mixture must not boil dry, for example, but there is no need for a reflux condenser. Dissolve the dry unsaponifiable matter at once in 5 ml. of cyclohexane and proceed as before with colour and ultra-violet tests, calculating the results as if the original oil were in the solution.

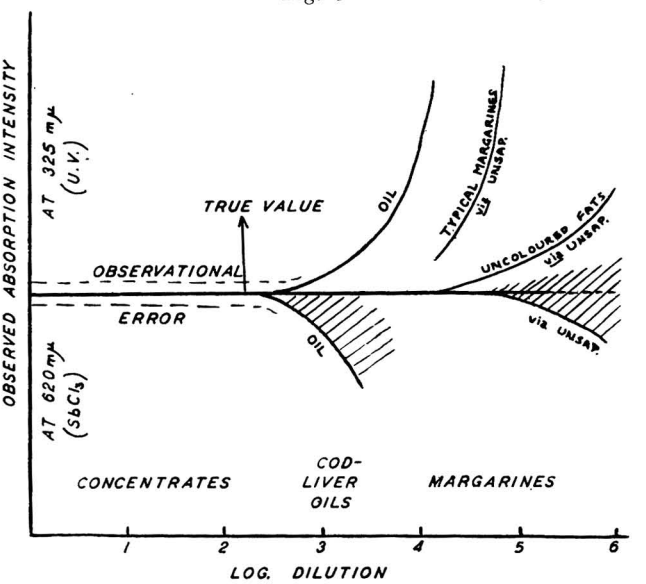
EXAMINATION OF MARGARINES.—While for concentrates the ultra-violet absorption provides the most reliable single criterion of potency, for margarines this method of testing is, in the present state of knowledge, useless owing to the preponderant effect of irrelevant absorption (Table II, Fig. 3). Results may be anything up to several hundred per cent. too high.

† Optional with less than 0·2 g. of oil.

For margarines, the most trustworthy test is the measurement of the absorption at 620$m\mu$ on adding antimony trichloride reagent to a solution of the unsaponifiable matter in cyclohexane or chloroform. A convenient procedure is as follows:

From 8 to 10 g. of margarine are boiled for 10 to 15 minutes with 30 ml. of alcohol containing 4 ml. of 60 per cent. potassium hydroxide solution. The mixture is diluted with 60 to 80 ml. of water and extracted three or four times with 50 ml. of freshly distilled ether. The combined extracts are washed with 50 ml. of water, 50 ml. of dilute potassium hydroxide or carbonate solution, and three times with 50 ml. of water, all at 30° C. As a precaution it may sometimes be advisable to re-extract the washings, in view of the low initial potency, which leaves no margin for possible loss. After removal of the ether (without porous pot, etc.), a few drops of alcohol are added and

Fig. 3



Diagramatic representation of the effect of the diluent oil or fat on $E(^{1\%}_{1\,cm.}\ 325m\mu)$ and $E(^{1\%}_{1\,cm.}\ 620m\mu)$ values, showing how saponification postpones deviation from the truth, and how at high dilutions the antimony trichloride reaction becomes more reliable than the ultra-violet absorption.

the wet residue is blown until dry two or three times at 100° C. under nitrogen or carbon dioxide. The dried unsaponifiable matter is immediately made up to 2 ml. with alcohol-free dry chloroform and the $E^{1\%}_{1\,cm.}$ 616–620$m\mu$ value is determined as if the solution contained 400–500 per cent. (w/v) of margarine, the intensities being matched as before at as near an optical density ($\log I_0/I$) of 2·0 as possible.

For a quick, though less accurate, test, the ethereal extractions may be reduced to two, washing confined to one portion of 50 ml. of water, and the ether dried with sodium sulphate. Such a test will detect gross irregularities and can be carried through in half an hour.

At its best, the test amounts in practice to a determination of the final dilution required to produce a standard effect, and proportionality is not stretched too far. In this respect the method resembles the "dilution test," but has the advantage that bleaching with charcoal can be avoided. We have found it exceedingly difficult to prevent a loss of 10 to 15 per cent. of the total vitamin A as a result of even the most cautious bleaching. The reaction colour of the unbleached material will probably not be blue, owing to the antimony trichloride reacting with the dye to give a relatively stable red complex; but as a rule the normal 620$m\mu$ band is sufficiently separated from other absorption to make estimation to about ±15 per cent. possible; carotenoids (up to more than the vitamin content) can be ignored.

As a refinement, a second reading may be made at 620$m\mu$ after the vitamin A band has faded, and the residual stable absorption, due to the dye and antimony complex, deducted from the gross absorption, due to dye plus vitamin. This correction, actually a slight over-correction, rarely exceeds 10 to 20 per cent. and provides a lower limit to the true value.

LIMITATIONS TO ESTIMATION OF POTENCY.—To these general indications of the technique recommended in the particular cases cited, it will be well to add some brief notes on the interpretation of the results. Limitations to accurate prediction of potency are imposed from three different directions:—(1) The ordinary errors associated with sampling, manipulation, and measurement. (2) Restrictions inherent in the character of the materials under examination. (3) Difficulties arising from the interpretation of the data, especially in relating data to biological activity.

It is not necessary here to insist on the importance of the first of these limitations, but it seems desirable to deal more fully with the other two. Reproducible results are not necessarily correct results. The most important of the limitations inherent in the materials themselves are:—(*a*) inhibition of colour reaction; (*b*) enhancement of colour reaction; (*c*) irrelevant ultra-violet absorption.

(*a*) The inhibition effect[11] is probably best seen in the ordinary blue-value determination on cod-liver oil, and is best overcome by saponifying and making the test on the unsaponifiable matter. Destruction of inhibitors by, for example, bromine, is not always satisfactory. Some inhibition must be expected with many of the margarine concentrates available, but its percentage effect becomes very much less marked with more highly potent preparations and undetectable in the average unsaponifiable fraction. The possibility of occasional unexplained losses on saponification, however, makes it advisable to test all materials directly whether they are also studied *via* the unsaponifiable matter or not.

Partial oxidation of an oil will lead at times to a modification of the blue colour by the introduction of a red constituent, and a quantitative visual comparison is then almost impossible; here again saponification usually overcomes the difficulty.

(*b*) Enhancement of blue colour may arise from the presence of cyclised or "spurious" vitamin A,[13] of vitamin A_2,[14] or of a constituent of whale oil, to which attention has been called.[15,16] With the exception of the last, these may safely be ignored in margarines and margarine concentrates, while the whale constituent fortunately does not sensibly affect the BV/UV ratio if the reagent is iron-free, and so is automatically allowed for by a lower conversion factor (below).

(*c*) Irrelevant absorption is of special importance in ultra-violet measurements, particularly when the Hilger vitameter or similar instruments are used, since obviously no distinction can be made between absorption at 325$m\mu$ due, respectively, to vitamin A and to other ingredients of the preparations. It is clearly important to know how much of the observed absorption is not due to vitamin A. The persistence or crest-to-trough depth of the absorption band provides a useful indication at a glance. Except with whale products, the irrelevant absorption likely to be encountered in most oils, and in margarine concentrates in particular, can be ascribed mainly to glycerides.* The whale-liver oil ingredient—which has also been seen in minor quantities in fish oils—cannot conveniently be removed. Its effect on the absorption spectrum is to displace the maximum from 325$m\mu$ towards 290$m\mu$ (315$m\mu$ is a common figure), but intensity readings are still made *at* 325$m\mu$ for calculation of potency. Although spectroscopically repugnant, this serves remarkably well in practice. Vitamin A_2 and "spurious" vitamin A together seldom contribute more than 5 per cent. to the total $E^{1\%}_{1\,cm.}$ 325$m\mu$ values. When they do, they are fortunately readily detected, and the material must be classified

* This amounts in fish oils to a fairly constant 0·1 to 0·3, a significant proportion with cod-liver oil (total absorption 0·5 to 1·5), but negligible in relation to, say, halibut oil.

as unsuitable for simple physical assay. Glycerides can be either allowed for as already described or, more generally, removed by saponification.

At the potency level of butter and margarines, even the residual absorption by other constituents of the unsaponifiable matter forms a serious proportion of the total absorption; the absorption not ascribable to vitamin A may in fact greatly exceed that due to the vitamin itself (Table II).

TABLE II

PERCENTAGE EFFECT OF IRRELEVANT ULTRA-VIOLET ABSORPTION
(Excluding A_2, spurious A, etc.)

	$E^{1\%}_{1\,cm.}$ 325$m\mu$	Per cent. irrelevant absorption	
		Direct	*Via* unsap.
Halibut, shark, etc., oils	50	0·2–0·6	negligible
5000 I.U. per g. concentrates ..	3·5	1–4	,,
Cod-liver oils	0·5–1·5	6–50	,,
Margarine	about 0·1–0·2	>90	up to 90

It is true that good results have been obtained with some natural rich summer butters by an ingenious modified technique,[17] but this cannot be applied to margarines (or many butters) in which a variable proportion of the non-carotenoid colouring matter remains after saponification and cannot by any means at present known be accurately allowed for, nor completely removed without loss of undefined quantities of vitamin A. Measurement of intensity before and after destruction of the vitamin by irradiation, etc., has been suggested; the effect on other constituents, particularly carotenoids, is, however, unpredictable. Possibly quantitative chromatography will provide an ultimate solution. Meanwhile, only the colour test remains as a practical alternative to the biological assay of margarine.

INTERPRETATION OF DATA.—Conversion of physical data to biological potency, expressed in terms of International Units, is accomplished by the use of suitable conversion factors. Those now used in this laboratory for converting $E^{1\%}_{1\,cm.}$ 325$m\mu$ values into I.U. per gram are:—(*a*) for fish-liver and visceral products: 1600; (*b*) for refined whale-liver products: 1200.

The first is the figure recommended by the International Conference on Vitamin Standardisation, 1934. The second is based on a large number of assays carried out in this department.

Concurrently with the ultra-violet factors, corresponding factors for the colour reaction receive automatic, if tacit, sanction (Table III). Whether the application

TABLE III

CONVERSION FACTORS—PHYSICAL DATA TO INTERNATIONAL UNITS

	Fish products I.U. per g.	Refined whale products I.U. per g.
$E^{1\%}_{1\,cm.}$ 325$m\mu$	1600	1200
$E^{1\%}_{1\,cm.}$ 620$m\mu$	510	380
L-620$m\mu$ value[4]	660	495
Carr-Price Blue Value	32	24
Moore Blue Units per g.	0·6	0·45
Dilution Strength	0·3–0·5	0·24–0·36

of any factor is justified depends on whether the material under test can be regarded as spectroscopically normal. Unless the various data conform reasonably (±10 per cent.) with the ratios for "pure" vitamin A (Table I), the material is abnormal and interpretation of results becomes open to question. Abnormal

materials, if spectroscopically similar, may, of course, legitimately be compared with each other, but not directly with vitamin A.

For all current margarines the appropriate factor for the $E^{1\%}_{1\,cm.}$ 620$m\mu$ value may be taken as 500 without exceeding the limits of error. It would be well, however, to avoid possible confusion with directly obtained biological data by referring to all potencies derived through conversion factors specifically as *Spectroscopic* International Units.

I wish to express my thanks to the Directors of Lever Brothers & Unilever, Ltd., for permission to publish this paper.

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Central Technical Department/Food
Lever Brothers & Unilever, Ltd.
Port Sunlight, Cheshire

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Discussion

Dr. F. H. Carr asked if Dr. Edisbury could give any figures comparing the dilution test with what he himself must have known to be the truth from biological tests. (Now published in Table III, p. 491.) If it would stand the test of comparison the dilution test would surely be of very great use to analysts. In Dr. Carr's view, the u.v. conversion factor of 1200 for whale oil, and even that of 1600 for vitamin A, must be the subject of controversy. While 1600 was the figure adopted by the International Committee on Vitamin Standards, in U.S.A. the factor for pure vitamin A was thought to be over 2000.

Mr. A. L. Bacharach said that Dr. Edisbury had several times referred to whale oil concentrates. He took it that Dr. Edisbury meant products made from whale liver oil and not whale body oil; it seemed to him particularly desirable at the present time to make the distinction clear.

Mr. J. R. Nichols asked if Dr. Edisbury had ever been unable to get a dilution value on margarines? With certain margarines he himself had found a fading of colour, leaving finally nothing but a pinkish shade. The dilution might be 1 in 5 or 1 in 50, and one could not tell the difference. Could the author say where he had gone wrong in getting such a result with margarine?

Dr. Edisbury, replying, said that, in his opinion, justification for the use of conversion factors turned on whether vitamin A was a single substance or a mixture of isomers, etc., spectroscopically similar, but biologically different. If heterogeneity was involved, one certainly got remarkably constant mixtures from a variety of sources, and the ultra-violet conversion factors recommended for fish and whale products were, with a few exceptions, regularly obtained experimentally. Assuming a unique vitamin A with $E(^{1\%}_{1\,c.m.}$ 325$m\mu$) 2000, and defining the International Unit as the activity of 0·6γ of β-carotene, any factor over 800 implied inefficient utilisation of the carotene standard by the test animals, while 1600 indicated the (fortunately consistent) effective use of one molecule of vitamin A out of the theoretically possible two from each molecule of β-carotene. He wondered whether the higher factors used in America originated through hyper-activity of the U.S.P. Reference Oil, or less than 50 per cent. efficient utilisation of carotene, when this subsidiary standard was initially "calibrated." American

factors were further exaggerated by ignoring the demonstrable deterioration of the U.S.P. oil. High factors were, of course, immensely popular with vendors. As regards the other questions, all whale products referred to were liver concentrates. Finally, the unsatisfactory colour obtained in margarine-testing was the chief reason for recommending the spectroscopic test, which, by measuring absorption intensity at one wavelength, avoided any question of matching dissimilar colours. In the simpler dilution test success depended on first bleaching out the dye. Fading was inevitable in either test; hence the need for rapidity.

Estimation of Vitamin D in Margarine

By N. T. GRIDGEMAN, B.Sc., H. LEES, B.Sc., and H. WILKINSON, Ph.D.

(*Read at the Meeting, May* 1, 1940)

It is now compulsory that all margarines sold in this country be vitaminised with vitamins A and D. The estimation of vitamin A by spectrophotometric methods has been discussed by Edisbury[1] (p. 484); in this paper we deal with the estimation of vitamin D.

There have been many chemical tests proposed for the estimation of vitamin D, but interference by other substances such as sterols, vitamin A and carotenoids, has so far made it impossible to apply them to margarine. Moreover, the potency of margarine (about 1 I.U. of vitamin D per gram—equivalent approximately to 0·025 p.p.m.) is very much lower than any which other investigators have estimated colorimetrically.

It is necessary therefore to use a biological method. There are three in general use, and all depend upon assessment of the antirachitic activity of vitamin D. They are the bone-ash method, the X-ray method and the line-test method. The first is prophylactic and depends upon assessing the degree of prevention, by vitamin-D administration, of the normal fall in the bone ash-content in rats weaned on to, and maintained on, a rachitogenic diet for a test period of 3 to 5 weeks. The other two methods are curative tests carried out on animals in which rickets has first been produced by a rachitogenic diet. In the X-ray method, the healing due to administration of vitamin D is assessed radiographically. In the last method, the line test, the healing that takes place between the cap and the shaft of the radius and ulna is also evaluated, but the assessment is dependent upon visual or photographic examination of stained sections of the bones.

In the assay of margarine a modification of the line-test method has been used in this laboratory for the last 7 years. The statistical error of this test has been calculated to be 76 per cent. to 132 per cent. ($P = 0{\cdot}99$) for ten pairs of rats. The technique, in which camera-lucida drawings of the stained distal ends of sagittal sections of the radii and ulnae are measured by a planimeter, was described by Morgan.[4] The diet given by him has been modified (Wilkinson[5]) to maize (flaked and ground), 84; meat meal, 12; salts, 4 per cent. The essence of the test consists in comparing the extent of healing of rickets effected over a ten-day period by daily doses of 0·25 or 0·5 I.U. of vitamin D Standard per rat, with that effected in a second group of rats receiving equivalent doses of the vitamin-containing material.

It is necessary that this material should be dosed to the rats in quantities or dilutions containing as nearly as possible either 0·25 or 0·5 I.U. even if, with a material of unexpectedly high or low potency, a re-assay has to be carried out. Appropriate doses of a margarine of unusually low potency, say of the order of 0·1 I.U. per gram, will constitute a significant fraction of the daily food-intake, and in consequence the consumption of the diet itself will be reduced to an extent detrimental to the healing mechanism. Lines on which a modified technique—involving a compensatory increase of the rachitogenic salt-mixture in the diet of the "margarine" rats—could be developed to meet such a contingency, have been

suggested elsewhere.[2] Extraction and assaying of the unsaponifiable fraction of the margarine fat should theoretically provide another solution, but in practice it has been found difficult to avoid vitamin-D losses during the chemical manipulation. Presumably margarine vitaminised much below normal will be encountered only infrequently, and therefore this necessarily tedious—and expensive—method of arriving at a reasonably accurate assay, will not often be called for. Nevertheless it would obviously be advantageous to have a vitamin-D test which could more easily be applied to margarines of all likely potencies.

This consideration, among others, led us to examine the merits of the bone-ash technique. This was before Henry and Kon[3] reported results, obtained by this method, on the antirachitic action of fat. While this may introduce a difficulty

TABLE I

Substance	Line-test ratio	Bone-ash test ratio
A	108	96
		96
B	110	98
		97
C	76	71
D	103	93
E	87	78
F	108	97
G	97	85
		88
H	69	50
	72	49
J	83	68
	93	68

into assaying material of low potency, we ourselves have encountered a further difficulty, which applies generally under the conditions of our experiments.

The bone-ash technique in use in this laboratory is based on the normal prophylactic technique described in the literature. The rachitogenic diet given above is employed during a test period of 3 weeks from the day of weaning. Dosage levels of 0·1 and 0·2 I.U. per day are generally used, although even lower levels can be used if a 5-week period is adopted. The ash content of the fat-free moisture-free tibia and femur is determined and used as an inverse measure of the rickets. A number of antirachitic substances have been assayed by both methods (line test and bone-ash) and five repeat assays have been performed. The results are expressed as the ratio:

$$\frac{\text{Potency of Test Substance Dose}}{\text{Potency of International Standard Dose}} \times 100,$$

and are given in Table I. The statistical errors of the ratios are not given.

The errors of both sets of tests were about the same. The results show that the bone-ash method allows very good repetition, possibly better, in fact, than the line-test method. However, the most notable feature is that in all the bone-ash assays the potency, expressed in the Table as a ratio, is lower by about 10 per cent. The materials which form the subject of the above Table cover a large range of potencies from 0·5 I.U. to 40×10^6 I.U. per gram.

In seeking to explain these differences we must bear in mind that the line-test measures a purely localised reaction, while the bone-ash test measures the results of a more generalised one. Throughout all this work the International Standard has been used. If the International Standard had been calciferol, pure vitamin D_2, which is one of the test substances included in Table I, then the assays obtained

by line-test and bone-ash methods would have been identical. It is possible that there is, in the International Standard, which is the only crude irradiated material used in this work, some substance that produces a greater response in the bone ash than in the line test. Two experiments have been carried out to examine this possibility.

The line test is necessarily curative and the bone-ash method is usually prophylactic, but by means of a prolonged line test it has been possible to carry out on the same animals both line-test and curative bone-ash assays. The results are given in Table II.

TABLE II

CURATIVE LINE-TEST AND BONE-ASH ASSAYS ON THE SAME ANIMALS

	Ratio: $\frac{\text{Potency of test substance dose}}{\text{Potency of International Standard dose}} \times 100$		$\frac{\text{Line test ratio}}{\text{Bone-ash ratio}}$
Expt.	Line test	Bone-ash test	
1	122	102	1·2
2	165	125	1·3

The results show that the ratio between the effects of two substances, one of which is the International Standard, is different for the line-test and for the bone-ash test. This indicates that the crude irradiated material constituting the International Standard has two different effects, according as it is used in the line-test or the bone-ash method.

SUMMARY.—Margarines of potency 1·0 International Units of vitamin D per gram can be assayed satisfactorily by the normal line-test method. Assays of the vitamin concentrates and the margarine made with them show that the margarine assay is not falsified by the obligatory feeding of fat to the test-animals. The assay of very low-potency margarines, however, entails the feeding of larger quantities of fat, which have been found to affect the responses of the test-animals. The more sensitive bone-ash assay has been suggested as a preferable method. This has been tested, but the two methods give somewhat different results. It is possible that the International Standard is not so effective in the line-test as it is in the bone-ash assay. Results indicating such a difference in activity have been obtained.

We wish to thank the Directors of Lever Brothers & Unilever, Ltd., for permission to publish these results.

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DISCUSSION

Mr. A. L. BACHARACH said that the paper threw a good deal of light on a very tricky problem. He thought that the suggested explanation of the fact that two different methods of assay gave significantly different results for the amount of vitamin D, in international units, present in oils, concentrates and calciferol, was probably the correct one. Nobody was really to blame for the fact that the International Standard Preparation behaved somewhat differently from pure calciferol; at the time the Preparation was made available, pure calciferol was not known. The authors' results differed from those of Dr. S. K. Kon in that they were unable to detect any antirachitic action of fat as such. This was fortunate, because it meant that they could feed their test sample direct to the animals without previous saponification to remove glycerides, and

this would obviously involve a considerable saving of time and would also avoid the risk of losing vitamin D.

Dr. H. E. Cox remarked that a few years ago Brockmann and Chen published a modification of the antimony chloride test which was supposed to give a quantitative determination of vitamin D. It was now known that as a quantitative test the method failed, but he would like to know whether the authors considered it of any qualitative value; if it could be used, it would be so much simpler than bone tests.

Dr. WILKINSON, replying, said that all he had wanted was a reliable method for the assay of margarine. Dr. Kon had used starch in his diet and had not added vitamin D, and therefore his experiments were not really comparable with those previously reported by Lees, Gridgeman and Wilkinson. Replying to Dr. Cox, he said that the spectrophotometric absorption due to one unit per gram would be so small that it would not be detectable in the test. In his laboratory they had not been able to apply the Brockmann-Chen test to margarine or margarine concentrates.

The Determination of Phosphorus in Titanium Steels*

BY A. T. ETHERIDGE, M.B.E., B.SC., F.I.C., AND D. G. HIGGS

IN a paper read before the Cleveland Institution of Engineers in 1920, Ridsdale[1] drew attention to the inaccurate results for phosphorus in cast iron caused by the presence of 0·1 to 0·4 per cent. of titanium. Apparently the low results obtained are due to the phosphorus being incompletely precipitated as phosphomolybdate. No explanation of the cause was suggested, but it seems probable that titanium may form a complex compound with the PO_4''' anion, thus competing with the formation of the molybdate complex. To prevent this interference Ridsdale put forward two remedies: (1) removal of titanium by cupferron precipitation; (2) modification of reagents, involving a double precipitation of the phosphomolybdate. Both methods appear to be accurate but are, of course, more cumbersome than the usual procedure for determining phosphorus in steel.

The matter has not received much attention in text-books apart from the statement that titanium, if present in quantity, must be removed before phosphorus is determined, but no attempt has been made to formulate the maximum percentage of titanium permissible for the accurate determination of phosphorus. This is probably due to the fact that titanium is rarely found in steel except in minute traces, which admittedly have no effect. Modern steel research, however, is gradually including the testing of alloys formed with elements not previously tried, and titanium is one of these. It has therefore become necessary to find a reliable method of determining phosphorus by a single precipitation without removing titanium. Preliminary work established the fact that the maximum amount of titanium consistent with this requirement is approximately 1 per cent. When the titanium-content exceeds that amount it cannot be held in solution in the usual nitric acid solvent (sp.gr. 1·2), so that under such conditions separation at the outset by a method on the lines described by Ridsdale (*loc. cit.*) appears to be the only practicable course. Considerable interest attaches, however, to the effect on steel of titanium of the order of 1 per cent. or less.

A series of tests was carried out on electrolytic iron (containing a mere trace of phosphorus, approximately 0·003 per cent.) to which were added known amounts of titanium and phosphorus. The standard titanium solution was prepared from potassium titanium oxalate, the oxalate radical being decomposed by boiling with conc. nitric acid, evaporating the solution to a small bulk and diluting with nitric acid (sp.gr. 1·2) to a known volume. The titanium-content of this solution was checked gravimetrically in the usual way. The standard phosphate solution was prepared from AnalaR sodium phosphate, a calculated amount being weighed

* A communication from the Research Department, Royal Arsenal, Woolwich.

and dissolved in nitric acid (sp.gr. 1·2) and diluted to a known volume with the same acid. The use of nitric acid of that strength as a solvent and diluent facilitates the preparation of the tests which are described below. For each test the usual two-gram weight of electrolytic iron was used.

In this first series the method described by Etheridge[2] was followed and the results are shown in Table I, Series I.

TABLE I

Titanium added Per Cent.	Phosphorus added Per Cent.	Phosphorus found*		
		Series I Per Cent.	Series II Per Cent.	Series III Per Cent.
1·00	0·020	0·011	0·018	0·021
1·00	0·030	0·025	0·029	0·030
1·00	0·040	0·032	0·035	0·041
1·00	0·050	0·042	0·045	0·051
1·00	0·060	0·048	0·055	0·061
1·00	0·070	0·059	0·065	0·070
1·00	0·080	0·068	0·073	0·079
1·00	0·090	0·074	0·084	0·091
1·00	0·100	0·083	0·096	0·100
0·50	0·040	—	0·039	—
0·50	0·060	—	0·057	—
0·50	0·080	—	0·077	—
0·25	0·040	—	0·039	—
0·25	0·060	—	0·058	—
0·25	0·080	—	0·078	—
0·10	0·040	—	0·039	—
0·10	0·060	—	0·059	—
0·10	0·080	—	0·080	—

* Corrected for phosphorus in the electrolytic iron used (0·003 per cent.).

It is obvious that the results thus obtained are low. It was suggested (by W. J. Agnew, of this Department) that vigorous stirring with a "policeman" rod immediately after the addition of the molybdate reagent, should be tried, this having been found beneficial in the somewhat similar interference of vanadium (*vide infra*). For this purpose tests were carried out in tall beakers to make possible the vigorous stirring referred to above. The results obtained are shown in Table I, Series II.

These results show that (1) the modification of vigorous stirring gives better results; (2) as expected, the results improve with diminishing titanium; (3) correct results cannot be obtained if the titanium exceeds 0·1 per cent.

At this point it occurred to us that Johnson's process[3] for vanadium steels, as modified in this Department, might also be effective for titanium steels. This process was originally put forward as a remedy for the well-known interference of vanadium. It requires the presence of nitric acid in much larger excess and of greater concentration than in the customary methods. Johnson operates on 1·63 g. of steel and requires the solution to stand overnight. Agnew has modified this process by using the usual two-gram. weight of steel and stirring vigorously after adding molybdate as previously described. Under these conditions it is unnecessary for the solution to stand overnight; on the contrary, the determination can proceed after the usual period of standing. Correct results have been obtained with vanadium steels by this method.

This modified process was used in a third series of tests and, as is shown in the last column of Table I, it gives correct results.

It is carried out as follows:—Two g. of steel are dissolved in 45 ml. of nitric acid (sp.gr. 1·2) in a tall beaker, digested if necessary (to decompose carbides) and oxidised with permanganate solution, the excess of which is destroyed with sodium or potassium nitrite as described by Etheridge.[2] Fifty ml. of conc. nitric acid

(sp.gr. 1·42) are added and the solution is boiled for one minute with a glass cover on the beaker. The beaker is removed from the hot plate, the cover and sides are rinsed lightly with hot water, 50 ml. of cold molybdate reagent* are added, and the mixture is vigorously stirred for some minutes with a "policeman" rod. After standing for approximately 30 minutes the precipitate is filtered off and dealt with as usual.

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March 18th, 1940

The Quantitative Electro-deposition of Tin from Chloride Solutions

Part I. Stannic Tin Solutions

BY F. G. KNY-JONES, M.Sc., A. J. LINDSEY, M.Sc., PH.D., A.I.C., AND A. C. PENNEY, M.Sc., A.I.C.

ADVANTAGES of using hydrochloric acid solutions for rapid electrolytic separation of metals have been set forth in previous communications from this laboratory.[1,2,3,4] The present paper describes a study of the conditions necessary for the quantitative deposition of tin in this way. Several analysts[5,6,1,7] have been unable to remove residual quantities (up to several mg.) of tin during electro-deposition from chloride solution, but no adequate explanation has been given; errors of similar magnitude can be found in the work of others. It has been suggested that small quantities of tin may be lost: (1) by formation of gaseous hydride at the cathode, (2) by volatilisation of stannic chloride during the preparation of the solution, (3) by re-solution of the deposit during washing, and (4) by mechanical loss of tin crystals from deposits of poor quality. We deal with these suggestions in turn.

Formation of Stannane.—This may occur to the extent of 0·01 per cent. of the evolved gases obtained by electrolysing a solution of tin sulphate between lead electrodes.[8] Schleicher and Toussaint[9] could not detect tin hydride under the conditions of electro-analysis, and this is in accord with the observation of Paneth and Rabinowitsch[8] that stannane is decomposed in presence of either tin or strong mineral acids.

Volatilisation of Stannic Chloride.—In our experience this does not occur in the preparation of solutions for analysis provided prolonged boiling with acid is avoided. For example, when a mixture of 2 g. of stannous chloride, 0·5 g. of potassium chlorate and 15 ml. of conc. hydrochloric acid was distilled in an all-glass apparatus the first 1 ml. of distillate contained no tin detectable with toluene dithiol[10] and the first 5 ml. contained only quantities up to 1 mg. When the same apparatus was used for the preparation of solutions from metallic tin as described below, no tin was volatilised. Addition of ammonium chloride or sodium chloride,[11] recommended by some analysts, is therefore unnecessary; moreover, as ammonium chloride promotes re-solution of the deposits (*vide infra*), it is better excluded from the electrolyte.

Solution of Deposit.—There is considerable risk of losing a portion of a tin deposit by re-solution during washing, this loss being promoted by electrolytic action between the tin deposit and the platinum electrode; as was to be expected

* Fifty-five g. of ammonium molybdate and 50 g. of ammonium nitrate, with 40 ml. of ammonia (sp.gr. 0·95) are dissolved in 700 ml. of hot water, and the solution is cooled, made up to one litre, and allowed to stand overnight; it should be filtered before use.

the loss is less when the cathode is coated with a less noble metal such as copper. Although no tin can be detected in the electrolyte after removal of the electrode, considerable amounts may be found in the washings. In our opinion this loss arises in the thin film of electrolyte covering the deposit just before and during washing. Thus in a series of about 25 experiments it was found that when a well-adherent deposit of tin was dipped for ten seconds into the electrolyte from which it had been separated there was a loss of about 1 mg., rising to 1·8 mg. when the temperature was raised from 20° to 40° C. and becoming still greater if the electrolyte made contact with the platinum as well as the deposit. Losses of tin during washing may be largely avoided by the analytical procedure described below.

Mechanical Loss of Deposited Tin.—Much attention has been given to the production of a good film. Large quantities of oxalic acid were recommended by Classen[12] and by Schoch and Brown,[5] the underlying principle being that with stannic tin the complex oxalate assists the formation of a good deposit by lowering the effective concentration of tin ions. As the same result may be achieved by lowering the cathodic current density, it would appear superfluous to add oxalates unless there is some other reason, such as in the separation of bismuth from tin and lead,[4] or in the fluoride separation of lead and tin.[13] It has been noted by Schleicher[14] and Lassieur[15] that the use of oxalate provides an anodic depolariser, but it is recognised by both that further addition of hydroxylamine is desirable to prevent any evolution of chlorine at the anode.

Engelenberg[16] lays stress on the importance of avoiding cathodic evolution of hydrogen, and recommends the use of a persulphate to prevent this. Svéda and Uzel[19] confirm the good effect produced by persulphate in presence of hydroxylamine on the deposit from stannous solutions, but find that larger quantities of hydroxylamine alone have a similar effect. The explanation offered is that hydroxylamine acts as both anodic and cathodic depolariser, being reduced at the cathode to ammonia.

In our investigations we have kept in mind the point that the tin in a solution for analysis may be in either stannous or stannic condition. We have confirmed the reduction of stannic ions during the deposition of antimony or antimony and copper,[18] and find that very little reduction occurs on the platinum electrode when tin alone is being deposited. We consider both hydroxylamine and hydrazine to be suitable anodic depolarisers and that a cathodic depolariser is not necessary provided that cathodic potential control is exercised. We cannot support the statement of Fischer and Schleicher that "hydrazine is unsuitable."[19]

PREPARATION OF SOLUTION.—The metal (tin or alloy) is attacked by a mixture of 10 ml. of hydrochloric acid (sp.gr. 1·16) and either 1 ml. of nitric acid (sp.gr. 1·42) or a little solid potassium chlorate, as little heat as possible being applied. When solution is complete a further 5 ml. of hydrochloric acid and the depolariser are added, and the solution is diluted to the required volume for analysis. If metals more noble than tin are first separated the final volume may be as much as 250 ml. We varied the volumes from 100 to 250 ml., the temperature from 20° to 70° C., and the quantity of depolariser from 0·5 to 4·0 g. In all the tests described here hydrazine (as sulphate or hydrochloride) was used as depolariser, but hydroxylamine is equally satisfactory.

In a number of experiments the electrolyte was neutralised just before the end to minimise loss during washing. The electrodes of Sand[1] were used, and all quoted potentials refer to his design of auxiliary saturated calomel electrode. In most of the tests the initial current was between 1·5 and 3 amperes and the electrolysis time for a quantity of 0·3 g. of tin was 30 to 35 minutes. "Chempur" tin was employed for most of the determinations.

Particulars of the determinations made are given in Table I. They show that in absence of ammonium salts the procedure followed gives very accurate results. The presence of ammonium salts gives rise to serious errors, but these can be

avoided by neutralising the solution just before the electrolysis is terminated. Experiments K3 to K6, in which 5 g. of oxalic acid was added to the solution, show that errors caused by this substance can be avoided by keeping the temperature above 40° C. during deposition.

TABLE I

Expt.	Temp. °C.	Depolariser	Volume ml.	Potential volt	Tin taken g.	Tin found g.	Error g.
Depositions on platinum electrode. Ammonium salts absent.							
K 1	30–35	A 4 g.	100	0·6–0·75	0·1505	0·1503	−0·0002
K 2	20	A 4 g.	250	0·6–0·8	0·1574	0·1576	+0·0002
K 3	20	A 4 g.	250	0·6–0·8	0·1511	0·1502	−0·0009
K 4	40–45	A 4 g.	250	0·6–0·85	0·1530	0·1531	+0·0001
K 5	40–45	A 4 g.	250	0·6–0·85	0·1535	0·1536	+0·0001
K 6	40–45	A 4 g.	250	0·6–0·85	0·1500	0·1497	−0·0003
Depositions on copper-plated electrode. Ammonium chloride (1–2 g.) present.							
P 7	32	A 4 g.	200	*	0·2415	0·2384	−0·0031
P 8	45	A 4 g.	300	*	0·2047	0·2006	−0·0041
P 9	30–35	B 4 g.	200	*	0·1342	0·1317	−0·0025
P 10	35	A 4 g.	200	*	0·2788	0·2786	−0·0002
P 11	40	B 4 g.	200	0·7–0·8	0·2048	0·2051	+0·0003 N
P 12	35	B 4 g.	200	*	0·2055	0·2051	−0·0004 N
P 13	40	A 4 g.	200	*	0·3011	0·3007	−0·0004 N
P 14	40	A 4 g.	200	*	0·2477	0·2472	−0·0005 N
Depositions on copper-plated electrode. Ammonium salts absent.							
L 15	35–40	B 4 g.	100	0·6–0·7	0·1920	0·1919	−0·0001
P 16	35	B 4 g.	100	*	0·1895	0·1888	−0·0007
P 17	30–35	B 4 g.	150	*	0·1912	0·1915	+0·0003
P 18	35	B 4 g.	100	*	0·1994	0·1983	−0·0011
L 19	35–40	B 4 g.	100	0·65–0·75	0·2068	0·2069	+0·0001
P 20	35	B 4 g.	150	*	0·1725	0·1727	+0·0002 N
P 21	35	B 4 g.	150	*	0·2442	0·2446	+0·0004 N
Depositions on platinum electrode. Ammonium salts present.							
K 22	35–40	B 0·5 g.	100	0·6–0·8	0·1504	0·1486	−0·0018
K 23	35–40	B 0·5 g.	100	0·6–0·8	0·1516	0·1502	−0·0014
K 24	70	B 1 g.	100	0·6–0·8	0·2456	0·2444	−0·0012
K 25	70	B 1 g.	100	0·6–0·8	0·2503	0·2502	−0·0001 N
K 26	70	B 1 g.	100	0·6–0·8	0·2427	0·2427	0·0000 N
K 27	70	B 1 g.	100	0·6–0·8	0·2514	0·2515	+0·0001 N

A = Hydrazine sulphate. B = Hydrazine hydrochloride. N denotes that the solution was neutralised at the end of the deposition. * Denotes that the potential was adjusted at the outset and later allowed to rise in order to preserve a constant current.

In Expts. K 3–K 6 inclusive, 5 g. of oxalic acid were added in order to simulate the conditions existing after bismuth has been removed by the method of Kny-Jones.[4]

SUMMARY.—Good results are obtained in the deposition of tin from stannic solutions when potential control is exercised and when ammonium salts are absent, or, if they are present, when the solution is neutralised just before the end of the electrolysis.

We wish to conclude by thanking Dr. Sand for his helpful criticism and advice.

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THE SIR JOHN CASS TECHNICAL INSTITUTE
JEWRY STREET
LONDON, E.C.3

April 6th, 1940

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.

OBSERVATIONS ON THE USE OF ELECTRODIALYSIS IN THE ANALYSIS OF SOILS

IN testing the applicability of Basu's electrodialysis apparatus[1] to the soils of Palestine, samples were collected (*a*) from the hilly land at Rehovoth in the south, and (*b*) from the marshy ground of Hederah in the north. As both soils were homogeneous, the samples were taken at depths of 0 to 120 cm. The first was a red sand soil poor in lime (less than 1·5 per cent.), and the second was a dark heavy soil also poor in lime (less than 0·5 per cent.). Tests for replaceable calcium were made (*a*) by leaching with *N* ammonium chloride solution (Wright),[2] and (*b*) by electrodialysis. Each sample was then mixed with 20 per cent. of calcium carbonate and the replaceable calcium was determined by Hissink's[3] method* and by electrodialysis. The results, as mg. equivalents of replaceable calcium per 100 g. of soil, are given in Table I.

TABLE I

Depth in cm.	Dialysis	Treatment with NH_4Cl	Mixed sample containing 20 per cent. of calcium carbonate: Hissink's method	Mixed sample containing 20 per cent. of calcium carbonate: Dialysis
Light soil				
0–30	4·00	4·20	4·40	No end-point reached
30–50	4·55	4·60	4·45	
50–100	4·50	4·50	4·30	
100–120	4·50	4·60	4·45	
Heavy soil				
0–30	29·80	30·00	30·20	No end-point reached
30–50	30·55	30·60	30·75	
50–100	30·75	31·00	31·05	
100–120	30·80	30·80	30·95	

These results indicate that whilst Basu's apparatus is of value for determining replaceable bases in Palestinian soils poor in lime, it is inadequate with (artificially prepared) highly calcareous soils.

I wish to thank the Government Analyst, Mr. G. W. Baker, for his advice, and Miss S. Adler for assistance in the analytical work.

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M. PUFFELES

GOVERNMENT CENTRAL LABORATORIES
JERUSALEM

February, 1940

* The soil is leached twice with *N* sodium chloride solution and the calcium in each extract is determined; the difference between the results is regarded as exchangeable calcium.

THE DETERMINATION OF BENZOIC ACID

SOME time ago we disagreed with another analyst as to the proportion of benzoic acid in a cordial. Both had followed the method of Monier-Williams (*Reports on Public Health and Medical Subjects*, No. 39, 1927; Abst., ANALYST, **1927**, **52**, 153, 229), and the only variation was that we had sublimed the benzoic acid for 2 hr. at 180° C., whereas the other analyst had sublimed it at 160° C. for 1 to 1½ hours, following the details given on p. 43 of the Report. Our conditions had been fixed as the result of a few control experiments, and, after further investigation, the other analyst was satisfied that he had failed to sublime the whole of the benzoic acid, and we later reached complete agreement.

Monier-Williams's recoveries of benzoic acid in control experiments were satisfactory, and the different conditions that we use for sublimation are necessitated by small, though apparently important, differences between our hot-air oven and that illustrated in his Report.

In our oven, which was purchased specifically for this work, there is a perforated plate about half way between the top and the bottom, and above this, about 5½ cm. from the top, is a flat solid plate. The thermometer is central and there are four holes for the sublimation tubes. These holes are much too wide for the test-tubes, and are flanged. The tubes are therefore held by well-fitting corks. Each tube is arranged so that the lower 4 cm. is inside the oven, and in this position the bottom of the tube is not in contact with the top plate, but about 1½ cm. above it. The thermometer bulb is placed at the same level and probably records the temperature of sublimation fairly closely. In the illustration of the apparatus used by Monier-Williams it would appear that the tubes are actually resting on a metal plate which, in all probability, is at a somewhat higher temperature than is recorded by the thermometer, and this presumably accounts for our differences.

In a more complete investigation, quantities of about 100 mg. of benzoic acid (A.R. quality) were weighed directly into clean, dry test-tubes, the acid was washed down with a little ether, and the solvent was evaporated with suction as in the Monier-Williams method, to leave residues in the same state as would be obtained by application of the method to a sample containing preservative. Acid-washed and ignited sand was then added, a disc of filter-paper was placed in position, and the sublimations were carried out at different temperatures and for different periods of time. The tubes containing the sublimates were dried and weighed, the benzoic acid was washed off with a little ether, and the tubes were re-weighed. The ethereal solutions were evaporated, the residues were dissolved in neutral 50 per cent. alcohol, and the solutions were titrated with $N/10$ sodium hydroxide solution which had been standardised against benzoic acid under the same conditions. The results for the gravimetric and volumetric determinations of the sublimed benzoic acid were in close agreement and are not given separately in the table below.

The lower portions of the tubes containing the sand were extracted with ether, and any unsublimed benzoic acid was determined by titration.

TABLE I.

Temp. of sublimation °C.	Time of sublimation Hours	Benzoic acid taken g.	Sublimate recovery Per Cent.	Recovered from sand Per Cent.
160	1	0·1005	44	47
	2	0·0945	77	16
	3	0·0923	97·5	1·3
170	1	0·0954	73	22
	2	0·0928	96	0·7
	3	0·1076	98	0·0
180	1	0·0912	90	5·4
	2	0·0887	99	0·0
	3	0·0884	98	0·0
190	1	0·0969	97	—
	2	0·0935	99	—
	3	0·0993	98	—

The results emphasise the necessity for control experiments in all analytical work. It is quite clear that unless the oven used for sublimation is arranged to agree in all respects with the one described by Monier-Williams, either a higher temperature or a longer time may be required for quantitative recovery of the benzoic acid.

R. W. SUTTON
O. HITCHEN

DERBYSHIRE COUNTY COUNCIL LABORATORY
COUNTY OFFICES
ST. MARY'S GATE, DERBY

June 26th, 1940

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 are submitted to the Publication Committee.

CITY OF SALFORD

Annual Report of the City Analyst for the Year 1939

Double Strength Self-raising Flour.—The bag containing the sample was labelled "double strength." The flour yielded 0·55 per cent. of carbon dioxide, a figure falling within the limits (0·4 to 0·6 per cent.) yielded by ordinary self-raising flours. The packers agreed to omit the words from the label.

Oil of Bitter Almonds.—Eight samples of oil expressed from different types of bitter almonds were examined to ascertain what variations might be expected.

Type of bitter almond oil	Clouding-point (Evers's modified arachis test) °C.	Bieber (B.P.) test	Iodine value (mean of 2 results)
Refined	−1·0 to −1·5	Very pale yellow	103·3
Cretan	0·0 to −0·5	Very pale buff	104·4
do.	0·0 to −0·5	do.	104·3
Mogador	0·0 to −0·5	Colourless	100·0
do.	0·0 to −0·5	do.	97·6
Sicilian	−0·5 to −1·0	do.	99·1
do.	−0·5 to −1·0	do.	99·4
Portuguese ..	0·0 to −0·5	Very pale brown	97·2

The yellow to brown colours obtained in the Bieber test were extremely faint and did not in any way resemble the pink colours given by mixtures of apricot kernel and almond oils. The figures for the clouding-point approximated closely to those given by Evers (Analyst, **1937, 62**, 99). It will be observed that three of the samples gave iodine values above the B.P. limits of 95 to 100 for almond oil.

Ammonia.—Two samples, from different retailers, each contained 15·6 per cent. by weight of NH_3. Proceedings were instituted and the vendors were fined for selling a poison without being on the List of Sellers under Part II of the Poisons List Confirmation Order, 1935.

Acidity of Soot Deposit.—The figures for the deposit in the special gauges in various parts of Salford are tabulated. The most noticeable feature is their acid nature, as shown by the *p*H values of the water collected. The monthly averages were as follows:

	Salford: Peel Park	Salford: Ladywell Sanatorium	Salford: Drinkwater Park	Marple: Nab Top Sanatorium
Acidity ..	0·55	0·46	0·42	0·23
*p*H	3·9	4·1	4·1	4·4

The *p*H due to carbonic acid in the air would be about 5·5; lower figures indicate an acid, and higher figures an alkaline deposit. Considering that Marple is well in the country and that its general figures are better than those obtained in Salford, its acid rainwater is noteworthy. It shows how widespread may be the drift of acid smoke from the cities. G. H. Walker

Ministry of Food

STATUTORY RULES AND ORDERS. 1940. No. 11*

Emergency Powers (Defence)

Food

The Feeding Stuffs (Maximum Price Order, 1940). Dated January 6th, 1940

THIS Order comprises 20 Articles with four Schedules fixing the maximum prices for the various feeding stuffs specified.

Article 1 defines the terms used in the Order:

"Compound Cake, Compound Meal or Compound Mixture" means a mixture, etc., containing not less than four of the feeding stuffs mentioned in Part A of the Third Schedule in proportions of not less than 5 per cent. of each, and which contains not less than 2·5 per cent. of oil and 12 per cent. of crude protein; but does not include any concentrate.

"Concentrate" means a feeding stuff which contains not less than four of the feeding stuffs mentioned in Part B of the Third Schedule, and which is manufactured for the purpose of being mixed with other feeding stuffs for the production of a complete ration for feeding to animals and which is properly balanced as to its protein, mineral and vitamin content.

"Flour Mill" means a mill where wheat is milled for human consumption.

"Home produced" in relation to any feeding stuffs means produced in the United Kingdom.

Definitions are also given of "Country flour mill," "distributing dealer," "grower," "importer," "sack," "ton" (= 20 cwt. of 112 imperial lb. each), and "wholesale dealer."

Article 2 provides that no specified feeding stuff shall be sold at a price exceeding the appropriate price (*i.e.* the price set out in the First Schedule, or, for compound cakes, as ascertained in accordance with Article 3). Specified sums may be added for kibbling, grinding, sacks, addition of grains or pulses, or of any meals.

Articles 3 to 11 specify the price per ton in excess of the costs of ingredients and manufacture that may be charged by the manufacturer for compound cake, meal or mixture, and the permissible reductions or increases in the appropriate prices for compound cakes, etc., feeding stuffs specified in the First Schedule, and dried sugar beet pulp.

Article 12 provides for the addition of transport charges to the appropriate price, and Article 13 deals with the apportioning of the cost of sacks. Particulars of all additional charges permitted by the Order must be supplied by the vendor on demand by the buyer in writing (Article 14). A reasonable extra charge may be made for credit (Article 15). Article 16 prohibits fictitious or artificial transactions.

THE FIRST SCHEDULE.—*Part A* tabulates maximum prices for wheat by-products, products from maize, barley, oats, pulses, and other cereals, rice bran, dried grains, etc., and sundry products (fish meal, whale meal, etc.).

Part B includes cotton-seed cake and meal, ground nut cake and meal, linseed cake and other oilseed cakes and meals.

Part C gives maximum prices for dried sugar beet pulp, plain or molassed, ex factories in different parts of England.

Part D gives maximum prices for maize, barley, oats, beans, peas, grain, lentils and locust beans; *Part E* the prices for imported wheat; *Part F* the prices for different kinds of hay, home-produced and imported.

The SECOND SCHEDULE tabulates the permissible charges per ton additional to the appropriate prices of the specified feeding stuffs, etc., when sold to a person buying otherwise than for re-sale.

The THIRD SCHEDULE, *Part A*, gives the list of feeding stuffs that may be mixed with those in Schedule I to produce compound cake, meal or mixture, *viz.*: meat and bone meal or meat meal or whale meat meal, molasses, home-grown wheat, barley, beans or peas (crushed or ground), and any other home-grown cereal or pulse (crushed or ground).

Part B gives the feeding stuffs that may be mixed with those in Schedule I to form concentrates: dried blood, meat meal, meat and bone meal, liver meal, whale meat meal, ground-nut cake meal, soya bean cake meal, wheat germ, cod-liver oil, any other vitamin potent oil, dried milk (whole, separated or buttermilk), dried whey, dried grass meal, dried clover meal, dried yeast, kelp.

The FOURTH SCHEDULE gives a list of 17 centres in England and Wales with the distances from them beyond which mills are classified as "country flour mills."

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* H.M. Stationery Office, York House, Kingsway, London, W.C.2. 1940. Price 5d. net.

STATUTORY RULES AND ORDERS. 1940. No. 1119*

The Compound and Mixed Feeding Stuffs (Control) (No. 2) Order, 1940. Dated June 29, 1940

THE Order contains 11 Articles and two Schedules. Article 1 defines the terms used in the Order:

"Compound or mixed feeding stuff" means any compound cake, compound meal, compound mixture or concentrate, or any other mixture of feeding stuffs.

"Feeding stuff" means any substance, other than hay or straw, which is used as food for livestock.

"Licensed concentrate" means a concentrate which a person is authorised to manufacture or prepare for sale in accordance with the terms of a licence granted under the Order.

"Livestock" means horses, mules, asses, cattle, sheep, pigs, goats, chickens, fowls, ducks, turkeys, guinea fowls, geese and carrier pigeons.

"Low protein oil seed cake or meal" means an oilseed cake or meal containing less than 22 per cent. of albuminoid.

"Protein rich substance" means a substance containing not less than 40 per cent. of albuminoid.

Article 2 orders that, except in accordance with the Minister's licence, no person shall:

(*a*) Manufacture or prepare for sale during each three calendar months beginning July 1st, 1940, more than two-thirds of the total quantity of compound or mixed feeding stuff which he manufactured or prepared during the corresponding period of three months between July 1st, 1938, and June 30th, 1939.

(*b*) Manufacture any compound or mixed feeding stuff other than one mentioned or in accordance with the conditions set out in the First Schedule, Part A or B.

(*c*) Use in the manufacture or preparation of any compound or mixed feeding stuff described in Part A of the Schedule any ingredient other than one specified in the Second Schedule.

Article 3 orders that any licence granted under Article 2 may prescribe the composition and quality of any compound or mixed feeding stuff, the name or description under which it may be sold and its maximum price.

Article 4 allows feeding stuffs which are the property of the keeper of livestock to be mixed under specified conditions, subject to the keeper making a written request.

Article 5 provides that every person selling a compound or mixed feeding stuff other than a mixture mentioned in Article 4, shall, if the weight is 2 cwt. or more, cause to be affixed to each container thereof or furnished to the buyer on an invoice a statement specifying the description of the compound or mixed feeding stuff as set out in the first column of Part A or B of the First Schedule or as set out in a licence granted under Article 2 (*b*), and the name of the person who manufactured or prepared the compound or mixed feeding stuff.

Article 6 provides that nothing in this Order relieves any person from the duty of furnishing a statutory statement in accordance with Sec. 1 of the Fertilisers and Feeding Stuffs Act, 1926.

Article 8 revokes:

(1) The General Licence dated September 3rd, 1939, as amended (S.R. & O., 1939, Nos. 1038 and 1777), made under the Control of Mills (Flour and Provender) No. 1 Order, 1939 (S.R. & O., 1939, No. 1037) so far as it is inconsistent with this Order.

(2) The Compound and Mixed Feeding Stuffs (Control) Order, 1940 (S.R. & O., 1940, No. 864), but without prejudice to any proceedings in respect of any contravention thereof.

By Article 9 the Order does not apply to Northern Ireland.

Article 10 provides that Article 2 (*a*) of the Order shall come into force on July 1st, 1940, and that the whole Order shall come into force on July 15th, 1940.

THE FIRST SCHEDULE, PART A.—COMPOUND CAKES AND MEALS.—Briefly summarised, the particulars, tabulated in four columns, are as follows:

National Cattle Food, No. 1 (Dairy ration or cattle or sheep fattening).—Oil, min. 4, max. 6; albuminoids, min. 19·5, max. 21; fibre, max. 9·5 per cent.

National Cattle Food, No. 2 (Ration for grass feeding or fattening).—Oil, min. 4, max. 6; albuminoids, min. 14, max. 17; fibre, max. 12 per cent.

National Cattle Food, No. 3 (Rearing).—Oil, min. 5, max. 6; albuminoids, min. 20, max. 22; fibre, max. 8 per cent.

National Calf Gruel.—Oil, min. 6, max. 12; albuminoids, min. 15, max. 24; fibre, max. 7 per cent.

None of the foregoing compounds must contain more than 2 per cent. of lime (as $CaCO_3$) or 1 per cent. of salt (as NaCl).

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* H.M. Stationery Office. Price 4d. net.

National Pig Food, No. 1 (Pig-nuts or pig meal for sows or weaners).—Oil, min. —, max. 3·5; albuminoids, min. 16, max. 18; fibre, max. 7 per cent.

Composition: Maize meal and/or barley meal and/or dried potato products, min. 35, max. —; wheat feed (other than bran), min. 20, max. —; wheat, min. —, max. 20; oats, min. —, max. 10; bran, min. —, max. 10; low protein oilseed cake and/or meal, min. —, max. 20; *fish and/or animal protein rich substances, min. 5, max. —; molasses, min. —, max. 5; *sundries, including vitamin potent substances and mineral matter, min. —, max. 10 per cent. (salt not to exceed 0·5 per cent.); plus sufficient vegetable protein to give the required albuminoid content.

National Pig Food, No. 2 (Pig nuts or pig meal for fattening).—Oil, min. —, max. 4; albuminoids, min. 13, max. 15; fibre, max. 7 per cent.

Composition: Maize meal and/or barley meal and/or dried potato products, min. 35, max. —; wheat feed, min. 25, max. —; ground oats and/or bran, min. —, max. 20; wheat, min. —, max. 20; low protein oilseed cake and/or meals, min. —, max. 25; *fish and/or animal protein rich substances, min. 2·5, max. —; molasses, min. —, max. 5; *sundries (salt not to exceed 0·5 per cent.), min. —, max. 10 per cent.; plus sufficient vegetable protein to give the required albuminoid content.

National Poultry Food, No. 1 (*Summer Laying*).—Oil, min. 3, max. —; albuminoids, min. 17, max. 19; fibre, max. 8 per cent.

Composition: Wheat feed and/or low protein oilseed cake and/or meal, min. 40, max. 65; cereals, min. 25, max. 50; *fish and/or animal protein rich substances, min. 5, max. 10; molasses (for pellets only), min. —, max. 5; *sundries (salt not to exceed 0·5 per cent.), min. —, max. 7·5 per cent.; plus sufficient vegetable protein to give the required albuminoid content. No decorticated or undecorticated cottonseed cake or meal may be used in this or any of the other poultry foods.

National Poultry Food, No. 1A (*Winter Laying*).—Oil, min. 3, max. —; albuminoids, min. 17, max. 19; fibre, max. 8 per cent.

Composition: Wheat feed and/or low protein oilseed cake and/or meal, min. 40, max. 65; cereals, min. 25, max. 50; *fish and/or animal protein rich substances, min. 5, max. 10; molasses (for pellets only), min. —, max. 5; *cod-liver oil containing not less than 85 I.U. of vitamin D; * sundries including vitamin potent substances and mineral matter (salt not to exceed 0·5 per cent.), max. 7·5 per cent.; plus sufficient vegetable protein to give the required albuminoid content.

National Poultry Food, No. 2 (*Summer Growing*).—Oil, min. 3, max. —; albuminoids, min. 14, max. 17; fibre, max. 8 per cent.

Composition: Wheat feed and/or low protein oilseed cake and/or meal, min. 40, max. 65; cereals, min. 25, max. 50; *fish and/or animal protein rich substances, min. 5, max. 10; molasses (for pellets only), max. 5; *sundries (salt not to exceed 0·5 per cent.), max. 7·5 per cent.; plus sufficient vegetable protein to give the required albuminoid content.

National Poultry Food, No. 2A (*Winter Growing*).—Oil, min. 5; max. —; albuminoids, min. 14, max. 17; fibre, max. 8 per cent.

Composition: Wheat feed and/or low protein oilseed cake and/or meal, min. 40, max. 65; cereals, min. 25, max. 50; *fish and/or animal protein rich substances, min. 5, max. 10; molasses (for pellets only), min. —, max. 5; *cod-liver oil containing not less than 85 I.U. of vitamin D; *sundries (max. salt 0·5 per cent.), min. —, max. 7·5 per cent.; plus sufficient vegetable protein to give the required albuminoid content.

National Poultry Food, No. 3 (*Battery*).—Oil, min. 3, max. —; albuminoids, min. 15, max. 18; fibre, max. 8 per cent.

Composition: Wheat feed and/or low protein oilseed cake and/or meal, min. 35, max. 65; cereals, min. 30, max. 50; *fish and/or animal protein rich substances, min. 5, max.10; molasses (for pellets only), min. —, max. 5; *cod-liver oil containing not less than 85 I.U. of vitamin D; *sundries (max. salt 0·5 per cent.), min. —, max. 15 per cent.; plus sufficient vegetable protein to give the required albuminoid content.

National Baby Chick Food.—Oil, min. 3, max. —; albuminoids, min. 16, max. 18; fibre, max. 6·5 per cent.

Composition: Wheat feed and/or low protein oilseed cake and/or meal, min. 35, max. 65; cereals, min. 30, max. 50; *fish and/or animal protein rich substances, min. 5, max. 10; molasses (for pellets only), min. —, max. 5; *cod-liver oil containing not less than 85 I.U. of vitamin D; *sundries (max. salt 0·5 per cent.), min. —, max. 20 per cent.; plus sufficient vegetable protein to give the required albuminoid content. Rye or rye products may not be used.

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* These ingredients may be in the form of licensed concentrates. Other substances with as high vitamin D content may be used in place of cod-liver oil. Such substances must be warranted in writing as fully effective for poultry in accordance with the Chick Test of the British Standards Institution.

PART B—CEREAL MIXTURES.

National Cereal Mixture, No. 1.—Barley meal, min. 15; fine wheat feed and/or middlings and/or pollards, max. 25 per cent.; maize meal, sufficient to complete mixture.

National Cereal Mixture, No. 2.—Cattle ground oats, min. 30; fine wheat feed and/or middlings and/or pollards, max. 15 per cent.; maize meal, sufficient to complete mixture.

National Wheat Provender Mixture, No. 1.—Barley meal and/or maize meal, min. 65; wheat meal, max. 35 per cent.

National Wheat Provender Mixture, No. 2.—Fine wheat feed and/or middlings and/or pollards, max. 30; maize meal, min. 35; barley meal, min. 20 per cent.; wheat meal, sufficient to complete mixture.

National Cooked and Flaked Wheat Mixture.—Cooked and flaked maize, min. 75; cooked and flaked wheat, sufficient to complete mixture.

National Cereal Mixture, No. 3.—Maize meal and barley meal; no conditions as to percentages.

National Poultry Corn, No. 1A.—Wheat, max. 30; oats, min. 20 per cent.; cut and/or kibbled maize, sufficient to complete mixture.

National Poultry Corn, No. 1B.—Wheat, max. 30; clipped oats, min. 20 per cent.; cut and/or kibbled maize, sufficient to complete mixture.

National Poultry Corn, No. 2A.—Wheat, max. 30; oats, min. 20 per cent.; whole maize, sufficient to complete mixture.

National Poultry Corn, No. 2B.—Wheat, max. 30; clipped oats, min. 20 per cent.; whole maize, sufficient to complete mixture.

National Chick Feed, No. 1.—Fine cut wheat, max. 45; cut groats, min. 10 per cent.; No. 4 fine-screened maize grits, sufficient to complete mixture.

National Chick Feed, No. 2.—Cut wheat, max. 30; No. 3 fine screened maize grits, min. 30; whole groats, min. 10 per cent.; dari and/or millet, sufficient to complete mixture.

National Chick Feed, No. 3.—Fine cut wheat, max. 50 per cent.; No. 4 fine-screened maize grits, sufficient to complete mixture.

National Chick Feed, No. 4.—Cut wheat, max. 60; whole groats, min. 10 per cent.; No. 3 fine-screened maize grits, sufficient to complete mixture.

THE SECOND SCHEDULE.—No ingredient other than those in the following list and those contained in the First Schedule to the Feeding Stuffs (Maximum Prices) Order, 1940, as amended, may be used in the preparation of compound or mixed feeding stuffs (see Article 2 (*c*) p. 505):—Sugar cane molasses, dried grass meal, dried clover meal, alfalfa meal, lucerne meal, dried liver meal, feeding dried blood, dried yeast, cod-liver oil or other vitamin potent substances, licensed concentrates, buck-wheat, home-grown wheat, dredge corn, home-grown barley, home-grown peas, malt, biscuit meal, mineral matter, spices, herbs, liquorice root, dried milk, dried buttermilk, whey powder, whey paste, groats, ground tapioca root (including tapioca flour, manioc meal, mandioca meal, cassava meal and tapioca ampas of feeding quality), kapok seed cake, kapok cake meal, dari, millet, rye. All the cereals mentioned may be whole, crushed or ground.

STATUTORY RULES AND ORDERS. 1940. No. 1238*

Order Dated July 11, 1940, Amending the Sausages (Maximum Prices) Order, 1940

THE following is a Summary of this Order made by the Minister of Food:

1. The Sausages (Maximum Prices) Order, 1940[a] (hereinafter referred to as the "Principal Order") is amended as follows:

(*a*) The definition of "Pork Sausages" in Article 1 of the Principal Order is replaced by the following definition:

"Pork Sausages" means sausages of which at least 90 per cent. of the meat content consists of pork.

(*b*) After the definition of "Kosher beef sausages" in Article 1 of the Principal Order the following definitions are inserted:

"Pork sausage meat" means sausage meat of which at least 90 per cent. of the meat content consists of pork.

"Beef sausage meat" means sausage meat which is ordinarily known and sold as beef sausage meat, and includes sausage meat the meat of which is not beef alone.

"Kosher sausage meat" means sausage meat manufactured as respects the meat therein from beef obtained from cattle slaughtered in accordance with the Jewish practice of slaughter.

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* H.M. Stationery Office, 1940. Price 1d. net.

[a] S.R. & O., 1939, No. 394 (*cf.* ANALYST, 1940, **65**, 358).

(*c*) At the end of Article 1 of the Principal Order the following paragraph is inserted:

"Any sausages other than pork sausage and Kosher beef sausages as defined by this Order shall be deemed to be beef sausages for the purposes of the Order, and any sausage meat other than pork sausage meat and Kosher sausage meat as defined by this Order shall be deemed to be beef sausage meat for the purposes of the Order."

(*d*) This allows a sum not exceeding 1d. per lb. to be added to the price of sausages (except Kosher beef sausages) filled in sheep casings and sold at not less than ten to the pound weight.

(*e*) In paragraph 2 of Article 3 the words "or any Order made by the Minister" is substituted for the word "Order."

(*f*) The word "or" is substituted for "of" in the last line of paragraph (5) of Article 5 in the Principal Order.

(*g*) The following Articles are inserted in the Principal Order immediately after Article 5.

5A. Where it appears to the Minister of Food that an offence has been committed in respect of which proceedings might be taken under the Defence (General) Regulations, 1939, against some person for an infringement of this Order and the Minister is satisfied that the offence of which complaint is made was due to an act or default of some other person and that the first mentioned person could establish a defence under Article 5 of this Order, the Minister may cause proceedings to be taken against that other person without first causing proceedings to be taken against the first mentioned person.

5B. This Order does not apply to cooked or canned sausages.

2. The Principal Order as thus amended took effect on July 15th, 1940.

Analysis of Commercial Fats and Oils

REPORT OF AMERICAN CHEMICAL SOCIETY COMMITTEE*

THE Committee on Analysis of Commercial Fats and Oils recommends the adoption by the American Chemical Society of its report on the following six methods:

TITRE.—Vertical stirring is recommended as more convenient, and giving a sharper end-point than horizontal stirring. A new (partial immersion) thermometer covering a wider range is specified. It has been found that a differential temperature of 10° C. between the titre-point and the bath is insufficient for low titres, and it is recommended that this be increased. The proposed modifications do not give results differing from those obtainable by the horizontal stirring method.

DETECTION OF TRISTEARIN IN LARD (BÖMER NUMBER).—A modification of the official A.O.A.C. method for the detection of beef fat in lard is described. The A.O.A.C. capillary tube method for melting-points yields consistent and satisfactory results with glycerides and fatty acids, and is therefore recommended for this determination in place of the use of a sulphuric acid bath.

With care, sufficient crystals of definite composition can be obtained by crystallisation from acetone at 30° C. In the modified method described, 20 g. of the filtered sample are treated in a centrifuge tube or cylinder with acetone at 30° C., and the solution is made up to 100 ml. with acetone, shaken and left for 18 hours at 30° ± 2° C. The tube is then centrifuged for 5 minutes, or if a cylinder is used the supernatant liquid is siphoned off. Another 20-ml. portion of acetone (at 30° C.) is added to the crystals and the mixture is centrifuged (or the liquid siphoned off) as before. The washing with 20 ml. of acetone is repeated, but this time the mixture is transferred to a filter-paper, and the crystals are washed with 5 small portions of acetone at 30° C. After removal of as much acetone as possible by means of a vacuum pump the paper is removed from the funnel and, with its contents spread out, allowed to dry thoroughly at a temperature below the m.p. of the glycerides. The mass is then finely divided and its m.p. determined in a sealed capillary tube.

The remainder of the glycerides is saponified by boiling for an hour with 100 ml. of 0·05 *N* alcoholic potassium hydroxide solution in a 500-ml. Erlenmeyer flask with a small funnel in its neck. The soap solution is treated with 100 ml. of water, evaporated on the steam-bath to remove alcohol, transferred to a 500-ml. separating funnel, diluted to about 250 ml., neutralised with hydrochloric acid (1 + 1), a slight excess of which is added, and shaken with 75 ml. of ethyl ether. The aqueous layer is drawn off, and the ethereal layer is washed until neutral to methyl orange and evaporated on the steam bath, and the fatty acids are dried for a few minutes at 100° C.

For determining the m.p., the capillary tube is dipped into the melted acids so that the sample stands about 1 cm. high in the tube. The open end is then sealed in a gas flame, and the tube is left for 30 minutes in ice water or overnight in a refrigerator (4° to 10° C.). The tubes containing the fatty acids and the glycerides are attached to the thermometer by a rubber band, and

* *Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 379–384.

the thermometer is suspended in a beaker of water (suitably agitated), so that the bottom of the thermometer bulb is about 3 cm. below the level of the water, the temperature of which must be at least 10° C. below the m.p. of the sample. The water is heated to give a temperature increase of about 0·5° C. per minute; the m.p. is the point at which the samples become clear and liquid.

If the m.p. of the glycerides, plus twice the difference between the m.p. of the glycerides and that of the fatty acids, is less than 73° C. the lard is regarded as adulterated. The Committee's investigation has indicated that 10 per cent. of beef fat can be detected with certainty, and that amounts down to 5 per cent. may be found. The method is not applicable to hydrogenated pork fats.

SMOKE, FLASH AND FIRE POINTS.—It is recommended that the latest revision of methods of the American Society for Testing Materials be adopted. *Smoke Point.*—The sample is heated to within about 75° F. of the smoke point (the temperature at which a thin bluish smoke is continuously given off) in a Cleveland open flash cup (A.S.T.M. designation D92–33), after which the flame is regulated so that the temperature of the sample rises at a rate not less than 9° or more than 11° F. per minute. The flash cup fits into a circular opening in an asbestos board resting on a ventilated metal heating plate of specified dimensions in which there is also a central circular opening. The source of heat is centred under the opening in the plate and must be of a type not to produce local over-heating, and a cabinet of specified dimensions surrounds the heating device. An A.S.T.M. open flash thermometer is used.

FLASH AND FIRE POINTS.—The same apparatus, including the thermometer, is used, but without the cabinet. Exact details of the procedure are given.

VILLAVECCHIA TEST.—The following modified (A.O.A.C.) test is recommended:—Ten ml. of the sample are mixed with an equal volume of hydrochloric acid (sp.gr. 1·19) and shaken for 15 seconds with 0·1 ml. of the Villavecchia reagent (2 ml. of furfural in 100 ml. of 95 per cent. ethyl alcohol). If any colour is observed in the lower layer which separates, 10 ml. of water are added, and the mixture is again shaken. If the colour persists the test is positive; if it disappears sesame oil is not present.

MODIFIED GARDNER BREAK TEST FOR SOYA BEAN OIL.—Only two minor changes in the method are recommended: The use of a tall beaker helps to prevent loss by frothing, and the thermometer (A.S.T.M. Open Flash) recommended will ensure better control of the temperature.

HYDROXYL VALUE,—It is recommended that the method for calculating the value should be included in the description of the A.O.A.C, method.

COLOUR READING.—The Committee has approved the use of the 2·5-cm. (1-inch) column in the Lovibond system of colour reading for samples of oils and fats which cannot be read in a 13·335-cm. (5·25-inch) column.

International Union of Chemistry

RULES FOR NAMING INORGANIC COMPOUNDS*

A. GENERAL.—*Names and Formulae.*—When there is no risk of uncertainty, formulae should be used for complicated compounds. As a rule, indications of stoichiometric proportions are not necessary when systematic names are used. Trivial names (*e.g.* saltpetre, caustic soda) which are not incorrect are permissible, but incorrectly formed names (*e.g.* sulphate of magnesia) should not be used even in technical literature.

B. BINARY COMPOUNDS.—I. *Position of Constituents.*—The electropositive element, *e.g.* in salts and salt-like compounds should come first (in English and German names). The electronegative constituent ends in *"ide."*

II. *Indications of the Proportions of Constituents.*—Stock's method of indicating valency (Roman figures in brackets) is advocated; *e.g.* "Copper (I) Chloride" for CuCl; "Copper (II) chloride" for $CuCl_2$. The terminations "ous" and "ic" should be entirely discarded. Valency of elements should be indicated by Roman figure just above the symbol on the right hand side (*e.g.* Cu^{I} salts; Fe^{II} compounds). Greek numerical prefixes without hyphens are used to indicate stoichiometric composition ("mono" can usually be omitted). "Octa" should be used for 8 and "Ennea" for 9. Greek prefixes above 12 are replaced by Arabic figures (without hyphens). Arabic figures are also used for fractions, although ½ may be expressed by "hemi." The functional system may be used instead of the stoichiometric (*e.g.* nitrous anhydride). The formulae are preferable to awkward names, *e.g.* $Na_{12}Hg_{13}$ rather than "12 sodium 13 mercuride."

III. *Designation of Intermetallic Compounds.*—Formulae alone should be used and, if possible, should give the exact number of atoms. If the composition is variable a stroke should be placed over the formula; *e.g.* $\overline{AuZn}$. Compounds whose composition is not constant are termed "non-Daltonian" compounds, to distinguish them from "Daltonian" compounds of constant composition.

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* Report of the Committee (W. P. Jorissen, *Chairman*, H. Bassett, A. Damiens, F. Fichter, and H. Remy) for the Reform of Inorganic Nomenclature, June 3rd, 1940. 28, Rue St. Dominique, Paris.

IV. *Indicating the Mass, Atomic Number and State of Ionisation on the Atomic Symbols.*—The number of atoms is shown in the right-hand lower index; the state of ionisation in the right-hand upper index; the atomic number in the left-hand lower index; the mass in the left-hand upper index. Example:

$$^{35}_{17}\mathrm{Cl}^{-1}_{2}$$

V. *Group Names.*—Compounds of the halogens are to be termed *halogenides* (not haloids or halides). The elements oxygen, sulphur, selenium and tellurium may be termed *chalkogens* and their compounds *chalkogenides*.

The alkali metals should not be termed "alkalis" nor the alkaline earth metals "alkaline earths," but these terms are permissible abbreviations in compounds, *e.g.* alkali chloride.

C. TERNARY, QUATERNARY, ETC., COMPOUNDS.—The preceding rules for binary compounds apply also to compounds of more than two elements. Radicals with special names are treated like the elementary constituents of a compound. For example, $Fe(SCN)_3$ = Iron (III) thiocyanate. Sulphur replacing oxygen in an acid radical should always be indicated by "thio" (not "sulpho"), but such names as "lead chlorofluoride" and "lead sulphochloride" are permissible for mixed salts.

The terms "alumino-," "boro-," "beryllo-," etc., silicates should be applied only to silicates which contain Al, B, Be, etc., in place of Si.

D. OXYACIDS.—Well-established names for most of the important simple oxyacids have been in use for a long time, and their alteration is unnecessary. The following names should be used for the more important acids of S, N, P and B:

H_2SO_2 = Sulphoxylic acid; $H_2S_2O_4$ = dithionous acid; H_2SO_3 = sulphurous acid; $H_2S_2O_2$ = thiosulphurous acid; $H_2S_2O_5$ = pyrosulphurous acid; H_2SO_4 = sulphuric acid; $H_2S_2O_3$ = thiosulphuric acid; $H_2S_2O_7$ = pyrosulphuric acid; H_2SO_5 = peroxy(mono)sulphuric acid; $H_2S_2O_6$ = dithionic acid; $H_2S_xO_6$ = polythionic acids; $H_2S_2O_8$ = peroxydisulphuric acid.

$H_2N_2O_2$ = hyponitrous acid; H_2NO_2 = nitroxylic acid; HNO_2 = nitrous acid; HNO_3 = nitric acid; HNO_4 = peroxynitric acid.

H_3PO_2 = hypophosphorous acid; H_3PO_3 = phosphorous acid; $H_4P_2O_5$ = pyrophosphorous acid; $H_4P_2O_6$ = hyphophosphoric acid; H_3PO_4 = (ortho)phosphoric acid; $H_4P_2O_7$ = pyrophosphoric acid; HPO_3 = metaphosphoric acid; H_3PO_5 = peroxy(mono)phosphoric acid; $H_4P_2O_8$ = peroxydiphosphoric acid.

H_3BO_2 = borous acid; $H_4B_2O_4$ = hypoboric acid; H_3BO_3 = orthoboric acid; HBO_2 = metaboric acid; $H_2B_4O_7$ = tetraboric acid.

In notes on decisions in these lists it is pointed out that there was no justification for the use of the name "hydrosulphurous" acid for the compound $H_2S_2O_4$ after it had been shown that the salts have the composition $R_2S_2O_4$ and not RHS_2O_4.

The name "hyposulphurous acid" would be justified for H_2SO_2, but the well-established name "sulphoxylic acid" should be used. The names "hyposulphurous acid" and "hyposulphite" should be discarded.

A distinction must be made between acids or salts derived by substitution from hydrogen peroxide and those derived from the highest oxidation stages of some elements; the latter are correctly termed *per*-salts, whilst the former are named peroxy acids and peroxy salts.

The acid H_2NO_2 has been termed "hydronitrous acid," but is more correctly called "nitroxylic acid" by analogy with sulphoxylic acid.

The term "ortho" is usually applied to the most hydroxylated acid known either in the free state or as salts or esters.

Pyro- and meta-acids are derived from ortho-acids by removal of water in stages. Pyro-acids have lost 1 mol. of H_2O from 2 mols. of ortho-acid (*e.g.* $H_2S_2O_7$, $H_2S_2O_5$, $H_4P_2O_7$, $H_4P_2O_5$). The rule does not apply to the polyboric acid $H_2B_4O_7$ (= $2B_2O_3.H_2O$), which contains less water than metaboric acid HBO_2 (= $B_2O_3.H_2O$). To avoid breaking the rule, the acid $H_2B_4O_7$ should be termed tetraboric acid in agreement with the terminology suggested for isopoly acids.

Acids derived from oxy acids by replacement of O atoms by S atoms are to be termed thio-acids and their salts thio-salts; *e.g.* H_2CS_3 = trithiocarbonic acid.

When the hydrogen ion is considered to occur in the form $[H_3O]^+$, it is advisable to call it the *hydronium* ion (not *hydroxonium* ion).

E. SALTS.—I. *General.*—The name of the metal or electropositive radical should always precede that of the acid radical which terminates in -ate, -ite, or -ide. Names such as nitrate of silver are permissible, but the name of the metal oxide must not be used in place of that of the metal. For mixed or double salts the rules given under C apply.

Salts of nitrogen compounds.—If these are regarded as co-ordination compounds they are to be designated as -onium or -inium compounds; *e.g.* hydrazinium chloride. If, however, they are regarded as addition compounds, the rules derived for these will apply (see F. V).

II. *Acid Salts.*—The rational names are formed by using "hydrogen" for the H atoms present. The hydrogen is to be named last of the electropositive constituents. Formulae should be used for salts of complicated composition.

The term *acid salts* (monacid, diacid, etc.) may be used to emphasise the type of compound rather than its composition; also the expressions primary, secondary, tertiary, etc., salts.

III. *Basic Salts.*—If salts can be considered as addition compounds of hydroxides to neutral salts they are termed *hydroxy salts* (*e.g.* Cd(OH)Cl = cadmium hydroxychloride). But when the hydroxyl group is bound in a complex, Werner's nomenclature of *hydroxo-* or *ol*-groups should be used. Salts in which oxygen atoms, as well as acid radicals, are attached to the metal are termed *oxy* salts, although the names of radicals with special designations may also be used (*e.g.* BiOCl = bismuth oxychloride or bismuthyl chloride).

F. HIGHER ORDER COMPOUNDS.—I. *Complex (Co-ordination) Compounds.*—Stock's method of indicating valency has been extended to the Werner nomenclature. For *complex cations* the Roman figures for valency are placed in brackets after the name of the element to which they refer; *e.g.* $[Cr(OH_2)_6]Cl_3$ = hexaquochromium (III) chloride. For complex anions of salts or acids the valency of the central atom is given in brackets after the name of the complex which ends in -ate; *e.g.* $H_4[Fe(CN)_6]$ = hydrogen hexacyanoferrate (II). It is unnecessary to give the valency of the central atoms of neutral compounds (non-electrolytes). Atoms or groups co-ordinated in the complex are to be mentioned in the following order: (i) *acidic groups*, such as cyano (CN), cyanato (NCO), oxalato (C_2O_4) and hydroxo (OH); (ii) *neutral groups*: aquo (H_2O), substituted amines [$C_2H_4(NH_2)_2$ = en], and last of all ammine (NH_3).

II. *Isopolyacids and their Salts.*—It is proposed that the empirical formulae should always be resolved into the Base anhydride:Acid anhydride ratios, thus avoiding unsettled questions of constitution. Alternatively, the composition referred to the simplest empirical formula, is given by means of Greek numerical prefixes, as in B II. The following examples are illustrative:

NaB_5O_8 Sodium pentaborate $Na_2O.5B_2O_3$ Sodium (1:5) borate
$Na_8Si_3O_{10}$ Tetrasodium trisilicate $4Na_2O.3SiO_2$ Sodium (4:3) silicate
$Na_{10}Mo_{12}O_{41}$ Decasodium dodecamolybdate $5Na_2O.12MoO_3$ Sodium (5:12) molybdate
$Na_2W_2O_7$ Disodium ditungstate $Na_2O.2WO_3$ Sodium (1:2) tungstate
$NaVO_3$ Sodium (mono)vanadate $Na_2O_3.V_2O_5$ Sodium (1:1) vanadate.

III. *Heteropolyacids and their Salts.*—The formulae are resolved into their constituent base and acid anhydrides, the simplest formula which expresses the analytical composition being used. Examples are:

$2R_5PO_5.17WO_3$ = 17-Tungstodiphosphate or $5R_2O.P_2O_5.17WO_3$ = 17-Tungsto-2-phosphate.
$R_8SiO_6.12WO_3$ = Dodecatungstosilicate or $4R_2O.SiO_2.12WO_3$ = 12-Tungstosilicate

IV. *Double Salts.*—The order of cationic constituents should be that of decreasing electropositive character. Constituents common to both salts should be mentioned only once.

V. *Hydrates, Ammoniates and other Addition Compounds.*—The collective names: hydrate, peroxyhydrate (not perhydrate) and ammoniate should be applied to compounds containing molecules of H_2O, H_2O_2 or NH_3. If the molecule forms part of a complex, the compounds are to be named *aquo, peroxyhydrato* and *ammine compounds* (see F. I). Addition compounds containing PCl_3, NOCl, H_2S, C_2H_5OH, etc., are better shown by a formula than by a special name.

Fruit and Vegetable Preservation Research Station, Campden

ANNUAL REPORT FOR 1939

THE Director (Mr. F. Hirst, M.Sc.) in his introduction to the Report points out that since the beginning of the war the National Mark Scheme has been allowed to lapse, and that unless that scheme is revived or some alternative method of control is introduced there will be no standards for quality in canned fruits and vegetables. It would be to the ultimate good of the industry that there should be some means of quality control.

Investigations, described in detail in the Report, include the following:

DRAINED WEIGHT OF ENGLISH CANNED FRUITS AND VEGETABLES.—In continuation of previous work, *Annual Report*, 1938, p. 17; ANALYST, 1939, **64**, 596) the Director and W. B. Adam give results showing the effect of the size of the mesh of the draining sieve on the drained weight. With a sieve with 8 openings to the inch the weights (expressed as percentage of filled weight) were on the average greater by 0·5 per cent. than those obtained with a mesh with 15 openings to the inch. For the sake of uniformity the suggested standards might well be based on the larger mesh.

The National Mark filling weights for A2 cans are 55 to 56 per cent. of the aqueous capacity of the cans for currants and berry fruits (except gooseberries), 59 per cent. for gooseberries, and 60 per cent. for stone fruits. The experiments described show that the filling weights of most soft fruits could be inoreased. It is suggested that for gooseberries, strawberries, raspberries, loganberries and blackberries the filling weight could be 59 per cent. of the capacity of the can, for blackcurrants, 58 per cent., and for cherries, plums and damsons, 61 per cent. The new standards, however, should be based, not on these filling weights, but on the final drained weights.

The following average drained weights (expressed as percentages of the aqueous capacity of the cans) were (i) observed and (ii) calculated in tests on 6000 cans of different sizes packed under the National Mark in recent years:—Gooseberries, (i) 56, 57, 55; (ii) 53, 56, 57. Strawberries, (i) 39, 37, 36; (ii) 34, 35, 36. Raspberries, (i) 46, 44, 45; (ii) 42, 44, 45. Loganberries, (i) 46, 45; (ii) 43, 45. Blackcurrants, (i) 52, 49; (ii) 46, 49. Blackberries, (i) 47, 44; (ii) 43, 45. Sweet cherries, (i) 52, 49; (ii) 51, 54. Greengages, (i) 56; (ii) 56. Golden plums, (i) 55, 51, 51; (ii) 49, 52, 53. Purple plums, (i) 55, 49; (ii) 50, 51. Victoria plums, (i) 55, 49; (ii) 52, 53. Damsons, (i) 54, 54; (ii) 54, 55.

The tests on vegetables were less comprehensive than those for canned fruits, each of the statistics being calculated from only 20 to 70 cans. The following average (i) observed and (ii) calculated drainage figures are recorded:

Beans (dwarf), whole, (i) 53, 52; (ii) 55, 54; sliced, (i) 56, 56; (ii) 59, 60. Beetroot, whole, (i) 66, 68; (ii) 67, 69; sliced, (i) 67, 71; (ii) 66, 67. Broad beans, ungraded, (i) 65; (ii) 69. Carrots, whole, (i) 60, 62; (ii) 62, 63. Celery, hearts, (i) 57, 63; (ii) 62, 65. Peas, (i) 61, 62, 63; (ii) 64, 65, 65. Potatoes, whole, (i) 63, 67; (ii) 67, 70.

The problem of standardising methods of determining drained weights has recently been studied by the U.S. Food and Drug Administration (Bonney, *J. Assoc. Off. Agric. Chem.*, 1939, **32**, 370), and it is advisable also to explore the possibility of establishing minimum standards for English canned fruits and vegetables.

Study of Hydrogen Swells.—Recent work has shown that corrosion in lacquered cans occurs chiefly in the seams, where the metal is exposed. Adjustment of the minor constituents of the steel (*e.g.* sulphur, phosphorus, copper) to their optimum proportions might effect improvements. Experiments indicated that the time taken for high-copper low-phosphorus steels to form 10 per cent. of hydrogen swells would be roughly double that of steels of the present composition. The results of Hoar, Morris and Adam (*J. Iron and Steel Inst.*, 1939, **140**, 55P) suggest that steel with a copper-content of 0·16 to 0·22 per cent. and a phosphorus-content below 0·04 per cent. is the most suitable for tinplate for lacquered cans for fruit, and it ought to be possible for manufacturers to supply such tinplate.

Sulphur compounds, present as ultramarine in "blued" sugar or as sulphite, act as accelerators of corrosion. Un-blued sugar should therefore always be used for canning. It is not known definitely at what concentration of sulphur dioxide corrosion is appreciably hastened, but possibly 10 to 20 p.p.m. in the sugar might have this effect.

The use of the post-lacquered (or flush lacquered) can affords almost complete protection against hydrogen swells for about 3 years under normal storage conditions and virtually eliminates the unsightly blue discoloration of red or purple fruits.

Fruit Gumming of Victoria Plums.—The results of the tests (by the Director and W. B. Adam) showed no significant differences between plums grown on light or heavy soils. On the whole there was less gum when the crops were heavy and the fruit small. The percentage of surface gum fell as the fruits ripened, but early ripening fruits showed more gum than under-ripe fruits. Gumming was distributed unevenly over the trees and differed appreciably on neighbouring trees.

Behaviour of Traces of Sulphur in Canned Fruits.—In the experiments (by G. Horner) the development of sulphide in presence of sulphite was followed by distilling the acidified fruit in an atmosphere of carbon dioxide, collecting the hydrogen sulphide in neutral silver nitrate solution, and titrating this with standard alkali. In lacquered cans the amount of sulphide present after 5 to 6 months did not exceed a trace, but in plain cans amounts up to 0·8 p.p.m. were recorded. The difference in the behaviour of the two types of can may be due to the unequal rates at which the changes from sulphur to hydrogen sulphide and from the latter to metallic sulphide take place.

Hydrogen Ion Concentration of Canned Vegetables.—Further progress in this investigation by W. B. Adam is recorded. In all the results described the quinhydrone electrode was used in combination with a saturated KCl-calomel half-cell, the E.M.F. being measured by means of a Tinsley potentiometer connected with a null-point galvanometer. The reading was taken about 30 seconds after adding the quinhydrone.

The preliminary results show that the *p*H value of immature peas does not alter appreciably during the later stages of ripening, although smaller sizes tend to have lower values than the larger grades. The *p*H of harvested peas is not affected by the stage of maturity at which they are cut. There is a fall in the *p*H of the covering liquid during processing, but with peas and beans the value subsequently rises as buffering substances are extracted. The following averages and ranges of final *p*H values for the principal English canned vegetables are given; the numbers of cans examined ranged from 10 to 82.

Beans, broad, 5·90–6·13 (av. 6·01); beans, dwarf, 5·33–5·99 (av. 5·59); beetroot, 4·94–5·61 (av. 5·39; carrots, 4·88–5·46 (av. 5·12); celery, 5·15–5·62 (av. 5·36); peas, immature, 5·67–6·37 (av. 6·12); potatoes, new, 5·68–6·24 (av. 5·82); spinach, 5·43–6·25 (av. 5·83); macedoine, 5·16–5·76 (av. 5·34).

Heat Penetration in Rotating Cans.—To accelerate the passage of heat towards the centre of cans methods of mechanical agitation are used, including automatic reel-and-spiral pressure cookers and continuously rotating batch sterilisers. A rotating thermo-couple has been designed for measuring the rate of heat penetration in all sizes of cans, and is described and

illustrated in a communication by W. B. Adam and J. Stanworth. It was found that heat penetration in canned beans in tomato sauce was very slow. Thus in stationary cans it required 300 minutes to reach a central temperature of 235° F. with a processing temperature of 240° F., but when the cans were rotated at 5 rev. per 10 seconds, with 20 seconds stop, the same central temperature was reached in 12½ minutes. The results indicated that with A10 cans the safe processing time may be reduced from 3¾ hours to half-an-hour by rotating the cans.

The new apparatus may also be used in testing other products to control the destruction of the spores of *Byssochlamys fulva*.

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(Supplementing the series published in the ANALYST up to 1933, **58**, 340, and bringing the Bibliography up to date.)

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E. B. D.

British Standards Institution

THE following Standard Specification has been issued:*

No. 914—1940. BRITISH STANDARD TESTS FOR LABORATORY PORCELAIN.

The tests have been prepared by a committee representative of makers and users of laboratory porcelain appointed by the Technical Committee on Scientific Glassware and Laboratory Ware. Several of the tests are based on those adopted by the Sub-Committee of the Institute of Chemistry and published in 1920 (*J. and Proc. Inst. of Chem.*, 1920, [3], 210–214). A new rapid test of the resistance to heat and cooling has been introduced.

The tests are divided into five sections: (1) Appearance, shape, weight, etc. (2) Tests for porosity of body and imperfections in glaze (dye test). (3) Resistance to heat and sudden change of temperature. (4) Constancy of weight and resistance of glaze to high temperatures. (5) Resistance of glaze to acid and alkali.

The test for porosity consists in soaking the specimens, some whole, others broken, in a 0·5 per cent. aqueous solution of eosin for 18 hours and ascertaining the amount of staining, if any.

A special furnace is described and illustrated for testing the resistance of ordinary crucibles to heat. The crucible is placed bottom downwards in a cage suspended from the lid of the furnace and heated at the specified temperature for 15 minutes, after which it is allowed to drop into a bucket of water at 15° C., which is placed 6 inches below the end of the furnace. The crucible should not break or crack after being heated to 240° C. and suddenly cooled under the specified conditions.

In the high temperature tests clean broken pieces of the porcelain are gently ignited in crucibles of the same make, and the vessels are cooled, weighed, and heated for 2 hours in a muffle at 950°–1000° C. There should be no change in weight and no adhesion of the pieces to one another or to the crucible.

Resistance of the glaze to acid and alkali is determined by testing the dishes in a specified manner with hydrochloric acid of constant b.p. for 4 hours, with *N* sodium carbonate solution for 4 hours at 100° C., and finally with 5 per cent. caustic soda solution under the same conditions. The losses, expressed as mg. per sq. dcm., should not exceed 1 mg. for HCl, 10 mg. for Na_2CO_3 or 60 mg. for NaOH.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Colorimetric Determination of the Preservative Value of Hops. Standard Colour Values of Some Hybrid Hops. A. A. D. Comrie. (*J. Inst. Brewing*, 1940, **46**, 255–256.)—It is pointed out that in the author's colorimetric method for the determination of hop preservative values (ANALYST, 1939, **64**, 828) the mixed α- and β-resins present must be considered in terms of the "α-resin equivalent," *i.e.* the effective sum of the resins when the usually-accepted preservative value of 1/3 of that of the α-resin is allotted to the β-resin. Thus α and β per cent. of α- and β-resin, respectively, in a hop would correspond with an "α-resin equivalent" of $(\alpha + \beta/3)$ per cent. Similarly, the "standard colour value" (*S.C.V.*) of a hop is, by definition (*loc. cit.*), the colour value of 0·8 mg. of the α-resin equivalent as it exists in the hop. Since, however, it has been found that the *S.C.V.* for the α-resin from all sources hitherto examined is a constant, (95) under the conditions of the experiment, whilst the *S.C.V.* for the β-resin depends on the nature of the hop in question, this means that the *S.C.V.* for the mixture will also vary according to the nature of the hop. For ordinary English varieties, however, this variation has been found to be negligible, and an average value of 82 for the α-resin equivalent $(\alpha + \beta/3)$, could be used. The new hybrid variety "Brewers' Gold" proved an exception (*S.C.V.*, 95), and further determinations have therefore been made on some of the better-known hybrids raised at Wye College. Values found for the *S.C.V.* of the weight of hop containing 0·8 mg. of α-resin equivalent were, Brewers' Gold, 96; Bullion Hop, 95; Quality Hop, 94; Brewers' Favourite, 91; Early Promise, 93; and Fillpocket, 92. These high figures appear to be due to the high *S.C.V.* of the β-resins (see above), but this in turn does not result from the influence of the species *Humulus americanus* in the inter-specific hybrids, because the wild uncultivated *H. americanus* (var. *neomexicanus*) grown at Wye had an *S.C.V.* of only 82, whilst a further hybrid similar to those above had an *S.C.V.* of 84. It is suggested that if these hybrid hops come into commercial cultivation a *S.C.V.* of 94 should give results in fair accordance with the accepted formula for the gravimetric method, $10(\alpha + \beta/3)$; comparisons of the preservative values obtained by the gravimetric and colorimetric methods confirm this for the above 6 hops. J. G.

* To be obtained from the Publications Department, British Standards Institution, 28, Victoria Street, London, S.W.1. Price 2s. net, post free 2s. 3d.

Fatty Acids and Glycerides of Solid Seed Fats. IX. *Mimusops Heckelii* (Baku) Kernel Fat. D. Atherton and M. L. Meara. (*J. Soc. Chem. Ind.*, 1940, **59**, 95–96.)—*Mimusops Heckelii*, N.O. *Sapotaceae*, is a tree widely distributed in the closed forest areas of the Gold Coast. The nuts, of average weight 20 g., consisted of about 60 per cent. of hard pericarp enclosing 40 per cent. of kernel containing 52 per cent. of fat with the following constants:—saponification equiv. 295·4, iodine value, 52·7; acid value, 2·4; unsaponifiable matter, 0·4 per cent. The component fatty acids in the neutral fat were determined by distillation of the methyl esters; another portion of neutral fat was crystallised from acetone, and the three fractions of increasing solubility were examined. The proportions of fatty acids were computed to be: palmitic, 4·4; stearic, 36·0; arachidic, 0·5; hexadecenoic, 0·3; oleic, 58·5; linolic, 0·3 per cent. by weight. The component glycerides were approximately: stearodiolein, 41–47; oleodistearin, 32–26; palmitodiolein, 14–6; triolein, 10–12; oleopalmitostearin, 2–8; fully saturated palmitodistearin 1 mol. per cent. From this analysis and those of other fats, *e.g.* shea butter, it is concluded that when oleic acid approaches 60 per cent. of the mixed fatty acids in a seed fat, triolein is usually present in proportions which, although relatively small, are larger than those of the fully saturated components when the converse relationship holds, *i.e.* the rule of even distribution is less closely followed in the production of the unsaturated glycerides than of the fully saturated glycerides. D. G. H.

Component Fatty Acids and Glycerides of some *Myristica* Fats. D. Atherton and M. L. Meara. (*J. Soc. Chem. Ind.*, 1939, **58**, 353–357.)—The kernel fats of (*a*) *Virola surinamensis* from S. America and (*b*) *Pycnanthus Kombo* (*Myristica angolensis* Walw.) from Sierra Leone were examined. The composition of the nuts was: shell, (*a*) 17, (*b*) 14; kernel, (*a*) 83, (*b*) 86 per cent., and the kernels yielded to petroleum spirit (*a*) 71 and (*b*) 61·6 per cent. of dark brown solid fats. These had saponification equiv. (*a*) 246·1, (*b*) 249·9; iodine value, (*a*) 14·5, (*b*) 67·0; free fatty acids as oleic acid, (*a*) 17·2, (*b*) 18·7; iodine value of neutral fat after removal of highly unsaturated resin acids, (*a*) 9·9, (*b*) 32·3. The component acids, estimated by ester fractionation, consisted of (per cent. wt.) decoic, (*a*) 0·5, (*b*) 0·2; lauric, (*a*) 14·8, (*b*) 11·5; myristic, (*a*) 72·5, (*b*) 58·1; palmitic, (*a*) 4·9, (*b*) 9·1; tetradecenoic, (*a*) —, (*b*) 26·3; oleic, (*a*) 6·3, (*b*) 17; unsaponifiable, resins, (*a*) 1·0, (*b*) 3·4. Crystallisation from acetone gave fractions the major component glycerides of which were estimated approximately as:—tetradecenodimyristin, (*a*) —, (*b*) 33; trimyristin, (*a*) 43, (*b*) 24; laurodimyristin, (*a*) 31, (*b*) 17; oleolauromyristin, (*a*) 12; lauromyristopalmitin, (*a*) 10, and in (*b*) small amounts of oleotetradecenomyristin, myristoditetradecenoin and laurotetradecenomyristin. The free fatty acids of both fats contained higher proportions of unsaturated acids than those from the neutral glycerides. The kernel fat of *Pycnanthus Kombo* is at present unique in containing 24 per cent. of Δ^9-tetradecenoic (myristoleic) acid, an acid not previously reported in seed fats but present in fish and whale oils, and to a small extent in butter-fat. The isomeric dihydroxy acids, m.p. 123° and 81·5° C. respectively, were prepared by mild alkali and acid oxidation of this tetradecenoic acid. D. G. H.

Fatty Acids and Glycerides of the Seed Fats of *Allanblackia floribunda* and *Allanblackia parviflora*. M. L. Meara and Y. A. H. Zaky. (*J. Soc. Chem. Ind.*, 1940, **59**, 25–26.)—*Allanblackia floribunda* (*a*) from Nigeria, and *Allanblackia parviflora* (*b*) from the Gold Coast belong to the N.O. *Guttiferae*. The nuts of *A. parviflora* consisted of 35·5 per cent. of shell and 64·5 per cent. of kernel containing 69·4 per cent. of fat. The analytical constants of the two fats were:—saponification equiv., (*a*) on refined fat 297·7, (*b*) 295·3; iodine value (*a*) on refined fat, 35·4, (*b*) 37·2; free fatty acids as oleic acid, (*b*) 1·9 per cent. The fats were examined in the usual way by lead salt separation from alcohol and fractionation of the methyl esters, and the proportions of fatty acids were calculated as:—myristic, (*a*) —, (*b*) 1·5; palmitic, (*a*) 2·9, (*b*) 2·3; stearic, (*a*) 57·1, (*b*) 52·0; arachidic, (*a*) 0·2, (*b*) 0·3; oleic, (*a*) 39·4, (*b*) 43·9; linolic, (*a*) 0·4, (*b*) —. Crystallisation from acetone and examination of the resulting fractions gave data for calculating the glyceride composition to be approximately:—oleodistearin, (*a*) 76, (*b*) 60; stearodiolein, (*a*) 15·5, (*b*) 26–29; oleopalmitostearin, (*a*) 5·0, (*b*) 6–9 mol. per cent. Thus the rather small differences in the proportions of fatty acid components in the two fats give rise to greater differences in those of the major components of the glycerides. The fat of *A. parviflora* is seen to be very similar to that of *A. Stuhlmannii* (*J. Soc. Chem. Ind.*, 1931, **50**, 468T; Abst., ANALYST, 1932, **57**, 113). D. G. H.

Composition of Commercial Palm Oils. V. Partial Separation of Palm Oils by Crystallisation as an Aid to the Determination of the Component Glycerides. T. P. Hilditch and L. Maddison. (*J. Soc. Chem. Ind.*, 1940, **59**, 67–71.)—Two more palm oils have now been examined (*cf. J. Soc. Chem. Ind.*, 1935, 54, 77T; Abst., ANALYST, 1935, **60**, 328). The two oils: (*a*) a plantation oil from the Cameroons and (*b*) a native oil from Grand Bassa, differed in their respective contents of palmitic and oleic acid, and after preliminary separation by acetone into glyceride fractions of relatively simple composition were found to contain the following component fatty acids: myristic, (*a*) 1·1, (*b*) 0·6; palmitic, (*a*) 45·1, (*b*) 37·6; stearic, (*a*) 4·1, (*b*) 3·7; hexadecenoic, (*a*) 0·8, (*b*) 1·4; oleic, (*a*) 38·6, (*b*) 50·3; linolic, (*a*) 10·3, (*b*) 6·4 per cent. by weight. The component glycerides were

approximately (mol. per cent.) "oleo" dipalmitin, (*a*) 43, (*b*) 31; palmitodi"olein," (*a*) 3, (*b*) 41; "oleo" palmitostearin, (*a*) 11, (*b*) 10; tri"olein," (*a*) 6, (*b*) 12; tripalmitin, (*a*) 5, (*b*) 3; dipalmitostearin, (*a*) 3, (*b*) 3; stearodi"olein," (*a*) perhaps up to 1, (*b*) perhaps traces. Comparison of these figures with those obtained before by progressive hydrogenation (*loc. cit.*) shows that the oils form a series in which the proportion of palmitic acid rises whilst that of oleic acid falls. Summarising, the chief components of palm oils are regarded as "oleo" dipalmitin and palmitodi-"olein" in amounts varying according to the proportions of palmitic, oleic and linolic acids in the whole fats. These together usually amount to 70–75 per cent. of the palm oil, "oleo" dipalmitin preponderating in oils of high palmitic acid content and conversely. The minor components are "oleo" palmitostearin (about 10–15) linoleodi"olein" and/or tri"olein" (6–15, varying with the oleic + linoleic acid content of the palm oil), and tripalmitin + dipalmitostearin (3–9 per cent.) varying with the palmitic acid content of the palm oil. D. G. H.

Fat of Land Crabs (Seychelles Islands). T. P. Hilditch and K. S. Murti. (*J. Soc. Chem. Ind.*, 1939, 58, 351–353.)—The fat, extracted from land crabs in the Seychelles, consisted of 101·3 g. of a soft yellow solid with saponification equiv. 234·4, iodine value 19·1, free fatty acids, as oleic acid, 1·0, and unsaponifiable matter 0·3 per cent. The fat was systematically crystallised from stated volumes of acetone at 0° C. for 1 day, the mixed fatty acids from each fraction were converted into the methyl esters, and each group was distilled at 0·1 mm. pressure through an electrically heated and packed column. From the data obtained the component acids were computed to be: octoic, 1·5; decoic, 5·3; lauric, 47·5; myristic, 14·0; palmitic, 13·1; stearic, 1·7; tetradecenoic, 0·7; hexadecenoic, 2·2; oleic, 5·3; linolic, 1·5; unsaturated C_{20-22} acids, 2·2 mol. per cent. The fat contained 66·3 per cent. of fully saturated glycerides of which the component acids included octoic, 3·2; decoic, 7·1; lauric, 54·8; myristic, 20·5; palmitic, 12·7; stearic, 1·7 mol. per cent. The striking resemblance between the fatty acids and those of seed fats of the palm family, and the almost entire absence of the characteristics of a typical marine animal fat are probably accounted for by the fact that these crabs feed on coconuts. D. G. H.

Alkaloids of some *Chondrodendron* Species and the Origin of *Radix Pareirae Bravae*. H. King. (*J. Chem. Soc.*, 1940, 737–746.)—Differences in the optical rotatory power of bebeerine from different samples of the drug *Radix Pareirae Bravae* are attributed to its origin in two very similar species, *Chondrodendron platyphyllum* and *Ch. microphyllum*. These yield *l*- and *d*-bebeerine, respectively, the latter occurring in the drug on the English market. From these species, from *Ch. candicans* (the British Guiana species) and from the drug, alkaloids were obtained as follows:—*Ch. platyphyllum* (Rio) root (924 g.):—*d*-iso-chondrodendrine (sulphate 43·1 g. plus base 1·7 g.), *l*-bebeerine (9·0 g.), *Ch. platyphyllum* (Bahia), root: (720 g.), *l*-bebeerine (hydrochloride 55·3 g. plus base 1·50 g.), *d*-isochondrodendrine (hydrochloride 1·1 g. plus base 0·65 g.); stems (538 g.): *l*-bebeerine (28·32 g.); leaves (300 g.): *l*-chondrofoline, *d*-isochondrodendrine, *l*-bebeerine (total crude alkaloid 3·4 g.) *Ch. microphyllum* (Bahia), root (845 g.): *d*-isochondrodendrine (sulphate 18·3 g. plus base 1·42 g.), *d*-bebeerine (hydrochloride 15·4 g. plus base 1·5 g.). *Ch. candicans* (British Guiana), stems (1500 g.): *d*-isochondrodendrine (hydrated sulphate 17·7 g. plus base 1·48 g.), *d*-bebeerine (4·93 g.). *Pareirae Bravae*: *d*-bebeerine *d*-isochondrodendrine, *d*-isococlaurine. The widely differing proportions of alkaloids in the same species are ascribed to climatic and seasonal causes. The new alkaloids *l*-chondrofoline and *d*-isococlaurine are related to bebeerine and isomeric with coclaurine respectively. Chondrofoline, $C_{33}H_{36}O_6N_2$, is phenolic and contains three methoxyl groups; methylation yields an amorphous O-methyl methiodide and methochloride closely resembling the corresponding products from bebeerine, while other reactions give products identical or enantiomorphic with those from bebeerine. The alkaloid does not give the Millon reaction and contains only one phenolic group. Isococlaurine, which was isolated from a relatively large amount of *Radix Pareirae Bravae*, is phenolic and contains one methoxyl group. The derivative on complete methylation, *d*-O-dimethyl-N-methylcoclaurine methiodide, is the optical enantiomorph of the similar product from natural *l*-coclaurine. The alkaloid gives the Millon reaction and does not give the catechol reaction. Probable structural formulae for the two alkaloids are given. The study of *d*-isochondrodendrine has enabled probable structures to be assigned to protocuridine and neoprotocuridine, isomeric phenolic alkaloids of pot-curare; many bisbenzylisoquinoline alkaloids can also be classified as either bebeerine or isochondrine types. E. B. D.

Separation and Determination of Isomeric Menthols. R. T. Hall, J. H. Holcomb, Jr., and D. B. Griffin. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 187–188.)—The synthetic menthol used to replace Menthol U.S.P. (*l*-menthol from *Mentha arvensis*) consists of varying mixtures of the other isomers with some of the U.S.P. product, the number and proportion of isomers depending upon the method of synthesis. When *d*-citronellal (from citronella oil) is the initial material a mixture of 3 isomers, *viz.* *d*-neomenthol, *d*-isomenthol and *l*-menthol, is obtained, and the large proportion of *l*-menthol in this particular mixture makes its commercial separation possible. The analytical reactions of these isomers have been studied. Most methods for the determination of total menthol depend

upon acetylation followed by hydrolysis of the acetylated product with alcoholic potassium hydroxide solution. Saponification with a solution of potassium hydroxide in diethylene glycol (Redemann and Lucas, *Ind. Eng. Chem., Anal. Ed.*, 1937, **9**, 521; Abst., ANALYST, 1938, **63**, 62) reduces the time of hydrolysis to one-fourth or one-fifth. The rates of both the acetylation and the hydrolysis vary for the different isomers. Acetylation for 2 hours is sufficient, and low results obtained for some of the isomers were invariably due to incomplete hydrolysis. Hydrolysis of the esters of *l*-menthol and *d*-isomenthol with 0·5 *N* alcoholic potassium hydroxide was practically complete within 30 minutes, but the *d*-neomenthol ester required 3·5 hours. The more rapid hydrolysis effected by the diethylene glycol solution of potassium hydroxide did not affect the accuracy of the results; again, however, the *d*-neomenthol ester (and consequently the isomeric mixture) required a longer time for hydrolysis than the esters of the other isomers. A. O. J.

Biochemical

Estimation of Phosphorus. R. J. L. Allen. (*Biochem. J.*, 1940, **34**, 858–865.)—The method of Fiske and Subbarow (*J. Biol. Chem.*, 1925, **66**, 375; *cf.* ANALYST, 1926, **51**, 205), as modified by King (*Biochem. J.*, 1932, **26**, 292; Abst., ANALYST, 1932, **57**, 532), was not found quite satisfactory when the analysis depended on the measurement of absolute colour density. The following method, in which amidol is substituted for 1-amino-2-naphthol-4-sulphonic acid, is proposed instead. Not more than 20 ml. of the solution to be tested (containing about 0·4 mg. of phosphorus in the form of phosphate) are transferred to a 25-ml. volumetric flask, and 2 ml. of 60 per cent. perchloric acid are added, followed by 2 ml. of amidol reagent (2 g. of amidol and 40 g. of pure sodium bisulphite are dissolved in glass-distilled water and diluted to 200 ml.), and 1 ml. of 8·3 per cent. ammonium molybdate solution. The solution is made up to volume and, after standing for 5 to 30 minutes, its extinction coefficient is measured. The amount of phosphorus is calculated by reference to a standard curve prepared by similar treatment of standard phosphate solutions. To measure the amount of total phosphorus, an appropriate amount of the material is digested over a micro-burner in a micro-Kjeldahl flask with 2·2 ml. of perchloric acid. When the liquid is colourless (a few drops of hydrogen peroxide may be added to facilitate the oxidation), the flask is allowed to cool, and the contents are washed into a 25-ml. volumetric flask and treated with amidol and ammonium molybdate solutions as before. Some materials gave turbid or coloured solutions, and the following further treatment was then adopted. The blue solution, obtained by allowing the solution of phosphate to stand for 5 to 30 minutes with the perchloric acid and amidol and ammonium molybdate solutions, is treated with 1 ml. of 10 per cent. oxalic acid solution. The mixture is transferred to a separating funnel and gently shaken with about 10 ml. of isobutyl alcohol. The aqueous phase is re-extracted with a further 5 ml. of isobutyl alcohol, the combined extracts are diluted to 25 ml. with ethyl alcohol, and the extinction coefficient of the solution is measured within 24 hours. The amount of phosphorus is calculated by the use of a standard curve. F. A. R.

Quantitative Estimation of Glycuronic Acid and its Conjugated Compounds by means of the Naphthoresorcinol Test of Tollens. W. Mozolowski. (*Biochem. J.*, 1940, **34**, 823–828.)—Numerous workers have endeavoured to make Tollens' naphthoresorcinol test applicable to the quantitative estimation of glycuronic acid, but without success. The following method has been found to give satisfactory results:—A 0·2 per cent. aqueous solution of naphthoresorcinol is allowed to stand overnight and filtered; the reagent must be used within a day. The solution under examination, made up to 2 ml. if necessary, is mixed in a test-tube with 2 ml. of the naphthoresorcinol reagent and 2 ml. of conc. hydrochloric acid are added from a burette. The tube is heated for 30 minutes in a boiling water-bath and cooled in ice-water for 10 minutes. To each sample 2 ml. of ethyl alcohol are added, and the mixtures are transferred to 100-ml. glass-stoppered measuring cylinders, the test-tubes being washed out 3 times with 5 ml. of pure ether. The cylinders are shaken 10 times in 30 seconds, and the absorption of the ethereal layer is measured in a Pulfrich photometer, using a 2-cm. cell and filter S57; a control solution prepared by treating distilled water in the same way is used in the other cell. The concentration of glycuronic acid is only proportional to the extinction coefficient, however, within the range 0·0125 to 0·05 mg., so that a preliminary test must be made when the concentration is unknown. Samples of 2·0, 1·0 and 0·5 ml. are respectively made up to 2·0 ml. and treated as described above. If the amount of glycuronic acid present lies between 0·005 and 0·5 mg., the extinction values obtained will be proportional to the volumes taken. If such proportionality does not exist, the solution to be examined must be diluted 5 or 10-fold and the procedure repeated until proportionality is reached. In this way satisfactory results were obtained on pure solutions of glycuronic acid, glycurone, benzoylglycuronic and bornylglycuronic acids. The method can also be applied to blood and urine, but account must be taken of interfering substances, and in urines containing such substances the estimation can only be carried out if the concentration of glycuronic acid is sufficiently high to allow of adequate dilution. True values can, of course, only be obtained when there is proportionality within a given series. F. A. R.

Blood Sugar Levels in Rats receiving the Cataractogenic Sugars Galactose and Xylose. W. J. Darby and P. L. Day. (*J. Biol. Chem.*, 1940, **133**, 503–509.)—A modification of the method devised by Folin and Malmros (*J. Biol. Chem.*, 1929, **83**, 115) for the estimation of blood sugar is described; this can be carried out on 0·025-ml. samples of blood. Blood from the tail-vein of the experimental animals was drawn to the 0·025 ml. mark of a special diluting pipette, and the solution was diluted to the 2·5 ml. mark with dilute tungstic acid solution (20 ml. of 10 per cent. sodium tungstate solution are diluted to 800 ml. and mixed with 20 ml. of 2/3 *N* sulphuric acid, and the solution is diluted to 1 litre). After being mixed, the solution was expelled into a clean dry 15-ml. centrifuge tube and centrifuged. Two ml. of the supernatant liquid were transferred to a test-tube graduated at 12·5 ml., 2 ml. of a standard solution containing 0·01 mg. of glucose per ml., were introduced into a similar tube, and to each were added 1 ml. of 0·4 per cent. potassium ferricyanide solution and 0·5 ml. of a carbonate-cyanide solution (prepared by mixing 150 ml. of 1 per cent. sodium cyanide solution with a solution of 8 g. of anhydrous sodium carbonate and diluting to 500 ml.). The solutions were heated in boiling water for 8 minutes, cooled for 2 minutes, and then treated with 2·5 ml. of colloidal ferric iron solution (prepared by allowing 20 g. of soluble gum ghatti, supported by a wire screen, to soak overnight in 1 litre of water, and adding to the extract a solution of 5 g. of anhydrous ferric sulphate in 75 ml. of 85 per cent. phosphoric acid diluted with 100 ml. of water. About 15 ml. of 1 per cent. potassium permanganate solution are added to destroy certain reducing materials present in gum ghatti). Finally, the contents of each tube were diluted to the 12·5 ml. mark and mixed, and the colours were compared in a colorimeter with a yellow filter. F. A. R.

Glutamic Acid of Normal and Malignant Tissue Proteins. A. C. Chibnall, M. W. Rees, E. F. Williams and E. Boyland. (*Biochem. J.*, 1940, **34**, 285–300.)—Kögl and Erxleben (*Z. physiol. Chem.*, 1939, **258**, 57) claimed that malignant tissues contain a number of their constituent amino-acids in a partially racemised form, whereas normal tissues contain amino-acids of normal rotation. The difference was said to be particularly striking in respect of the glutamic acid present. Thus acid hydrolysis of normal lung or heart tissue yielded *l*(+) glutamic acid, showing the normal rotation (in 9 per cent. hydrochloric acid) of $[\alpha]_D$ + 31·6°, whilst hydrolysis of various kinds of tumours gave rotations as low as +4·6°, indicating the presence of as much as 40 per cent. of *d* (−) glutamic acid. The method used by Kögl and Erxleben for isolating glutamic acid as its hydrochloride was the cuprous oxide method of Abderhalden and Fuchs (*Z. physiol. Chem.*, 1908, **57**, 339), which has now been shown to give comparatively small yields of glutamic acid with preferential salting out of racemic glutamic acid hydrochloride. Thus even from normal tissue protein preparations, such as gliadin, small first crops of partly racemised glutamic acid hydrochloride were obtained. Glutamic acid was isolated from seven different malignant tissue protein preparations and two normal tissue protein preparations by a modification of Foreman's method (*Biochem. J.*, **1914, 8,** 463). Good yields of the acid were obtained, and the degree of racemisation was in every instance small, the amount of the *d* (−) antipode from the malignant tissue being only about 0·2 per cent. It is concluded that Kögl and Erxleben's claims cannot be sustained and that racemisation of amino-acids is not characteristic of the proteins of malignant tissue. The modified Foreman method used in the present investigation was as follows: The dry protein was hydrolysed with 20 per cent. hydrochloric acid for 22 hours, and the excess of hydrochloric acid was removed by repeated evaporation *in vacuo*. The resulting syrup was treated with cream of lime, the excess of lime was removed, and the calcium salts were precipitated from the solution by the addition of 8·5 volumes of absolute alcohol. The calcium salts were dissolved in water, re-precipitated with alcohol and re-dissolved in water. Calcium was removed from the solution by means of oxalic acid, and the filtrate was evaporated to a syrup after addition of hydrochloric acid. On standing overnight much glutamic acid hydrochloride crystallised out, and was recrystallised from water. The mother liquors were boiled with sulphate-free copper carbonate, the excess was removed by filtration, and the filtrate was concentrated. Impure copper aspartate separated and was purified by re-dissolving in hydrochloric acid and re-precipitating with copper carbonate. The filtrate from the copper aspartate was freed from copper by means of hydrogen sulphide, and the calcium salts of the remaining amino-acids were isolated and treated as before, yielding a further crop of glutamic acid hydrochloride. This procedure was applied to all mother liquors, and ultimately nearly the whole of the glutamic acid was isolated. F. A. R.

Sucrase Activity in the Barley Plant. H. K. Archbold. (*Biochem. J.*, 1940, **34**, 749–763.)—Component parts of the barley plant were collected from the time of emergence of the sixth leaf until harvest, frozen in solid carbon dioxide, and stored in the frozen condition until required. The sucrase content of each portion was estimated by powdering the frozen material, allowing it to warm up to room temperature and squeezing out the sap. Aliquot portions of the green suspension thus obtained were incubated with a sucrose solution in presence of an acetate buffer solution; after neutralising with sodium carbonate solution, the optical rotation of the solution was measured in a polarimeter, and the amount of sucrose hydrolysed was calculated. The optimal *p*H for barley sucrase was found to be

4·8. The lowest level of activity was found in the stems, a slightly greater activity in the roots, whilst the leaves and leaf-sheaths were about twice as active as the stems. In the ear, activity increased rapidly with growth and declined again after full emergence. The maximum value for the ear was higher than for any other part of the plant. The total activity of the plant increased until ear emergence and then decreased. F. A. R.

Fermentation Process in Tea Manufacture. V. Cytochrome Oxidase and its Probable Rôle. VI. Effect of Dilution on the Rate and Extent of Oxidations in Fermenting Tea Leaf Suspensions. E. A. H. Roberts. (*Biochem. J.*, 1940, **34**, 500–516.)—V. Subsequent work has revealed that the mechanism previously proposed (*cf.* ANALYST, 1939, **64**, 616) for the fermentation process of tea is untenable. This hypothesis postulated that the oxidation of ascorbic acid precedes that of tannin during fermentation. It has now been shown that tea leaf at the end of fermentation still retains a considerable part of its enzymatic activity, taking up oxygen if fresh substrate, in the form of a green leaf infusion, is added; ascorbic acid, on the other hand, is oxidised very slowly by fermented tea leaf, the rate of oxygen uptake being much lower than that recorded at the beginning of fermentation. Moreover, the failure to absorb oxygen is not due to inhibition by tannin fermentation products. It would appear, therefore, that the oxidation of ascorbic acid by fermenting tea leaf is a side-reaction brought about by the primary oxidation product of tea tannin:

o-quinone + ascorbic acid
⟶ catechol + dehydroascorbic acid

and that ascorbic acid oxidase is not responsible for the first stage of fermentation. Previously the author had found it impossible to reconcile his results (obtained in Assam) and his hypothesis with the results obtained by Lamb (*Tea Res. Inst. Ceylon, Ann. Rep.*, 1937, 65), working in Ceylon. By repeating the experiments at a lower temperature, comparable with the temperature prevailing in the hills in Ceylon, Lamb's results have been confirmed. The new results provide conclusive proof that an oxidase system occurs in tea leaf and is readily inactivated by alcohol at temperatures of about 30° C., and furnish additional evidence against the ascorbic acid theory. The properties of the oxidase now shown to be present are in close agreement with those of cytochrome oxidase. Thus (1) both enzymes are readily destroyed by alcohol and acetone, (2) both are firmly attached to tissues, (3) the oxygen uptake of each is markedly accelerated by an increase in the oxygen tension and (4) neither is very specific (unlike ascorbic acid oxidase and catechol oxidase); both oxidise catechols, tea tannin, *p*-phenylenediamine and ascorbic acid. On the assumption that cytochrome oxidase is the enzyme responsible for the uptake of oxygen, it becomes necessary to modify the scheme formerly proposed, substituting cytochrome *c* (Fe″- and Fe‴-haematin denote the reduced and oxidised forms respectively and CoH_2 and Co denote the reduced and oxidised forms of cozymase) for ascorbic acid as the oxygen carrier:

(1) Fe″-haematin + O_2 $\xrightarrow{\text{cytochrome oxidase}}$ Fe‴-haematin + H_2O_2

(2) Tea tannin + Fe‴—haematin → *o*-quinone + Fe″-haematin

(3) Tea tannin + H_2O_2 $\xrightarrow{\text{peroxidase}}$ *o*-quinone

(4) *o*-Quinone → condensation products

(5) Fe‴-haematin + CoH_2 → Fe″-haematin + Co

(6) *o*-Quinone + CoH_2 → tea tannin + Co

(7) Co + substrates $\xrightarrow{\text{dehydrogenases}}$ CoH_2 + reaction products

VI. The rate of oxygen uptake by suspensions of fermenting tea leaf in water increases as the dilution increases; if the logarithm of the concentration is plotted against the logarithm of the oxygen uptake, a straight line results. From this graph it is possible to calculate the oxygen uptake for any given concentration, such as that existing on the fermenting room floor under factory conditions. The agreement between the calculated and the observed values was found to be fairly close. The variation in the rate of uptake with enzyme concentration is not due to diffusion effects, since tea juice itself shows a similar behaviour; nor is it due to variations in the concentration of substrate. The most likely explanation appears to be that it is due to variations in the degree of dispersion of the coenzymes required for carbohydrate breakdown. This view is supported by the observation that where coenzyme dilution can have no effect, as in the simple oxidation of tea tannin or pyrogallol by the cytochrome system, the reaction is independent of enzyme concentration, but direct evidence for this explanation is as yet lacking. The fermentation of tea in the factory does not follow the same course as fermentation in dilute aqueous suspension, peroxidase playing a negligible role in dilute suspension. Thus the essential reactions in the factory are (3), (5) and (6) above, whilst in dilute suspension the essential reactions are (2) and (6). F. A. R.

Vitamin A, Carotene and Xanthophyll Content of the Yolk of Hens' Eggs. B. Sjollema and W. F. Donath. (*Biochem. J.*, 1940, **34**, 736–748.)—Different groups of hens were given the same basal diet with different sources of carotene and vitamin A. The amounts of vitamin A, carotene and xanthophyll in the eggs laid by these hens were estimated after saponification of the yolks and extraction with peroxide-free ether. Half of the extract from each yolk was evaporated to

dryness, the residue was dissolved in chloroform, and the solution was treated with antimony trichloride solution; the total blue colour due to the three components was measured in a Pulfrich step-photometer. The other half of the extract was evaporated to dryness, the residue was dissolved in 75 ml. of petroleum spirit (b.p. 60° to 80° C.) and the solution was shaken several times with 85 per cent. alcohol until no further colour was extracted. The petroleum spirit solution, containing the carotene, was washed with water, dried and distilled. The residue was dissolved in a suitable volume of petroleum spirit, and the colour of the solution was measured in the step-photometer, filter S47 being used. The combined alcoholic extracts, containing the xanthophyll, were diluted with 5 volumes of water and extracted with petroleum spirit. The extract was washed, dried and concentrated to a suitable volume, and the colour of the solution was measured as before, with the use of filter S47. The carotene and xanthophyll contents were estimated by reference to standard curves prepared with the aid of pure specimens of these two substances, and the amount of vitamin A was calculated by subtracting from the total blue value, a blue value equivalent to the total carotene and xanthophyll found to be present. The results indicated that once the birds had been in production for some time the amounts of vitamin A, carotene and xanthophyll in the eggs depend chiefly on the diet. Yellow maize and green fodder, either fresh or dried, were the best sources of the vitamin A and carotene of the yolk. Yellow maize alone, constituting 25 per cent. of the ration, produced yolks with a vitamin A content of 150 I.U. per yolk, whilst the addition of lucerne resulted in even higher vitamin A contents, up to a maximum of 320 I.U. per yolk. A greater amount of xanthophyll than carotene was consistently found to be present, so that the yellow colour is chiefly due to the former. F. A. R.

Estimation of Riboflavin. I. New Biological Method. M. M. El Sadr, T. F. Macrae and C. E. Work. II. Estimation of Riboflavin in Milk: Comparison of Fluorimetric and Biological Tests. K. M. Henry, J. Houston and S. K. Kon. III. Statistical Analysis of the Data. J. O. Irwin. (*Biochem. J.*, 1940, **34**, 601–612.)—Up to the present the biological assay of riboflavin has been hampered by the difficulty of providing adequate amounts of all the members of the vitamin B_2 complex other than riboflavin. The problem of providing such a supplement has now been solved by treating aqueous extracts of whole liver at *p*H 5 with Norit carbon; this removes all the riboflavin quantitatively, and the resulting filtrate contains adequate amounts of all other B_2 vitamins recognised to be essential for the rat. By means of this supplement a growth response curve to graded doses of riboflavin was constructed. By use of the curve assays have been made of the riboflavin-content of full cream spray-dried milk and evaporated milk, and the results have been compared with those obtained by fluorimetric assays of the same samples of milk. One sample of spray-dried milk was found to contain 15·2γ of riboflavin per g. by the biological test and 10·0γ by fluorimetric assay. For another sample of spray-dried milk, the corresponding values were 9·3 and 10·3, and for an evaporated milk 2·6 and 2·9. The agreement was satisfactory when the milk was fed at a level not exceeding 10γ of riboflavin daily. F. A. R.

Estimation of Nicotinic Acid in Animal Tissues, Blood and Certain Foodstuffs. I. and II. E. Kodicek. (*Biochem. J.*, 1940, **34**, 712–723, 724-735.)—The nicotinic acid contents of a wide range of animal tissues, foodstuffs and medicinal preparations were estimated by a colorimetric method almost identical with that described by Harris and Raymond (*cf.* ANALYST, 1940, **65**, 183), involving the use of cyanogen bromide solution and *p*-aminoacetophenone. Some of the most important results obtained, expressed as γ per g. of fresh material, are as follows: liver (ox) 170, liver (sheep) 200, adrenals (sheep) 135, heart, muscle, kidney, pancreas, spleen, lung, brain (ox or sheep) 30 to 75, salmon 84, herring 40, cod 30, roe (herring or turbot) 21 to 23; liver extract (Eli Lilly "343"), 1090, marmite 640, yeast 74 to 91; egg-white $<0·5$, egg-yolk 10, milk (November to December) 0 to 5 (average 3), dried milk 25. F. A. R.

Combined Ascorbic Acid in Plant Foodstuffs. I. J. C. Pal and B. C. Guha. (*J. Indian Chem. Soc.*, 1939, **16**, 481–495.)—Aqueous, alcoholic and ethereal extracts of cabbage, germinated kancha mung (*Phaseolus mungo*) and the Indian fruit bel (*Aegle marmelos*) were prepared. The ascorbic acid contents of the aqueous extracts were estimated by titration with 2:6-dichlorophenolindophenol before and after boiling in an atmosphere of nitrogen; it was found that the titration value was increased by the heat treatment. Even greater increases in the apparent ascorbic acid contents were observed when the alcoholic extracts were heated. With the ethereal extracts no reduction of the indicator occurred before heating, but an appreciable quantity occurred after heating. It was concluded that part of the ascorbic acid in these plant foodstuffs is present in a combined form ("ascorbigen"), from which the free vitamin is released by heating. By contrast, an alcoholic extract of mango showed a decrease in ascorbic acid content on heating, and therefore contained no "ascorbigen." The increased titration value observed after heating extracts of cabbage, mung and bel, cannot be attributed to the destruction of ascorbic acid oxidase as suggested by Van Eekelen; for the phenomenon was observed, first, with ethereal extracts that were shown not to contain the enzyme, and, secondly, with alcoholic extracts heated at 30 to 40° C. for 10 minutes, a temperature not sufficiently high to destroy the enzyme.

Ascorbigen can be obtained practically free from ascorbic acid by extracting the tissue with ether. It is readily hydrolysed by dilute acid at room temperature, and probably, therefore, by the gastric juice in the stomach.

II. P. N. Sen-Gupta and B. C. Guha. (*Ibid.*, 496–504.)—Dried cabbage was extracted with different solvents with the object of obtaining a concentrate of ascorbigen. Chloroform was found to be best for this purpose, and although by no means all the ascorbigen was extracted, the product was free from uncombined ascorbic acid. Dehydroascorbic acid was also absent, since treatment of the extract with hydrogen sulphide in the cold did not produce reducing substance. When the chloroform extract was evaporated and the residue extracted with water, all the ascorbigen was removed but only 20 per cent. of the total solids, so that this procedure effected a 5-fold concentration. This aqueous extract was used to test the effect of ascorbic acid oxidase on the reducing substances formed by heating ascorbigen. It was found that 60 to 70 per cent. disappeared on treatment with the enzyme, suggesting that, whilst the main product was ascorbic acid, some other non-specific reducing substance was also formed. Finally, the chloroform extract was fed to scorbutic guinea-pigs; the results showed not only that ascorbigen was active, but that its activity is comparable with that of an equivalent amount of ascorbic acid.

F. A. R.

Concentration of Ascorbigen from Cabbage. B. Ghosh and B. C. Guha. (*J. Indian Chem. Soc.*, 1939, **16**, 505–510.)—The method previously used for obtaining a concentrate of ascorbigen (*cf.* preceding abstract) was not satisfactory, as the substance is not completely extracted by chloroform. Alternative methods were accordingly investigated, starting with cabbage juice. Glacial metaphosphoric acid produced a precipitate, but this contained only a small proportion of the ascorbigen together with some protein, whilst most of the ascorbigen remained in the filtrate. Other precipitants were tried with much the same result. The effect of various adsorbents was next tried, and it was found that charcoal adsorbed 60 per cent. of the ascorbigen from cabbage juice. The adsorbate was eluted by boiling for 1 hour with a 3 : 7 mixture of chloroform and absolute alcohol, and the pasty mass left after evaporation of the solvent contained combined ascorbic acid together with other material. The ascorbigen was further purified by extraction with a minimum quantity of water, centrifuging and evaporating the centrifugate to dryness in a desiccator. The product from 1 kg. of fresh cabbage contained the equivalent of 4·43 mg. of ascorbic acid. No further concentration could be effected by electro-dialysis, but some purification was effected by tungstic acid precipitation. The concentrate gave a strong Molisch reaction, reduced Fehling's solution, did not respond to the xanthoproteic, biuret or Millon tests, but gave positive glyoxylic acid and Pauly reactions; it contained nitrogen and sulphur but no phosphorus. F. A. R.

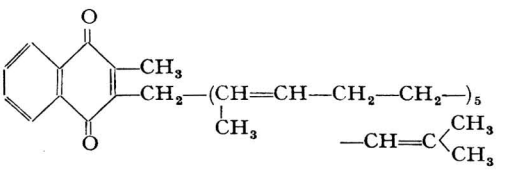
Estimation of Vitamin C in Foodstuffs. P. N. Sen-Gupta and B. C. Guha. (*J. Indian Chem. Soc.*, 1940, **16**, 549–555.)—Trichloroacetic acid extraction followed by titration with 2 : 6-dichlorophenolindophenol tends to give a value for total vitamin C that is too low, because first some activity is lost by the action of ascorbic acid oxidase before this is destroyed by the trichloroacetic acid, and secondly, biologically potent ascorbigen does not reduce the dye unless split by heating. The use of mercuric acetate, as recommended by Emmerie and van Eekelen (*Biochem. J.*, **1934**, **28**, 1153), while eliminating interfering substances, causes some loss of ascorbic acid. The method now proposed consists in heating an aqueous suspension of the foodstuff in hydrogen sulphide followed by treatment with ascorbic acid oxidase. The enzyme was prepared according to the method of Tauber, Kleiner and Miskind (*J. Biol. Chem.*, 1935, **110**, 211; *cf.* ANALYST, 1935, **60**, 629), and purified by precipitation twice from aqueous solution by acetone; its potency was checked against pure ascorbic acid. Ten g. of the foodstuff were suspended in 50 ml. of water and treated with hydrogen sulphide on the water-bath. After removal of hydrogen sulphide with carbon dioxide or nitrogen, the mixture was treated with 2·5 ml. of 20 per cent. trichloroacetic acid. After centrifuging, the volume was made up to 100 ml. Ten-ml. aliquot portions were treated with 1 to 2 drops of sodium hydroxide solution to bring the *p*H to 5·6, followed by 2 ml. of *M* sodium acetate buffer solution (*p*H 5·6) and 3 ml. of the enzyme solution. The mixture was incubated at 40° C. for 30 minutes, made up to a definite volume and titrated against the dye solution. The difference in the titration value before and after incubation with the enzyme gave the amount of "true" ascorbic acid, comprising free ascorbic acid, dehydroascorbic acid and ascorbigen. This method was used for the estimation of ascorbic acid in 30 Indian foodstuffs, and in nearly every instance higher results were obtained by this method than by simple trichloracetic acid extraction.

F. A. R.

Estimation of Vitamin D in Food Substances containing Phosphorus. K. H. Coward and E. W. Kassner. (*Biochem. J.*, 1940, **34**, 538–541.)—It is well known that when rats are fed on a high calcium—low phosphorus diet, they develop rickets which can be largely, if not completely, healed by addition of phosphates to bring the Ca : P ratio more nearly equal to 1. Thus, unless the amount of phosphorus in the diet is relatively high, the effect produced by the addition of a foodstuff whose vitamin D-content is being estimated may be due partly to the vitamin D present and partly to the phosphorus. Experiments were therefore carried out to ascertain

the healing produced in rats fed on a rachitogenic diet of high calcium—low phosphorus content by (*a*) vitamin D alone, (*b*) phosphate alone, (*c*) vitamin D and phosphate together. The results indicate that the effect of giving both supplements is not additive, but multiplicative. It is concluded that the only practical method of assaying vitamin D in foodstuffs containing amounts of phosphorus sufficient to alter materially the Ca : P ratio is to saponify the material and carry out the assay on the unsaponifiable fraction. F. A. R.

Constitution of Vitamin K_2. S. B. Binkley, R. W. McKee, S. A. Thayer and E. A. Doisy. (*J. Biol. Chem.*, 1940, **133**, 721–729.)—Vitamin K_2 is a yellow crystalline solid, m.p. 53·5 to 54·5° C., previously isolated from putrefied fish-meal. It has now been shown to be 2-methyl-1 : 4-naphthoquinone substituted in the 3-position by a long unsaturated chain which may be considered to be two farnesyl radicles fastened head to tail. Its structural formula is:



This constitution was established by the ozonolysis of the diacetate of dihydro-vitamin K_2, which yielded 1 : 4-diacetoxy-2-methylnaphthalene-3-acetaldehyde (identical with the compound obtained by the ozonolysis of the diacetate of dihydro-vitamin K_1), levulinaldehyde and acetone. F. A. R.

Quantitative Test for Biotin and Observations regarding its Occurrence and Properties. E. E. Snell, R. E. Eakin and R. J. Williams. (*J. Amer. Chem. Soc.*, 1940, **62**, 175–178.)—An assay method is described for biotin; it depends on the growth of a strain of *Saccharomyces cerevisiae*. The yeast was grown on a basal medium consisting of sucrose 20 g., ammonium sulphate 3 g., potassium hydrogen phosphate 2 g., magnesium sulphate (hydrate) 0·25 g., calcium chloride (hydrate) 0·25 g., boric acid 1 mg., zinc sulphate 1 mg., manganese chloride 1 mg., titanous chloride 1 mg., ferric chloride 0·5 mg., copper sulphate (hydrate) 0·1 mg., potassium iodide 0·1 mg., *l*-aspartic acid 0·1 g., inositol 5 mg., β-alanine 0·5 mg., aneurin 20γ, and vitamin B_6 20γ, dissolved in 1 litre of distilled water. Two ml. of an aqueous solution of the material to be tested were sterilised and cooled, and 10 ml. of the basal medium were added containing 0·02 mg. of suspended yeast. The mixture was incubated at 30° C. for 16 hours and then diluted with 10 ml. of saturated aqueous chlorothymol solution and shaken for 3 to 5 minutes. The amount of yeast present was estimated by means of a thermocouple and galvanometer (Williams, McAlister and Roehm, *J. Biol. Chem.*, 1929, **83**, 315; Abst., ANALYST, 1929, **54**, 613), this method being found superior to measurement by means of a photoelectric colorimeter. Blank cultures and cultures containing known amounts of biotin (0·000025 to 0·00025γ) were run simultaneously with the unknowns, and the amounts of biotin in the unknown samples were read off from a standard curve. It was found that in parallel tests of a crude liver extract and a biotin solution, curves of identical character were obtained when the concentration was plotted against the weight of yeast produced; it is concluded that the test is highly specific for biotin. Vitamin B_6 must be present in the medium before any significant effect of biotin is observed. The following results (in γ per g.) were obtained, clear aqueous extracts being produced by autoclaving the material for 10 minutes at 15 lb. pressure with a large volume of water, except that casein was hydrolysed with sulphuric acid: cane molasses 1·7 to 2·1, beet molasses 0·06 to 0·9, whey 0·12, egg white 0·05, egg yolk 0·37, yeast 0·13, casein 0·125, potato 0·01, autolysed liver 3·9. F. A. R.

Bacteriological

Thermal Death Time of Micro-organisms encountered in the Brewing Industry. S. S. Epstein and F. D. Snell. (*J. Inst. Brewing*, 1940, 175–178.)—Twenty-one cultures were studied, including 5 *sarcina* (*Pediococcus*) strains, 2 *Lactobacillus* strains, 2 *Acetobacter* strains, 8 yeasts and 4 cultures from infected bottled beer, storage ale, storage beer and draught beer. The pure strains of bacteria were cultivated in sterile beer at 77° F. until turbidity developed and then kept at room temperature for six days before testing. The yeasts were grown in brewers' wort under the same conditions. The object of using cultures 6 days old was to simulate practical conditions, the cultures having by this time attained maximum resistance to heat. The size of the inoculum was 10,000 to 30,000 organisms per ml. The results of these studies indicate that the standard practice of pasteurisation at 140° F. for 20 minutes should render beer free from all living organisms commonly encountered in the brewing industry. The order of heat resistance from the greatest to the least was found to be:—*Lactobacillus*, *Pediococcus* (*Sarcina*) and *Acetobacter*. None of the bacteria tested was able to survive an exposure to 60° C. (140° F.) for ten minutes. Of the yeasts, the order of heat resistance was: —*S. pastorianus*, *S. ellipsoideus* and brewers' culture yeasts, *Mycoderma* and *Torula*. Yeast spores were killed at 58° C. (136·4° F.) within 20 minutes and at 60° C. within 15 minutes. D. R. W.

Mechanism of the Microbial Oxidation of Ammonia. Part II. G. Gopala Rao and W. V. Sundara Rao. (*J. Indian Chem. Soc.*, 1939, **16**, 681–690.)—The inhibitory action of capillary active substances such as

the urethanes, nitriles, alcohols and ketones, and of the cyanides on the microbiological oxidation of ammonia has been investigated. The tests were made in flasks containing 100 ml. of culture medium, one ml. of enriched culture of the nitrite-forming bacteria, and varying volumes of the test substance; the whole was made up to 200 ml. and portions of the liquid were withdrawn from time to time for the determination of nitrites. The tabulated results show that these substances inhibit the respiration of the bacteria for a time, but that ultimately nitrite begins to appear, the length of time before its appearance increasing with the concentration of the added narcotic. These experiments lead to the conclusion that oxidation of ammonia is a surface catalytic reaction taking place at certain active centres on the surface of the bacteria. Recent work of Quastel and Wooldridge shows that a suspension of bacteria behaves in a manner identical with any colloidal system possessing catalytic properties, and the fact that resting bacteria are able to bring about reversible reactions whose equilibrium points are independent of the amount or conditions of the organism supports this view. Some bacteria, such as *B. coli*, have a wide range of activation, effective for as many as 56 substances. According to Quastell this is associated with specific patches on the surface of the bacteria, which first adsorb and then activate the substance. The more elementary nitrifying bacteria cannot utilise any of the complex organic molecules; they oxidise ammonia and make use of the exothermic reaction for their life activities and for the chemo-synthesis of carbohydrates from atmospheric carbon dioxide. Whereas *B. coli*, *pneumococci* and *staphylococci* are presumed to have a variety of specific patches on their surface the nitrifying bacteria are presumed to have only one kind. In the author's opinion the inhibitory action of urethane, alcohols, etc., is not specific and these so-called poisons act by competing with the substrate for the space available for adsorption. With the cyanides, however, the resulting inactivation is presumed to be due to the centres containing an iron-rich complex, the function of which is completely inhibited by combination with the CN' ion. The results here recorded show that in the same series of compounds the concentration required for marked inhibition is lower with compounds of higher molecular weight. Thus with ethyl alcohol it is $M/10$ with acetone $M/20$, with octyl alcohol $M/500$ and with benzophenone $M/4000$.

D. R. W.

Gas Analysis

Starch-iodide Method for Ozone. C. E. Thorp. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 209.)—Many of the reagents proposed as substitutes for potassium iodide (*cf.* Benoist, ANALYST, 1919, **44**, 183; Briner and Perrottet, *Helv. Chem. Acta*, 1937, **20**, 293; Abst., ANALYST, 1937, **62**, 415) have been found satisfactory, but potassium iodide is preferred as a quick and convenient method for the detection of ozone. When ozone in admixture with air was passed through neutral potassium iodide solution and the iodine liberated on acidification was titrated with standard sodium thiosulphate solution the greatest sensitivity obtained was the detection of 0·0013 mg. of ozone per ml. of 2 *N* potassium iodide solution. Since analyses of air containing 0·1 p.p.m. by weight of ozone are frequently required, 9·9 litres of air would have to be passed through each ml. of potassium iodide solution before the ozone could be detected in this way. Various methods have been tried to increase the sensitivity of the potassium iodide reagent (Baskerville and Crozier, *J. Amer. Chem. Soc.*, 1912, **34**, 1332; Abst., ANALYST, 1912, **37**, 587; Ernst, *Biochem. Z.*, 1931, 232, 346). The use of thiocyanate ions (Ernst, *loc. cit.*) may increase the sensitivity towards some compounds, but appears to decrease it towards ozone. The use of free acid to lower the *p*H of the potassium iodide solution introduces another error, owing to the formation of hydrogen peroxide. The use of a buffer solution greatly increased the sensitivity without introducing the error caused by free acid. Five ml. of an aqueous solution of 5 g. of aluminium chloride hexahydrate and 1 g. of ammonium chloride per litre are added to each 100 ml. of potassium iodide solution, and the mixture should not be acidified during the subsequent titration. The sensitivity of the mixture is 0·00062 mg. of ozone per ml. of potassium iodide solution and the sensitised solution is stable for over three hours. The ozone sample is drawn through 100-ml. gas-washing bottles each fitted with a Jena glass disc (Thomas, *Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 193) until a deep colour of iodine is formed in the first bottle. The sodium thiosulphate solution for the titration should not exceed 0·01 *N*, and a 2 ml. micro-burette is recommended for greater accuracy. For ozone concentrations of less than 0·5 p.p.m. the gas-washing bottles should be of the semi-micro type, and the volume of the test solution should not exceed 10 ml. There must be no exposed cork or rubber stoppers; ground glass connections are preferable, but neoprene or rubber or cork coated heavily with shellac or lacquer may be used. Only ultra-violet light will produce pure ozone. The product of other types of commercial ozoniser will contain hydrogen peroxide and oxides of nitrogen. To remove these the ozone should be passed through absorption tubes containing chromic acid and potassium permanganate.

A. O. J.

Determination of Carbon Disulphide in Air. F. F. Moorhead. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 373–374.)—A copper-diethylamine reagent is used, *viz.* 1 ml. of a 1 per cent. solution of diethylamine in 2-methoxyethanol mixed with 2 drops of 0·1 per cent. cupric acetate solution in 2-methoxy-

ethanol. A yellow or brownish colour is produced with carbon disulphide. The reaction is more sensitive and more suitable for colorimetric work than the xanthate reaction. Methoxyethanol is better than ethanol as reaction medium, as the colour is more stable. The method involves drawing a sample of air (100 ml. is usually sufficient) through the reagent. A suitable absorption tube consists of a 6 to 7 cm. length of glass tubing, 10 mm. in diameter, containing glass beads which are retained by constrictions at the ends. The tube is used in a vertical position, the lower end being sealed to an upturned length of capillary tubing in which a bulb is blown, and the upper end sealed to a right-angled leading tube. The reagent is placed in the absorption tube connected at the upper end to an aspirator, which conveniently consists of a tap funnel containing water. After aspiration, the contents of the absorption tube are rinsed into a 2-ml. measuring flask and made up to a definite volume with the solvent. For the colorimetric comparison, which is made after about 20 minutes in a Klett (Duboscq type) colorimeter with 2-ml. cups, a standard solution of carbon disulphide in methoxyethanol is added to the reagent. The method is capable of determining 1 to 30γ of carbon disulphide. If hydrogen sulphide is present the air should be passed through 5 per cent. aqueous copper sulphate or lead acetate paper and then through a desiccating agent before entering the carbon disulphide absorption tube. S. G. C.

Agricultural

Electrodialysis of Soils. Influence of Exchangeable Bases on the Recovery of Manganese by Electrodialysis. R. C. Hoon and C. L. Dhawan. (*J. Indian Chem. Soc.*, **1940, 17**, 195–204.)—Recovery of manganese from soils by electrodialysis is affected by the presence of other bases in the exchange complex. Three natural soils were freed from exchangeable bases and converted into manganese soils (*cf.* Prince and Toth, *Soil Science*, **1938, 46**, 83). The manganese-contents of the artificial soils were determined (i) directly by the bismuthate method, (ii) in the leachates, obtained from the soils with *N*-neutral ammonium acetate solution, and (iii) by electrodialysis for 5 hours. Sets of (manganese + base) soils were prepared from each artificial soil by addition of different amounts of sodium, potassium, calcium and magnesium hydroxides. The soils were then shaken with water for 48 hours and electrodialysed. Results were tabulated for base added to soil, *p*H of 1 : 5 suspensions, and percentage recovery of the other base. A few natural soils, from good and bad areas, containing different amounts of manganese were also electrodialysed for 5 hours. Manganese was determined separately in the electrodialysate recovered in the first hour and in the collective electrodialysate of the next four hours; the total recovery in 5 hours and the percentage of total manganese in it were calculated. In the artificial soils, the manganese-sodium sets yielded the maximum amount of electrodialysable manganese and the minimum amount as deposit on the cathode, etc. In these and in the manganese-potassium sets there was on the whole a tendency for the manganese recovered in the deposit to decrease with increasing *p*H. In the manganese-magnesium sets the least manganese was recovered in the electrodialysate and mainly in the deposit. In the manganese-calcium deposit on the cathode, the manganese-content decreased as the *p*H increased to about 8·0, then increased slightly. The results showed that soils with high *p*H values and high manganese contents yield more manganese on electrodialysis than those with low *p*H values and low manganese contents; all, or almost all, the electrodialysable manganese is obtained in the first hour. It is concluded that at high *p*H values when the dominant base is sodium, more manganese comes out in the soluble form and is thus available to plants; this availability may be reduced when the dominant base in the exchange complex is calcium or magnesium.

E. B. D.

Determination of Rotenone in Derris Root. T. M. Meijer and D. R. Koolhaus. (*Ind. Eng. Chem., Anal. Ed.*, **1940, 12**, 205–209.)—Analyses of the same sample of powdered derris root by different workers showed that differences of about 4 per cent. in the nominal value of the rotenone-content of the dry sample and large differences in the moisture-content may occur. A study of the methods proposed for the determination of rotenone in derris root showed that several gave results concordant with those obtained by the method here described. Methods in which the extraction is complete and sufficient attention is paid to the crystallisation of the rotenone or rotenone and carbon tetrachloride solvate, should give similar results. Suitable solvents for extraction are ether, benzene, chloroform, ethyl acetate and trichloroethylene. Carbon tetrachloride should not be used, especially with samples of high rotenone-content, as Seaber (*J. Soc. Chem. Ind.*, 1937, **56**, 168T; Abst., ANALYST, 1937, **62**, 630) and Jones and Sullivan (*J. Econ. Entomol.*, 1938, **21**, 148) have shown. At least 75 per cent. of the powdered root must pass through an 80-mesh sieve. The powder (50 g.) held in a Soxhlet extractor without a thimble by means of a cotton-wool plug, was extracted with ether for at least 65 hours. The ether was distilled off in a 100-ml. centrifuge tube, and any rotenone separated during the extraction was transferred to the tube and the flask was rinsed out with ether until the liquid in the tube measured 25 ml. The tube was kept at room temperature for a day and in a refrigerator for a further 2 days. The mother liquor was poured into a flask, the remaining rotenone was broken up with the addition of 10 to 15 ml. of ether, and the tube and flask were placed in the refrigerator for another day. The tube was centrifuged at 3500 r.p.m. for

3 to 5 minutes and the supernatant liquid was added to the mother liquor. The centrifuge tube was then dried at 70° C., and finally at 100° C. *in vacuo*, and its contents were weighed. The purity of the rotenone was ascertained by means of an empirical table correlating its melting-point with its purity. If the m.p. was below 140° C. the product was centrifuged with 10 ml. of ether and its m.p. again determined. Sometimes substances with m.p. very near that of rotenone separate (Meijer and Koolhaus, *Rec. Trav. Chim. Pays-Bas*, 1939, **58**, 207), and for this reason the purity of the product was ascertained also from its optical rotation (Seaber, *loc. cit.*). For each ml. of the mother liquor and washings 4·2 mg. of rotenone were added as a correction for solubility, and any additional rotenone that separated from the mother liquor was added to the crude rotenone. The mother liquor was evaporated in a 500-ml. tared round-bottomed flask on the water-bath and finally *in vacuo* at 40° C. The residue was dried over lime for 2 days and weighed, and the weight of resin so obtained when added to the weight of crude rotenone gave the total ethereal extract. Moisture was determined by heating 2 to 3 g. of the powdered sample to constant weight at 105° C. To determine unextracted rotenone the resin was treated first with boiling petroleum spirit and then with boiling cyclohexane, and the rotenone was separated from the residue as the carbon tetrachloride solvate. An amount corresponding with about 9 per cent. of the rotenone originally found was recovered from the resin, especially when the sample had a high ethereal extract and a low rotenone-content. Determination by a chromatographic method gave a similar result. Jones and Graham (*J. Assoc. Off. Agr. Chem.*, 1938, **21**, 148) have proposed a method in which the ground root is extracted with chloroform and the rotenone determined by conversion into the carbon tetrachloride solvate. Extraction with chloroform at room temperature is practically complete. The value for pure rotenone found by the ether-extraction method was almost equal to that found for crude rotenone by the method of Jones and Graham, and, as the solvate obtained in the latter method is fairly pure, the value should not be lowered by a correction for purity. A satisfactory uniform method might be based on the Jones-Graham method, but it must be adapted to samples in which the ratio of rotenone to total extractive is high. This may be effected by grinding samples so that at least 75 per cent. passes through a 200-mesh sieve, or by extracting the powdered root several times with chloroform. Jones (*Ind. Eng. Chem., Anal. Ed.*, 1937, **9**, 206) has determined rotenone by conversion of the carbon tetrachloride solvate into the dichloroacetic acid solvate. Since each molecule of dichloroacetic acid is associated with a molecule of rotenone, the solvate may be titrated with standard alkali solution. Derris root ground to pass a 200-mesh sieve lost a considerable amount of its apparent rotenone-content when heated to 60° C. and still more at 80° C. Heating the sample to reduce the moisture-content before analysis is therefore to be discouraged. A. O. J.

Organic

Rapid Qualitative Test for Alcoholic Hydroxyl Groups. F. R. Duke and G. F. Smith. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 201–203.)—The red colour of solutions of hexanitrato or hexaperchlorato potassium or ammonium cerate in alcohol suggested the use of these substances as reagents for the detection of the alcoholic hydroxyl group. A solution of 400 g. of hexanitrato ammonium cerate in a litre of 2 *M* nitric acid is approximately 0·7 *M* in nitrato cerate ion. A solution of perchlorato ceric acid in 6 *M* perchloric acid is available commercially and is approximately 0·5 *M* in perchlorato cerate ion; it is prepared by electrolytic oxidation of cerous perchlorate in perchloric acid solution. With this solution (unlike the hexanitrato solution) the concentration of the acid is not an important factor. The procedure is as follows:—The cerate reagent (1 ml.) is diluted with 2 ml. of water, and 1 or 2 drops of the compound to be tested (or its saturated aqueous solution) are added. A red colour indicates an alcohol. In testing compounds insoluble in water 1 ml. of the cerate reagent is mixed with 2 ml. of dioxane, and 1 or 2 drops of the compound (dissolved if necessary in the least amount of dioxane) are added. Dioxane solutions cannot be used with the perchlorato cerate reagent owing to reduction of the reagent. Acids, aldehydes, ketones, esters and hydrocarbons do not interfere with the test. Amines raise the *p*H to such an extent that insoluble cerium compounds may be precipitated. Aromatic amines and phenols interfere by forming characteristic colours and precipitates. Easily oxidised substances (*e.g.* oxalic acid) interfere by reduction of the reagent, and organic dyes may cause colour interference. Alcohols containing up to 10 carbon atoms are included in the test. Primary, secondary and tertiary alcohols give instantaneous reactions. Hydroxycarboxylic acids also react. With glucose, sucrose and dextrin the colour is rapidly lost by oxidation. Solutions containing 2 to 4 per cent. of butyl alcohol react with the nitrato cerate reagent, and solutions of 1 to 2 per cent. with the perchlorato cerate reagent. The more reactive the alcoliolic hydroxyl group the more sensitive the test; *e.g.* benzyl and allyl types are especially sensitive. The oxidation potential of the nitrato cerate reagent is much lower than that of the perchlorato cerate reagent (Smith and Getz, *Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 191). For this reason the colour with the nitrato reagent is more permanent. Tertiary alcohols form much more stable coloured solutions than primary and secondary alcohols, and this may serve as a means of differentiation. The colour obtained with the four butyl alcohols was studied quantitatively in a photoelectric

colorimeter. The intensity was found to increase progressively with the four isomeric alcohols in the order iso-, secondary, normal, tertiary. In all instances the colour was more intense with the perchlorato cerate reagent and more stable with the nitrato cerate reagent, and was most pronounced with the tertiary alcohol. By comparison of methyl alcohol with nonyl alcohol the intensity of colour was shown to diminish with the longer chain. The colour produced by aqueous solutions of methyl alcohol is greater than that produced by solutions in dioxane. The use of perchloric acid with organic compounds in the manner described introduces no danger in the application of the test provided that the solutions are not heated. A theoretical explanation of the colour formation is given. A. O. J.

New Reaction of Formic Acid. T. L. Davis and W. P. Green. (*J. Amer. Chem. Soc.*, 1940, **62**, 1274–1276.)—Interaction of bromine and formic acid produces a substance which, in presence of bromine, reacts with aniline to form derivatives of urea. A mixture of 12 g. of bromine and 30·7 g. of anhydrous formic acid was kept for 2 days, then run slowly from a separator into a solution of 108·3 g. of aniline in 300 ml., of benzene, which was mechanically stirred. The solution (which contained a precipitate) was made alkaline, steam distilled to remove aniline and brominated aniline, and extracted with ether. On evaporation, the extract left 0·2644 g. of a crude product which on fractional sublimation yielded impure carbanilide and a brominated carbanilide fraction. Treatment of the second fraction with aniline followed by sublimation and repeated recrystallisation also yielded carbanilide. It is considered probable that dibromodihydroxy-methane is formed in presence of excess of bromine, by addition of bromine to the tautomeric form of the acid—dihydroxy bivalent carbon. E. B. D.

Separation of Hydroxy from Non-hydroxy Fatty Acids by means of a Dibasic Acid Anhydride. F. E. Kurtz and P. S. Schaffer. (*J. Amer. Chem. Soc.*, 1940, **62**, 1304–1305.)—Separation of hydroxy fatty acids from non-hydroxy acids by the usual methods is difficult when the differences in their physical properties, or in those of their derivatives, is small. A better method is to heat the mixed esters of the mixed acids with a dibasic acid anhydride, dissolve the reaction mixture in petroleum spirit and extract the derivative of the hydroxy ester with alkali. Owing to its solubility in esters, maleic anhydride is recommended for separating hydroxy acids from saturated acids, but with unsaturated acids partial saturation occurs. For these, succinic acid may be used, but it requires addition of a solvent; with dioxane low yields are obtained; pyridine gives better results but forms a tarry precipitate on prolonged heating, though much less than with maleic anhydride. With either reagent more than one separation is usually required. With castor oil acids (about 85 per cent. of hydroxy acids) two separations give hydroxy acids practically free from non-hydroxy acids. E. B. D.

Purification of High Molecular Weight Fatty Esters. L. O. Buxton and R. Kapp. (*J. Amer. Chem. Soc.*, 1940, **62**, 986.)—The following procedure is recommended for removing substantially all of the fatty acids that have not reacted in the preparation of their esters. The alcohol is distilled from the esterification mass, and the residual mixture of free fatty acids, crude ester and catalyst is dissolved in 2 to 5 parts by weight of solvent such as ethylene dichloride to 1 part of ester. Ten to 20 g. of the solution are dissolved in a mixture of alcohol and ether and titrated with standard 0·5 *N* alcoholic potassium hydroxide solution. On the basis of this titration value an equivalent weight of concentrated (preferably 38 per cent.) aqueous potassium hydroxide solution is added slowly, with constant stirring, to the ester solution. The potassium salts of the fatty acids that have not reacted and of any mineral acid present as catalyst are filtered off without suction and washed with a small quantity of fresh ethylene dichloride to remove traces of neutral ester. The filtrate is distilled without drying. The esters so prepared have acid values of 0·5 to 1·0 and can be further purified by vacuum distillation. The method has been used to obtain quantitatively the yields of methyl and ethyl esters of lauric, oleic, linolic, stearic and ricinoleic acids from the original esterified mass prepared with the respective crude acids, and also for the preparation of relatively pure mono- and dinaphthenates of diethylene glycol. E. M. P.

Thiocyanogen Value of Linolenic Acid. J. P. Kass, H. G. Loeb, F. A. Norris and G. O. Burr. (*Oil and Soap*, 1940, **17**, 118–119.)—The thiocyanogen value of α-linolenic acid was studied under the conditions of the determination recommended by the American Oil Chemists' Society (*Ind. Eng. Chem., Anal. Ed.*, 1936, **8**, 233). The thiocyanogen solutions, which varied in concentration from 0·16 to 0·18 *N*, were freshly prepared for each series of determinations and were dispensed from a 25-ml. automatic pipette protected with a calcium chloride tube. All glassware was dried for one hour at 105° C., and the reaction temperature was maintained at 19° ± 1° C. by keeping the reagents and reaction mixtures in a large water-jacketed incubator. Ethyl linolenate (iodine value, Wijs, 247·3; theory 248·5) was prepared by debromination of purified hexabromostearic acid (from linseed oil) with zinc and 7·5 *N* ethyl alcoholic hydrogen chloride according to the method of Rollet (*Z. physiol. Chem.*, 1909, **62**, 422). In determinations in which the excess of 0·16 *N* thiocyanogen solution used varied between 150 and 290 per cent., whilst the time of contact was either 20, 24 or 28 hours, it was found that the thiocyanogen value of ethyl linolenate approximated to 151·2, corresponding with 167·3 for linolenic acid (of theoretical

iodine value 273·7) instead of 182·5 as assumed by Kaufmann and Keller (*Z. angew. Chem.*, 1929, **42**, 73). Experiments on mixtures of corn (maize) oil acids with varying proportions of α-linolenic acid (28–30 hours' contact with the 0·16 *N* reagent) showed that the observed thiocyanogen values agreed with those calculated on the assumption that the linolenic acid has a thiocyanogen value of 167·3 and not 182·5, subject only to slight deviations from the calculated values with increasing concentrations of linolenic acid in the mixed acids. The authors suggest the substitution of the empirically determined value of 167·3 for the thiocyanogen value of linolenic acid in equations used in calculating the proportion of this acid by thiocyanometric analysis. The empirical value is also shown to vary somewhat with the concentration and excess of the thiocyanogen reagent and with the period of its contact with the unsaturated acids or esters (*cf.* Hilditch and Murti, ANALYST, 1940, 444, footnote). T. P. H.

Separation and Characterisation of Petroleum Acids. H. G. Schultze, B. Shive and H. L. Lochte. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 262–266.)—Preliminary investigation of alkali wash from the refining of light burner oil showed that of the numerous combinations of physical constants suggested for the rapid characterisation of fractions the product of the refractive index and the density was the simplest and most useful. Calculation of the value of $n_D^{20} \times d_4^{20}$ for a large number of reported aliphatic, unsaturated and naphthenic acids, phenols and hydrocarbons showed that the aliphatic acids have products ranging from 1·280 to 1·350 with most values between 1·300 and 1·310, the naphthenic acids products range from 1·390 to 1·470 with most values between 1·410 and 1·440, the products of phenols have values above 1·500, and those of hydrocarbons values below 1·300 and usually below 1·280. The only exception found among the acids containing the cyclopentyl group—the typical naphthenic acids—was 1,2,3-trimethylcyclopentane carboxylic acid (Noyes and Burke, *J. Amer. Chem. Soc.*, 1912, **34**, 174). Its constants yield the value 1·313, which is within the range of the typical aliphatic acids. The acids liberated from a large quantity of conc. alkali were distilled under reduced pressure and the fractions so obtained were submitted to further fractionation. The fraction of the desired boiling range was separated into strong and weak acid components by fractional neutralisation, and further separation was effected by fractional neutralisation of the strong acids with sodium carbonate. Repetition of this procedure with various non-phenolic fractions effected the separation of the strongest organic acids, which were selected for more detailed study. During some of the later fractionations a solid substance separated in the condenser, and this was ultimately identified as dimethyl maleic anhydride which has since been isolated from a separate source (Hancock and Lochte, *J. Amer. Chem. Soc.*, 1939, **61**, 2448). The following procedure for the separation of acids by means of their silver salts was found satisfactory:—The silver salts were fractionally precipitated in the usual manner, each fraction was dissolved in ammonia, and the solution was fractionally neutralised with *N* nitric acid. When the acids from the first fractions were liberated from their silver salts by treatment with dehydrated phosphoric acid and subsequent distillation, a main fraction of dry colourless acids was obtained. By repetition of the silver salt separations a pure compound was obtained, and, by analysis of its *o*-phenylenediamine and *p*-nitrobenzyl derivatives, it was ultimately identified as *n*-valeric acid. By similar methods *n*-butyric acid, caprylic acid and *n*-octanoic acid were isolated and identified. When the final filtrate from the silver salt method was acidified and steam-distilled the presence of *o*-nitrophenol was detected. Although the yield was small, the formation of this substance was interesting because the absence of phenol in petroleum acids has often been reported. Fractions for which the product of density and refractive index indicated the presence of naphthenic acids were selected and submitted to further silver salt separations and conversion into acyl chloride and amido derivatives. Finally the amides of *p*-hexahydrotoluic acid and some of its isomers were isolated and identified. Although formerly the naphthenic acids in petroleum were thought to consist of cyclohexyl acids, the tendency in the last decade has been to assume that naphthenic acids are all cyclopentyl acids, since only very little indication of cyclohexyl acids had been obtained and none had been isolated. The amount of the pure solid isomer of *p*-hexahydrotoluic acid obtained in this investigation was very small, but other isomers appeared to be present in larger amounts. Since the acids isolated were obtained from a mixture of straight run and cracking process products, the cyclohexyl acids may have been formed during the cracking process. A. O. J.

Colorimetric Determination of Primary Mononitroparaffins. E. W. Scott and J. F. Treon. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 189–190.)—When samples of air containing nitroethane were passed through dilute sodium hydroxide solution and the alkaline solution was reduced by means of iron, tin or zinc after acidification with sulphuric acid, quantitative yields of ethylamine were not obtained, although the reduction of aqueous solutions of nitroethane was satisfactory. When the alkaline solutions were added to an excess of hydrochloric acid containing ferric chloride a pink colour was formed, and a method of determining as little as 0·5 mg. of nitroethane in 25 ml. was based on this reaction. An aliquot portion of the solution, containing 1 to 20 mg. of nitroethane in 1 to 15 ml., is neutralised and treated with 1·5 ml. of 20 per cent. sodium hydroxide solution in a 25-ml. flask. After 15 minutes the solution is acidified with 6 ml. of dilute hydrochloric acid

(1 + 7) and 0·5 ml. of 10 per cent. ferric chloride solution is added immediately. Comparison solutions containing approximately the same amounts of nitroethane are treated similarly. After standing for 15 minutes the solutions are made up to 25 ml. and the colours are compared. A colorimeter equipped with a 1·58 cm. Wratten filter No. 65A in B glass (Eastman Kodak Co.) is recommended for making the comparison. The optimum *p*H for the greatest intensity and stability of the colour is 1·25 to 1·30. Solutions containing more acid fade gradually, and those with a higher *p*H do not change from the initial brownish colour to the final deep red. With very small amounts of nitroethane colour interference is prevented by reducing the amount of ferric chloride solution. The colour should always be developed at room temperature; it fades rapidly when the solution is heated. Nitroethane solutions which have not been made alkaline do not react with the ferric chloride, and alkaline solutions allowed to stand for a short time after acidification no longer react. Apparently only the aci- form of the nitro-compound gives the reaction. Stable coloured complexes with ferric chloride are also formed by 1-nitropropane and 1-nitrobutane, and colorimetric methods have been based on these reactions. Ferric chloride reacts with 2-nitropropane and with 2-nitrobutane, but the colours fade rapidly. Nitromethane does not react but may be determined by means of its colour reaction with vanillin and ammonia (Manzoff, *Z. Unters. Nahr. Genussm.*, 1914, **27**, 469; Abst., ANALYST, 1914, **39**, 264). The complex formed with nitroethane was isolated but proved too unstable for purification and analysis. Spectrophotometric examination of the coloured solutions showed that for the ranges of concentration used in the test the absorption followed the Lambert–Beer law. The method gave satisfactorily accurate results with samples of air containing known amounts of nitroethane, and was also successfully applied with slight modifications to the determination of nitroethane in tissues. A. O. J.

Identification of Organic Compounds. II. Piperidyl Derivatives of Aromatic Halogenonitro Compounds. M. K. Seikel. (*J. Amer. Chem. Soc.*, 1940, **62**, 750–756.)—The characteristics of the piperidyl derivatives of aromatic halogenonitro compounds and the rate of formation of the derivatives can be used to identify the aromatic bodies. The two following general procedures were worked out: (A) To 0·5 g. of the halogenonitro compound add 1·5 ml. (1·0 ml. for bromo compounds) of piperidine. Observe phenomena such as spattering (stir to prevent local over-heating), boiling, colour formation, and the time elapsing before piperidine hydrohalide separates; these are partly dependent on the quantities used. Heat the resulting solution or mixture under reflux in an oil-bath for one hour, cool, add water and filter. If the product separates as an oil, the following crystallisation methods should be tried in order: (*a*) place the mixture in ice for an hour with scratching (with few exceptions this method is sufficient); (*b*) decant the aqueous layer containing excess piperidine and piperidine hydrohalide and wash the oil several times with water; (*c*) freeze in dry-ice. If no crystallisation is obtained, abandon the experiment. The yields are 90 to 100 per cent. and the crude product will melt sharply if the chlorine replacement is complete and if decomposition products due to excessive reactivity are not present. Recrystallise from 85 per cent. alcohol, using excess if the m.p. is low. Certain high-melting insoluble compounds, which are indicated in tables, require 95 per cent. alcohol. The yield of piperidine hydrohalide, which is an index of the completeness of the reaction, can be determined qualitatively by evaporating the aqueous filtrate of the crude product and weighing the residue. If a nitro group is replaced, the residue is a reddish acetone-soluble gum, giving a nitrosamine reaction with diphenylamine reagent. (B) Dissolve 0·5 g. of the compound in 5 ml. of boiling alcohol (more if necessary), add 1·5 ml. (1·0 ml. for bromo-compounds of piperidine) and heat for fifteen minutes on the steam-bath. If the product does not separate on cooling or icing, force it out with water, crystallising any oil obtained as described under procedure A. If water has not been added before filtering, wash the precipitate with water to remove possible contaminating piperidine hydrohalide. The yields are only 70 to 90 per cent., but the crystallisation from alcohol effects purification. The times of reaction in both of these procedures may be altered to suit individual materials, and the following procedures may also be applied. (C) Mix the two reactants and add water after a definite time (which is indicated for those substances studied). (D) Add ice-cold piperidine to the solid compound (also iced), continue icing until the initial violent reaction has moderated and then allow the mixture to stand in a water-bath at room temperature, stirring frequently, until the spontaneous evolution of heat ceases (1 to 2 hours). (E) Dissolve the compound in the minimum quantity of cold alcohol (20 to 40 ml. for 0·5 g.), add piperidine and after the specified time at room temperature add water to precipitate the product. To prepare monopiperidyl derivatives when di-piperidination is rapid exactly two mols. of piperidine must be used. Thirty-seven halogenated nitrobenzenes were studied, and the conditions for the preparation of their piperidyl derivatives are given. Proofs of the identity of certain of the derivatives are given. E. M. P.

New Colour Reaction for Diarylamines. E. M. Meade. (*J. Chem. Soc.*, 1940, 1808.)—The amine is dissolved in a little anisole and a solution of methylmagnesium iodide in anisole is added, followed by benzoyl chloride. If a diarylamine were originally present a pronounced red colour is produced. The test is particularly useful for following the course of

N-substitution of diarylamines; for example, it will detect 1 per cent. of unchanged diphenylamine in a sample of N-methyldiphenylamine. The following substances gave a positive reaction: diphenylamine, 4′-methoxy-4-methyldiphenylamine, 4:4′-dimethoxydiphenylamine, phenyl-β-naphthylamine, *o*- and *p*-methoxyphenyl-β-naphthylamines; the following failed to give the reaction: N-methyldiphenylamine, N-methyl, -acetyl-, and -benzoyl-4:4′-dimethoxydiphenylamines, aniline, methylaniline, dimethylaniline, benzylaniline, and *p*-anisidine. E. M. P.

Estimation of Lignin in Tannin Materials. J. G. Shrikhande. (*Biochem. J.*, **1940, 34**, 783–789.)—When the lignin present in plant tissues was estimated by the 72 per cent. sulphuric acid method after pre-treatment with 5 per cent. sulphuric acid, surprisingly high results were obtained with certain types of materials, such as tea leaf prunings. It appeared as though the pre-treatment, instead of removing extraneous material that interfered with the lignin estimation, rendered it insoluble. The high results obtained coincided with the presence in the materials of tannins, especially of catechol tannins, which are precipitated by acids and thus rendered insoluble. A modified method of pre-treatment, to free the material from tannins, caffeine, chlorophyll, proteins and pentoses, all of which were shown to interfere with the estimation, was worked out. Two g. of the oven-dry material (powdered to pass an 80-mesh sieve) are extracted in a Soxhlet apparatus with 200 ml. of 95 per cent. alcohol for 4 hours. The residue is boiled under reflux with 150 ml. of water for 1 hour, the extract is filtered off, and the residue is hydrolysed with 150 ml. of 5 per cent. sulphuric acid for 1 hour. The product is filtered off on a weighed sintered glass crucible, washed free from acid, dried and weighed. Portions (0·2 g.) of this material are weighed into 1-litre beakers and treated with 20 ml. of 72 per cent. sulphuric acid. The powder is well mixed with the acid and allowed to stand overnight at 20° to 22° C. The contents of the beaker are then made up to 800 ml. with water and boiled for 2 hours, the volume being kept constant by the frequent addition of water. The suspension is filtered through a Gooch crucible with a No. 42 Whatman filter paper, and the precipitate is dried, weighed, and then ignited, the lignin content being calculated on an ash-free basis. F. A. R.

Inorganic

Determination of Arsenic in Organic and Inorganic Compounds. D. T. Lewis and V. E. Davis. (*J. Chem. Soc.*, 1939, 284–286.)—For the gravimetric determination of arsenic the authors favour its precipitation as uranyl ammonium arsenate, a slimy gelatinous precipitate quite insoluble in acetic acid and convertible by ignition into uranosic oxide U_3O_8. The arsenate solution is treated with 10 ml. of 4 *N* ammonia, and acetic acid until a faint odour is perceptible; it is boiled, and an excess of uranyl acetate solution is added. The precipitate, which becomes coarser after some hours' standing, is collected, washed, and ignited, leaving a moss-green residue of slightly reduced oxide. This is dissolved in a little strong nitric acid, which is cautiously evaporated; the final residue is ignited over a Bunsen burner, leaving U_3O_8, which is weighed. Arsenite is not readily oxidised to arsenate by nitric acid, but dropwise addition of potassium bromate solution at 70° C. effects rapid oxidation. For the simultaneous determination of arsenite and arsenate, the arsenite in the solution is first titrated with bromate, after which the total arsenate is determined by precipitation with uranyl acetate, arsenate being computed by difference. Organic arsenicals may be oxidised by the Carius method, but some decompose explosively in contact with fuming nitric acid. A safe and efficient method consists in introducing 0·1 to 0·3 g. of the substance into a Kjeldahl flask containing a cold mixture of 20 ml. of strong sulphuric acid, 10 g. of potassium nitrate and 0·25 g. of pure starch. After gentle warming the heat is gradually increased, and the acid is boiled until all of the organic matter has been oxidised. After cooling, 40 ml. of cold water are cautiously added, followed by bromate solution until the liquid is coloured by bromine. The solution is neutralised with strong ammonia, filtered, slightly acidified with acetic acid and treated with uranyl acetate solution, and the determination is completed as before.

[It would seem that incorporation of filter-pulp with the uranyl ammonium arsenate precipitate would expedite filtration, washing, and the expulsion of arsenic by ignition.—ABSTRACTOR.] W. R. S.

Determination of Chromium in Chrome-tanned Leather. R. M. Lollar. (*J. Amer. Leather Chem. Assoc.*, **1940, 35**, 443–452.)—In the method described below, the ash is treated with perchloric acid, and the interference of ferric iron in the iodimetric titration of chromate is prevented by addition of phosphoric acid. The weighed sample (2 g.) is ignited in a nickel crucible, finally in a muffle. The ash is transferred to a conical 125-ml. flask, and gently boiled with perchloric acid of constant b.p. (about 71 per cent.) until the colour of the solution changes from green to orange. This may take from a few minutes to several hours. The acid solution, while still hot, is cautiously diluted with water and poured into a 400-ml. beaker, and the flask is rinsed with water (total volume, 150 to 200 ml.). The liquid is boiled for a short time to expel any chlorine, left to cool completely, and acidified with 25 to 30 ml. of phosphoric acid (1 : 1). After addition of 10 ml. of 10 per cent. sodium iodide solution the liquid is set aside for one minute out of the sunlight, and titrated with 0·1 *N* thiosulphate solution, starch being added towards the end. A rapid dilution of the hot perchloric acid is found necessary to prevent risk of formation

of hydrogen peroxide, which would reduce chromic acid. Potassium iodide in phosphoric acid solution gives a precipitate which masks the end-point; hence the sodium salt is used. The optimum concentration of phosphoric acid for the titration is 3 *N*; at higher concentrations ferric iron reacts upon the iodide, whilst insufficient phosphoric acid causes too slow a reduction of chromic acid by the iodide.

W. R. S.

Colour Test for Elemental Sulphur. H. Sommer. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 368–369.)—The material is extracted with hot pyridine, and the solution is filtered if necessary. One-tenth volume of 2 *N* sodium hydroxide solution is added and after brief shaking at room temperature the colour of the pyridine solution is noted at once. A light sky-blue colour is produced with 1 part of sulphur in 100,000 of pyridine; the colour is greenish-blue with 1 part in 10,000, dark olive-green with 1 part per 1000 and deep red-brown with a 1 per cent. solution. A range of blue colours is given by the use of saturated sodium bicarbonate solution instead of sodium hydroxide, when the mixture is boiled for a few seconds. The colour is unstable. Both crystalline and amorphous sulphur respond to the test. No colour is given by sodium sulphide or sodium thiosulphate. Elemental selenium or phosphorus produces no colour. Pyridine may be replaced by aniline or triethanolamine, but the sensitiveness is lower. Carbon disulphide inhibits the reaction and halogenated hydrocarbons, such as chloroform, produce purple colours with alkali-pyridine mixtures. The blue colour is probably due to colloidal sulphur. References to similar reactions are found in the earlier literature. S. G. C.

Cerate Oxidimetry. Stability of Solutions. G. F. Smith and C. A. Getz. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 339–340.)—Quadrivalent cerium in nitric and perchloric acid solutions provides oxidation potentials of 1·6 and 1·7 volt respectively, whereas in molar sulphuric acid solution the potential is 1·44 volt. At the higher potentials the field of usefulness of the reagent may be extended, provided that the stability of the solution is not unduly sacrificed. Eighteen solutions of the nitrato and perchlorato cerate ions were prepared and examined. Nitric and perchloric acid solutions of hexanitrato ammonium cerate in nitric and perchloric acids between 1 and 3 *M* were the most promising. A 0·1 *N* solution of commercial hexanitrato ammonium cerate in 1 *N* perchloric acid was sufficiently stable in ordinary light not to require re-standardisation for a period of 20 days. The stability of all solutions improved on keeping, as the accumulation of cerous ions lowered the oxidation potential.

S. G. C.

Potentiometric Determination of Hydroxylamine and Hydrazine in Alkaline Solution. H. T. S. Britton and M. Königstein. (*J. Chem. Soc.*, 1940, 673.)—The reaction of hydroxylamine and of hydrazine with both Fehling's solution and ammoniacal copper sulphate solution can be followed potentiometrically by using a system of the following type:

− Pt	100 ml. Fehling's solution + 0·1 *M* NH_2OH. HCl (or 0·1 *M* $N_2H_4.H_2SO_4$	Satd. KCl	*N* KCl	*N* KCl + HgCl	+ Hg

The complete reduction of the bivalent copper is indicated by a sudden reduction of potential. To prevent diffusion, the ends of the saturated potassium chloride bridge were plugged with asbestos and one end was immersed in a vessel containing *N* potassium chloride solution, in which was inserted also the tube leading from the normal calomel electrode. The titration vessel was fitted with an ebonite cap containing holes for two platinum electrodes, a thermometer, a tube leading from the burette, the end of the salt bridge, a mechanical stirrer, and a tube for the introduction of nitrogen. The whole cell was immersed in a water-bath kept at such a temperature that the solution in the titration vessel was at 90° to 92° C. Sodium tartrate was used instead of Rochelle salt in the Fehling's solution. When ammoniacal copper sulphate was used, ammonia was passed through the solution instead of nitrogen (*cf.* Britton and Phillips, ANALYST, 1940, **18**, 149).

E. M. P.

Detection of Thiocyanate, Iodide, Bromide and Chloride. D. Hart and R. Meyrowitz. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 318–320.)—Tests on a separate portion of the solution are made for each anion, without the use of silver as a group reagent. Provision is made against the presence of the following ions: ferricyanide, ferrocyanide, cyanide, sulphide, thiosulphate, arsenite and tartrate. *Thiocyanate.*—Large amounts of iodide interfere with the detection of thiocyanate by means of ferric iron. Addition of lead nitrate to the solution acidified with nitric acid precipitates much of the iodide; ferricyanide is precipitated on addition of cobalt nitrate; thiocyanate is tested for in the filtrate by means of ferric iron. *Iodide* is detected by shaking the solution with carbon tetrachloride and hydrogen peroxide after removal of interfering substances as follows:—Ferricyanide, ferrocyanide, sulphide and cyanide are precipitated from the solution, slightly acid with sulphuric acid, by the addition of cobalt acetate (in absence of the first two ions, cyanide and sulphide may be removed by simply boiling the acidified solution); arsenite is precipitated with hydrogen sulphide; thiocyanate is destroyed by boiling for 30 seconds in 6 *N* sulphuric acid. *Bromide* is identified by shaking the solution with successive portions of carbon tetrachloride and a little potassium permanganate until the aqueous layer remains pink; a yellow or brown colour in the carbon tetrachloride layer indicates bromide. Interfering ions are first

removed. Thiosulphate is decomposed by boiling the acidified solution; mercuric acetate is added, followed by cobalt acetate to precipitate ferricyanide; any tartrate is precipitated with lead acetate. The precipitate is filtered off, mercury in the filtrate is precipitated with hydrogen sulphide and the liquid is filtered again. Thiocyanate is decomposed as in the test for iodide, and iodide is removed by boiling with sodium nitrite in dilute sulphuric acid solution. *Chloride.*—Ferricyanide, ferrocyanide, cyanide and sulphide are removed as indicated above. Iodide, bromide, thiocyanate and thiosulphate are removed by steam distilling the solution containing 30 per cent. of conc. nitric acid. A small amount of bromide remains, but this is removed by shaking the solution with carbon tetrachloride and potassium permanganate; the colour of the excess permanganate is subsequently destroyed with sodium nitrite, and any colloidal sulphur remaining from the decomposition of thiosulphate is removed by shaking with black mercuric sulphide and filtering the liquid. Chloride is detected with silver nitrate.

S. G. C.

Microchemical

Micro-gravimetric Determination of Active Hydrogen by the Grignard Reagent. R. N. Evans, J. E. Davenport and A. J. Revukas. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 301.)—A micro-gravimetric Zerewitinoff method is described in which the evolved methane is burned to carbon dioxide and water, which are absorbed in microchemical absorption tubes and weighed. The method is applied to the analysis of impregnated paper insulating tapes. The second hydrogen in the water molecule is active toward the Grignard reagent. Preliminary tests with the method show that theoretical results may be obtained for the active hydrogen content of typical organic compounds at 25° C. in absence of certain types of peroxides. The apparatus is the same as that employed by the authors in the determination of water in impregnated paper insulating tapes (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 553) with slight variations, in that the reagent is introduced into the reaction chamber through a serum rubber stopper, commonly employed in medical laboratories, by means of a syringe fitted with a stainless steel needle, and that two reaction cells in series are used, so that the reagent siphons on to the sample without air contamination. The rate of gas flow is approximately 1 litre per hour for the combined gases at the exit end of the train. J. W. M.

Micro-determination of Potassium. I. A. Kaye. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 310–311.)—A variation of the cobaltinitrite method, using ceric sulphate instead of potassium permanganate in the titration, is applied to the determination of amounts of potassium ranging from 0·0200 to 0·1200 mg. About 0·5 ml. of the test solution is placed in a 15-ml. centrifuge tube, 0·5 ml. of sodium cobaltinitrite reagent (Kramer and Tisdall, *J. Biol. Chem.*, 1921, **46**, 339) is added dropwise with shaking, and the contents of the tube are left for 1 hour at 20°–25° C. The precipitate is centrifuged for 10 minutes at about 2000 r.p.m., the supernatent liquid is carefully removed and 5 ml. of water are added so as not to disturb the precipitate. Centrifuging and washing are carried out 3 times in all.

Reagent.—About 4·5 g. of anhydrous ceric sulphate are dissolved in 500 ml. of water to which 100 ml. of conc. sulphuric acid have been added. The solution is diluted to 1 litre and standardised with standard sodium thiosulphate. One ml. of this reagent is added for amounts of potassium less than 0·06 mg., the tube is heated in a water-bath for 2 or 3 minutes, until the precipitate dissolves. The solution is cooled to room temperature, 1 drop of 1 per cent. potassium iodide solution is added, and the liberated iodine is titrated with standard (0·002 *N*) sodium thiosulphate, a few drops of 0·2 per cent. starch solution being added near the end-point. At the same time 1 ml. of the ceric sulphate solution is titrated with the sodium thiosulphate. J. W. M.

Identification of Perchlorate, Persulphate and certain other Inorganic Acid Radicals with Zwikker's Reagent. A Sensitive Reaction for Copper. G. H. Wagenaar. (*Pharm. Weekblad*, 1940, **77**, 465–468.)—Zwikker's reagent (a mixture of 4 ml. of 10 per cent. copper sulphate solution, 1 ml. of pyridine and 5 ml. of water) will detect barbituric acid derivatives, saccharin and organic acids (*cf. id.*, 1931, **68**, 975; 1932, **69**, 1186; and ANALYST, 1934, **59**, 850). In tests with 31 acid radicals amorphous precipitates were obtained with iodides, cyanides, thiocyanates and molybdates. Perchlorates gave light purple to blue, square and hexagonal crystals, which coalesced as they grew (sensitiveness, 1:100); scratching was unnecessary, and chlorates did not interfere. The crystals from persulphates were blue, very regular right-angled prisms, which coalesced into rectangular groups if the solution was strong (sensitiveness, 1:1000); some small black squares were also observed; scratching helped in this instance. Thiosulphates (1:1500) formed groups of long radiating needles, which were unaffected by the presence of sulphites. Chromates (1:300) crystallised in yellow X-shaped groups of rectangular plates, and there were also some isolated narrow rectangles which usually had a rectangular indentation in each of the narrow sides, and sometimes in all sides, giving an H-shaped appearance. Dichromates gave amorphous or microcrystalline precipitates. The shape of the crystals obtained from permanganates was dependent on the concentration of the solution; they were aggregates either of square plates or of dark needles (1:3000). This last reaction also affords a sensitive test for copper, 1 drop each of pyridine water (1:10) and *N* potassium permanganate solution being added to the solution

under examination, when plates of irregular shape or small needles are formed (sensitivity, 1:25,000 of Cu^{++}). Certain organic substances also react, *e.g.* when a small crystal of sodium sozoiodolate is added to a drop of the reagent a brown precipitate, which forms star-shaped groups of fine crystals, is produced. Illustrations are given of the various forms of crystals. J. G.

Micro-titration of Selenium. G. Wernimont and F. J. Hopkinson. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 308.)—A simple and accurate volumetric method, using the dead-stop end-point electrometric titration (Pring and Spencer, ANALYST, 1930, **55**, 375; *et al.*) is applied to the determination of minute amounts of selenium, *e.g.* in urine. The precision is claimed to be $\pm 2\gamma$ in the range 2 to 150γ on a basis of a 100-ml. sample; the method is sensitive to 0·02 p.p.m. *Detail.*—To 100-ml. samples of urine (containing abnormal amounts of selenium) 25 ml. of conc. sulphuric acid are added, in 300-ml. Kjehldahl flasks, together with 0·7 g. of mercuric oxide and several glass beads. The flasks are heated until the water has evaporated and the organic matter has been destroyed (about 3 hours). The clear solutions are transferred to 300-ml. flat-bottomed flasks, 60 ml. of water being used for rinsing. Into each flask are introduced 25 ml. of conc. sulphuric acid, 50 ml. of 48 per cent. hydrobromic acid containing 1 per cent. (by vol.) of liquid bromine, and the ground joint is moistened with conc. sulphuric acid. The flasks are then attached to small Liebig condensers and the mixtures are distilled until 35 to 40 ml. of distillate have been collected. The distillate is treated with 5 per cent. sodium sulphite solution and then with 5 per cent. phenol solution to destroy the bromine. Standard (0·001 *N*) sodium thiosulphate solution is then added to give an excess of 2 to 3 ml. After addition of 1 ml. of freshly prepared 5 per cent. potassium iodide solution, the excess of sodium thiosulphate is immediately titrated with 0·001 *N* potassium iodate solution, the dead-stop method being used to determine the end-point. J. W. M.

Physical Methods, Apparatus, etc.

Machine for Testing the Fastness to Rubbing of Dyed Materials. D. A. Derrett-Smith and H. B. Bradley. (*J. Soc. Dyers and Colourists*, 1940, **56**, 261–265.)—The fastness to rubbing of a dyed material is defined as the extent to which it gives rise to a coloured mark when rubbed against a white material, and it is best determined by means of a machine which rubs under standardised conditions. Existing methods (*cf.* German Fastness Commission, "*Verfahren, Normen und Typen*," Verlag Chemie, Berlin, 1935; Forster, Ramachandran and Venkatamaran, *J. Soc. Dyers and Colourists*, 1938, **54**, 216) are discussed, and it is concluded that there is a need for a simple machine which produces a rubbing effect as near as possible to that produced by hand tests, under standard conditions and in a comparatively short time. In the machine now described a strip of plain weave bleached dress linen is held between a fixed and a detachable clamp over a flat aluminium platform, the detachable clamp being connected with the overhanging end of the cloth which is kept taut by means of a weight attached to this clamp; the weight and clamp together weigh 820 g. The dyed material to be tested is wrapped round a hard beech-wood block which is attached to a light metal carriage by means of screws and wing-nuts. The block also carries a detachable weight which ensures close contact between it and a small area of the dyed material. A heart-shaped cam driven by a geared electric motor and attached to the block-carriage by means of connecting rods, causes the block carrying the dyed material to travel 5·5 inches backwards and forwards along the bleached cloth, at the rate of 84 rubs per minute. Numerous tests on materials dyed with azo-colours are described, these being chosen because one of the factors governing their fastness is believed to be substantivity of the particular Brenthol used; the lower the latter, the greater the proportion of dye held mechanically in the final dyeing (*cf.* Blackshaw, *id.*, 1936, **52**, 135). It was established that with dyeings of relatively high fastness it is best to compare the development of the coloured mark on the bleached material after a definite number of rubs (*e.g.* 500) with each of the dyed materials to be tested. When the fastness is low (*e.g.* when a mark is produced after 50 rubs) the test is carried out in 3 stages, each of 20 rubs, the bleached cloth being moved along in the clamps in such a way that a separate record of the effect of each stage is obtained on the same sample; in this way it is possible to ascertain whether the dye is being rubbed off on to the bleached material at a uniform rate, or otherwise. Alternatively, a particular dyeing may be taken as a standard, measurements being made of the number of rubs necessary to produce the same depth of marking as is obtained from a given number of rubs on the standard. Actual numerical measurements of the coloured marks may be made with the Lovibond Tintometer. Damping enhanced the severity of the test, more colour being rubbed off. Yarn dyeings from Brenthol-BN were found to be faster than those from Brenthol-AS or -OT, which were similar in this respect; with piece dyeings a decreasing order of fastness was obtained from Brenthol-BN, -CT and -AS. Vat dyeings on linen were comparatively fast when dry, 200 rubs giving no discernible mark, although yarn dyed with indigo gave a faint mark after 100 rubs.
J. G.

Luximeter: Construction and Application. W. L. Carson. (*Gen. Elec. Rev.*, 1940, **43**, 91–92; *J. Text. Inst.*, 1940, **31**, A 341.)—The

Luximeter is a simple photo-electric instrument for the measurement of the transmission of light through liquids. It was designed originally for use in the phosphatase test for the pasteurisation of milk, but has many other applications in chemical analysis and industrial process control. Light from a 6- to 8-volt Mazda lamp, which can be maintained at a constant intensity, passes through a test-tube containing the sample to the photo-electric cell, which actuates a sensitive indicating instrument. The intensity of light required for a given test varies according to the opacity of the sample, and is controlled by means of a resistance in the lamp circuit. Readings taken on a light meter, which is similar to the common exposure-meter except that it is divided arbitrarily into 100 divisions, are plotted against the concentrations for solutions of known strength, and this curve is used to obtain the concentrations of unknown solutions from the corresponding readings on the light meter.

J. G.

Optical Method for the Determination of Quartz Particles in Felspar. G. H. McIntyre and M. Bozain. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 326–328.)—A 25-g. sample of the finely powdered felspar is mixed with sufficient gum arabic solution to make a stiff paste. This is dried in a mould and the cake is subsequently fused at a temperature gradually rising to 2500° F. to yield a translucent glass, in which the quartz particles remain suspended. The cooled button is pulverised to pass a 200-mesh sieve. A small sample is mixed with a drop of glycerin and pressed between glass microscope slides, and the number of quartz particles per unit area of field is counted in polarised light. Standards are prepared by a similar technique from felspar of low quartz-content intimately incorporated by grinding with known proportions of quartz. A curve is plotted of the number of particles per unit area of field in relation to the percentage of free quartz, and from this the proportion present in the test sample may be determined.

S. G. C.

Reviews

CHEMISTRY IN THE SERVICE OF MAN. By ALEXANDER FINDLAY. Fifth Edition. Pp. xx + 398. London, New York, Toronto: Longmans, Green & Co., Ltd. 1939. Price 8s. 6d. net.

This book, in its fifth edition and its thirty-fourth year, seems to me to exercise the same fascination as I recall from the now so remote-seeming past when, as a schoolboy, I first devoured the contents of its revealing pages. Then, as to-day, if less consciously, I was carried away by the author's enthusiasm for his subject, pride in his colleagues and patience with his readers. A perusal of the third edition, nearly ten years later, impressed one even more with the author's courage. Writing a book of this kind, intended primarily for the non-practitioner, if not for the layman, is rather like teaching an illiterate to read. There are two possible lines of approach. He can gradually be trained up to a point where he is familiar with all the words of one syllable, after having been taught his alphabet, by appropriate use of simple pictures and monosyllables. But there his education will end, for he has no grammar, no understanding of the structure of sentences and no possibility of understanding the subtler uses of language. The other method is to teach literacy itself, with all that this implies about knowledge of words and sentences and the logic of the written medium. Professor Findlay's method is analogous with the second, and in this respect differs from that of less courageous or less energetic writers. He does not hesitate to take his non-chemical reader—and to take him at the very beginning of the book—into discussions on radioactivity and atomic structure. By the end of the third chapter the phlogiston theory has been met and disposed of. And so it goes on, the reader being led rapidly, but with logic and clarity, through the wide ramifications of modern chemical science, with head in the exciting empyrean of theory, feet on the solid earth of practice.

Professor Findlay of all men clearly believes that accurate statement of facts justifies any amount of careful explanation, for it is the groundwork of the reader's understanding that he is attempting to build, and this itself must never be liable to disturbance through the existence and subsequent detection of faulty units of construction. He will, therefore, doubtless forgive a reviewer for calling attention to one or two minutiae. Modern commercial casein (p. 367) is certainly never

prepared by solution in ammonia and subsequent precipitation, but by careful control of the "starter" (in the self-souring process) or of the *p*H produced by the addition of hydrochloric acid (in the grain-curd process). It would have been well to point out that rennet casein differs chemically, as well as physically, from the acid casein used in the paper-making and paint industries. The food use of casein is so trivial that it is hardly worth mentioning.

Again, it is not correct to say, nor was it at the time Professor Findlay passed the proofs, that vitamin B_1 "is not yet an industrial product." Synthetic aneurin has been on the market for at least two years, in this country as well as in Switzerland, Germany and the United States. Finally, synthetic ascorbic acid, being made from optically active sorbose, is not racemic, but identical with the natural product.

But these are, as I have admitted, *trivia*, and only mentioned in the interests of the accuracy that Professor Findlay so clearly holds dear. The book remains what it has always been—a masterpiece of lucid and enthralling exposition. Every chemist should see to it that his children have this book. There is no doubt whatever that they will read it, and little less doubt that their father would be well advised to read it also, so that he may meet the cross-examination that is sure to follow.

A. L. BACHARACH

COLLOID CHEMISTRY (A TEXTBOOK). By H. B. WEISER. Pp. viii + 428. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. 1939. Price 24s. net.

Students of elementary colloid science are now well provided with textbooks from the pens of the recognised masters, and surely with the appearance of Professor Weiser's volume the bookshelf is complete. If one admits that there was room for another introductory text, then praise must be accorded to this most recent effort.

To quote the Preface: "The plan of the book is as follows: After an introductory chapter, which is concerned with the general aspects of the colloidal state of matter, appears a section of seven chapters dealing with the phenomena of adsorption at various types of interfaces. Following this fundamental section on adsorption, ten chapters are devoted to the formation and properties of lyophobic and lyophilic sols. This discussion is followed in turn by shorter sections on gels, emulsions and foams, and aerosols and solid sols. The three concluding chapters deal in a more comprehensive way with the application of colloid chemical principles to contact catalysis, dyeing and clay. An attempt has been made to render the presentation as clear and concise as possible by outlining the subject matter with frequent section and paragraph headings."

When the author, himself a notable investigator, tells us that various sections have been approved by Abramson, Bancroft, Briggs, Bradfield, Bartell, Bull, Foulk, France, Garrison, Milligan, Harkins, Holmes, McBain, Miller, and Williams, a challenge faces any reviewer. These names spell authority. Deeper interest is added to the usual pleasurable reading of such volumes.

The present reviewer unreservedly recommends this textbook as a very good introduction to colloid chemistry, written in agreeable style. Modern views are well treated on the whole, although space has not permitted detailed discussion on certain points deserving of fuller treatment, such as emulsions, hydrophilic sols, and, in particular, colloidal electrolytes. Adsorption phenomena are well treated in 113 pages, whilst sols occupy 149 pages.

Printing and binding leave nothing to be desired.

WILLIAM CLAYTON

INTRODUCTORY COLLEGE CHEMISTRY. NEIL GORDON and WILLIAM E. TROUT, JR. Second Edition. Pp. ix + 753. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. 1940. Price 21s.

Gordon and Trout's introductory textbook, now in its second edition, opens in a very elementary way with the chemistry of water and leads on, as far as possible by experiment, through the chemistry of the usual non-metallic elements (oxygen, hydrogen and nitrogen). On the way such subjects as molecular and atomic weights are dealt with. The electrical theory of matter is introduced at a very early stage and is used freely to explain various facts of chemistry. Theories of ionisation, including that of Brönsted, are discussed in considerable detail. After sections on sulphur and the halogens the Periodic Classification is treated fully and explained in the light of modern ideas of atomic structure. An account of carbon, phosphorus, the metalloids and the colloidal state completes the first half of the book.

In dealing with the metals the periodic classification is, surprisingly enough, discarded and the subject is treated from the point of view of the groupings in the common group analysis tables. This unorthodox approach is justified on the grounds that it permits of an experimental treatment. The book closes with a brief account of certain periodic groups, including those of the rare earths and the inert gases.

One of the most striking features is the apparent chaotic medley of methods used for presenting the facts. A closer examination, however, reveals very sound reasons for this. Introductory College Chemistry is what its title suggests—an introductory textbook—and the authors have tried to introduce the subject experimentally, wherever possible, but, where the nature of the subject demands it, have adopted the usual didactic method of statement. Thus, interposed between experiments, which must be performed by the student, unfinished equations, blank tables and unanswered questions, one finds very good accounts of such subjects as the law of mass action, ionisation, and atomic numbers. The book is naturally very elementary but, if rightly used, should form a satisfactory introductory course. It is well bound, free from errors, and altogether very attractive.

HAROLD TOMS

KINGZETT'S CHEMICAL ENCYCLOPAEDIA. Revised and Edited by RALPH K. STRONG, Ph.D., with a Foreword by Sir GILBERT T. MORGAN, O.B.E., F.R.S., D.Sc., LL.D. Sixth Edition. Pp. x + 1088. London: Baillière, Tindall & Cox. 1940. Price 45s.

"At a time of national emergency a chemical encyclopaedia becomes more than ever essential, for it gives a concise introduction to the most fundamental of all industries, the chemical trades and manufactures which subserve the community's needs for food and clothing, shelter and defence." So wrote the late Sir Gilbert Morgan in his foreword to this edition of *"Kingzett's Chemical Encyclopaedia."* This is surely sufficient justification for the publication of such a book at one of the most critical times in our national history, when, possibly for the first time, innumerable non-technical administrators find themselves compelled to wrestle with the problems of a highly technical science and its applications to various industries. For them, as well as for chemists and others engaged in the chemical industry, this encyclopaedia will furnish most of the general information they are likely to need. The references appended to many of the sections will be found useful when more detailed information is required.

Unfortunately, Sir Gilbert Morgan, who had undertaken the task of revising the Encyclopaedia at Mr. Kingzett's request, found himself unable to do so owing to ill-health, and the revision was undertaken by Dr. R. K. Strong. Sir Gilbert died before the book was published, but he was able to assist Dr. Strong by reading the manuscript and proofs.

In essence, the new edition is identical with the original work, and its aim is still that laid down sixteen years ago by Kingzett, ". . . to prepare an epitomised digest of chemistry and its industrial applications, in a form which should be useful as a work of reference by all classes of the community." Probably no other one-volume encyclopaedia has achieved this so successfully as the book under review, for it covers every conceivable aspect of chemical theory and practice and the applications of chemistry in industry and commerce. Even the economic aspect is dealt with in this new edition, although it is doubtful how far statistics, already four years old, will be of permanent value.

He would be a bold reviewer—and an omniscient one—who would dare to pass judgment on this book *in toto*, and the present reviewer has been content to judge the book by its references to that section of chemistry with which he is most familiar. For the rest, he can only express his unbounded admiration for the enormous mass of data collected within the confines of its covers.

A few errors are to be expected in a work dealing with such a vast and widely-dispersed literature, and due allowance must be made if the accounts do not always include the results of the latest researches. Nevertheless, it is a little surprising to find under the heading "Sterols," that "There are many isomers, it is supposed, of cholesterol, but the available data respecting them, although of biological importance, is very vague"; such a statement was doubtless true in **1933**, which is the last year referred to in the bibliography, but surely not in **1940**. Similarly, the subject "Hormones" is most inadequately treated, the latest reference being to a paper published in **1931**! Under the heading "Ergot," ergometrine, the most important alkaloid isolated, is not mentioned at all, yet it was isolated in **1934**. On the other hand, some of the sections, such as those on "Vitamins" and "Legal Matters," are up to date and contain quite modern references. Some of the descriptions also suffer from a certain lack of precision. Thus, many would regard the phrase "used in medicine" as hardly a sufficiently precise description of the uses to which such substances as codeine and methyl salicylate are put. Similarly, the regular use of empirical formulae, where structural formulae might have been included without much trouble, is to be deplored.

The following are some of the more important errors noted. On p. **10**, sulphanilic acid is stated to be the **1:3** isomer. "Corpus luteum" is described as a hormone on p. **259**. "Oestrone" does not appear at all, but is described under the older designation "Theelin," which is no longer used. "Lachrymator" is spelt "lacrimator." Finally, it is stated under "Anaesthetics," that the first person to use ether for this purpose was William Morton in **1846**, whereas the discovery is usually attributed to Crawford Long, who used it in **1842**.

There are also certain rather surprising omissions. Thus, under "Hydrogen Ion," a comprehensive description is given of the use of the hydrogen electrode, but the quinhydrone and glass electrodes are mentioned only in the bibliography. Again, sulphanilamide is included, but not prontosil, or any of the other members of the sulphonamide group which are nowadays of such great importance.

It will be realised, of course, that the subject matter of these criticisms is a mere fraction of the mass of sound information which constitutes the bulk of the book. The true test of the usefulness of such an encyclopaedia as this is the value which all chemists in every branch of the science place upon it, and there can be no doubt at all about the warm reception that this new edition will receive.

F. A. ROBINSON