THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held at 5.30 p.m. on Wednesday, April 3rd, 1940, at the Chemical Society's Rooms, Burlington House, the President, Dr. E. B. Hughes, in the chair.

Nominations were read in favour of Sir William Willcox, K.C.I.E., C.B., C.M.G., M.D., F.I.C., proposed by the Council for Honorary Membership, and the following applicants for Ordinary Membership:—J. A. Freeman, B.Pharm., B.Sc., F.I.C., Ph.C., M.P.S., and J. T. Stock, B.Sc.

The following papers were read and discussed:—"Hair Dyes. Part II. The Functions and Reactions of Phenols," illustrated by a cine-film in colour showing the process of hair-dyeing, by H. E. Cox, D.Sc., Ph.D., F.I.C., and "The Determination of Aluminium, Magnesium or Beryllium in Nickel Alloys," by R. C. Chirnside, F.I.C., L. A. Dauncey, B.Sc., and P. M. C. Proffitt.

THE SAUSAGES (MAXIMUM PRICES) ORDER, 1940
STATUTORY RULES AND ORDERS, 1940. No. 394

The Council has adopted the following recommendation of the Analytical Methods Committee of the Society.

For the purposes of the above Order "Meat Content" should be determined as follows:—

> The process consists in the chemical determination of water, protein (nitrogen × 6·25), fat and ash. The calculation of the meat content is made on the lines advocated by Stubbs and More (ANALYST, 1919, **44**, 125), with the exception that the factors for converting meat nitrogen into de-fatted meat should be 100/3·4 for beef or mutton, 100/3·6 for pork.

Detailed methods of analysis are given in the above-mentioned paper of Stubbs and More. An alternative method for the determination of fat may be employed, comprising digestion of 2 to 3 grams of the sample with about 20 millilitres of hydrochloric acid (1 volume of strong hydrochloric acid diluted with from 1 to 2 volumes of water) at a temperature just below boiling-point until the meat fibre is disintegrated and all the fat is liberated, with subsequent extraction of the fat with ether.

Obituary

SIR WILLIAM JACKSON POPE, K.B.E., F.R.S.

THE death of Sir William Jackson Pope, at the age of 69, inflicted a loss felt far beyond the bounds of his own country, for he had won a great position in the world of chemistry.

He was an outstanding product of the system of technical education established in this country in the latter part of the last century; technical colleges provided his chemical training, they gave him his earlier teaching appointments, and in them he laid the foundations of his great scientific reputation.

He was born in North London in 1870, the eldest of eight children. His parents were William Pope, a native of Biggleswade, and Alice Hall, of Prudhoe, Northumberland. They were married in 1869.

One of the earliest students at the Finsbury Technical College, he came at once under the stimulating influence of H. E. Armstrong, and when Armstrong was made Professor of Chemistry at the Central Technical College, Pope went with him and later became his assistant. A strong attachment existed between the two men; Armstrong followed with unbounded pride the career of his most distinguished pupil, and Pope retained an almost filial regard for his one-time teacher.

In 1897, at the age of 27, he received his first appointment, the Headship of the Chemical Department of the Goldsmiths' Company's Institute at New Cross—he was afterwards to become Prime Warden of the Company—and, four years later, left it to become Professor of Chemistry and Head of the Chemical Department at the new Manchester Municipal School of Technology, where he remained for seven years.

His election to the Professorship of Chemistry at Cambridge took place in 1908, on the retirement of Professor G. D. Liveing, and in the following year he was elected to a professorial fellowship at Sidney Sussex College.

During the world war he served as a member of the panel of consultants of Lord Fisher's Board of Invention and Research, and his energies were directed unsparingly towards finding solutions of the chemical problems with which the country was then faced. The strong practical sense with which his great knowledge was combined made his help particularly effective and, in connection especially with the sources of high explosives, aerial photography, and retaliation to the German use of poison gas, he rendered assistance of the highest value. His services received recognition in the K.B.E. conferred on him in 1919.

After the war Pope became more and more involved in the administration of chemical affairs, a sphere in which his keen and subtle mind inevitably brought him to the front. In 1918, in his presidential address to the Chemical Society, he had advocated the formation of a body to co-ordinate the activities of the societies representing the different chemical interests in this country. Largely through his efforts the body thus foreshadowed was soon afterwards brought into being as the Federal Council for Pure and Applied Chemistry, and he was its first chairman. At the same time he was engaged, with some of the more prominent

chemists of the allied countries, in bringing about the formation of the *Union Internationale de Chimie*, designed to co-ordinate in a somewhat similar fashion the chemical societies of different countries. From 1922 to 1925 he served as president of this organisation, and in June, 1923, the Fourth International Chemical Conference, attended by a distinguished gathering of British and foreign chemists, met in Cambridge under his leadership.

Another mark of the esteem in which he was held by his Continental colleagues was his selection to preside—during the period 1922 to 1936—over the Chemical Conferences held in Brussels under the Solvay Foundation. At these assemblies of the foremost authorities on the subject to be debated his linguistic powers were much in evidence, for during the discussions he had frequently to act as interpreter, translating German contributions into French or French into German, and he made admirable after-dinner speeches in French at the banquets with which the conferences concluded. In appreciation of his services he was, in 1937, created Grand Officier de l'Ordre de Léopold. Many medals, including the Davy Medal of the Royal Society, were awarded him, he received many honorary degrees, and he was made a foreign member of learned societies of many lands.

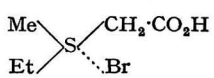
His first original scientific communication was a joint paper with Armstrong, in which it was shown that pinene could be characterised through its oxidation-product, sobrerol. In this, his talent for crystallography was manifested. The crystallographic characters of dextro-, laevo- and racemic sobrerol were described, and the terpene fraction of American turpentine was shown to consist essentially of a mixture of *d*-pinene with a smaller and variable proportion of *l*-pinene.

Pope had studied crystallography under H. A. Miers and became deeply interested in it. In all his earlier researches much of the work was devoted to securing crystallographic data, and the hours he spent in the dark room with his goniometer were probably among the happiest in his life. These crystallographic studies had an important influence on the development of his chemical work, for they enhanced that natural faculty for visualising spatial relationships which drew him inevitably into the field of stereochemistry where his greatest achievements were won.

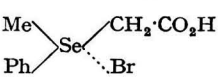
After the work on sobrerol was finished Armstrong asked him to examine the action of sulphuric acid on camphor in consequence of a communication by Marsh on this subject. Encountering experimental difficulties he sought the help of his colleague, F. S. Kipping, and thus began a scientific partnership which lasted several years and had an important effect on the future course of his work. The two investigators carried out an extensive examination of the sulphonation of camphor and its halogen derivatives, discovering the highly crystalline sulphonic derivatives of camphor and the series of halogenated camphors obtainable from them. They observed the phenomenon of *pseudo*-racemism, and among their other researches was a noteworthy investigation in which they showed that the addition of glucose or mannite to solutions of sodium chlorate (which crystallises in enantiomorphous forms) caused a remarkable disproportion between the numbers of *d*- and *l*-crystals deposited. The partnership was brought to an end by Kipping's departure to take up his professorship at Nottingham, and Pope shortly afterwards went to the Goldsmiths' Institute.

Here one of his first investigations was an attempt to resolve tetrahydropapaverine in order to get evidence of the presence of the asymmetric carbon which Goldschmiedt's papaverine formula would require. Tartaric acid, till then the only acid employed for resolving externally compensated bases, proved ineffective. He accordingly tried bromocamphorsulphonic acid, which he had got to know so well through his work with Kipping, and was immediately successful. Having got this indication of the efficacy of the camphorsulphonic acids—he attributed it to their strength and the exceptional crystallising power of most of their derivatives—he lost no time in applying them to one of the outstanding problems of the time—the resolution of asymmetrically substituted ammonium salts. Suitable material was already available in the benzyl-phenyl-allyl-methyl ammonium salts prepared, but found unresolvable by Wedekind. Pope, with Peachey, prepared the β-camphorsulphonate and, crystallising it from non-polar solvents, succeeded in effecting a resolution and obtaining the enantiomorphous optically active bromides and iodides. Thus for the first time an optically active compound owing its activity to an asymmetric atom other than carbon was prepared. This showed that the valencies of other elements besides carbon had sufficient configurational stability to give rise to observable optical activity when they were asymmetrically combined. It was a discovery which was at once recognised as one of first importance in stereochemistry, and it opened a wide field to investigation.

It was followed up by the resolution in rapid succession (with the collaboration of Peachey, Harvey and Neville) of asymmetric compounds of sulphur, selenium and tin. The sulphur compound examined was methyl ethyl thetine bromide (I), and this and the analogous methyl phenyl selenetine bromide (II)



(I)



(II)

were both resolved by means of bromo-camphor-sulphonic acid. Optically active tin was obtained by means of methyl ethyl propyl tin camphor- and bromocamphor-sulphonates.

These investigations were made at a time when the distinction between electrovalency and co-valency was imperfectly understood, and the optically active compounds of quadrivalent sulphur, selenium and tin were regarded as formally analogous to compounds of asymmetric quadrivalent carbon. It is to be noted, however, that Pope expressly pointed out that they were salts and must be held to ionise, and that in the optically active ions three radicals only were attached to the asymmetric atom.

About the time that these investigations were completed he left New Cross for Manchester. His work at the Municipal School of Technology at first followed the lines he had pursued at the Goldsmiths' Institute. The experiments on selenium and tin were continued, as well as investigations of the stereochemistry of cyclic bases, but he soon broke new ground.

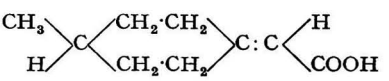
Using Grignard reagents, as for the alkyl tin halides, he prepared, with Peachey, trimethyl-platinic salts, and with Gibson, dialkyl gold halides, thereby showing for the first time that the noble metals were capable of combining with

organic radicals. With Read he began the investigation of asymmetric compounds of simple structure in order to determine what degree of molecular complexity was necessary to give rise to stable optical activity. Chloroiodomethane sulphonic acid, with only a single carbon atom in the molecule, and chloroiodo acetic acid with two, were eventually obtained in stable optically active forms, and many years later Read and McMath succeeded in finding a method for resolving chloro-bromo-methane sulphonic acid.

At this time, also, he was engaged with W. Barlow in preparing the elaborate memoirs in which the Barlow–Pope valency-volume theory of crystal structure were expounded. Although the fundamental postulate of the proportionality between valency and volume cannot now be maintained, there was much in these memoirs—as, for example, the way in which the consequences of close-packing were developed—that foreshadowed present ideas based on X-ray analysis.

The outstanding investigation initiated in the Manchester period was, however, that on methyl*cyclo*hexylidene acetic acid. Pope conceived the brilliant idea of producing a compound which should be optically active and yet contain no asymmetric atom in its molecule—at least, none in the ordinary sense of the term. The molecular type which he devised as best suited for this purpose could be


(III)

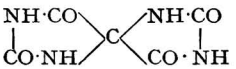

(IV)

regarded as derived from a substituted allene (III) by the expansion of an ethylenic residue into *cyclo*hexylidene, leading, with an appropriate choice of substituents, to the above-mentioned acid (IV).

For the production of this compound he secured the collaboration of W. H. Perkin, and a process for its synthesis was described in a joint paper in 1908. By a remarkable coincidence Marckwald and Meth were at the same time engaged on the synthesis of this compound for the same purpose. They prepared an acid which they believed to be methyl*cyclo*hexylidene acetic acid and resolved it into optically active components. It was, however, different from Perkin and Pope's acid. Since the latter was prepared by a process which definitely fixed its constitution, whilst Marckwald and Meth's synthesis allowed the alternative production of methyl*cyclo*hexene acetic acid, it was clear that Perkin and Pope's was the right compound, and Marckwald and Meth's the isomer with an ordinary asymmetric carbon atom. But although Perkin and Pope's process gave the right compound, it involved several difficult operations and could not easily be made to yield enough of the acid for resolution. Shortly afterwards, however, Wallach chanced upon a simpler synthesis, and the acid could then be readily obtained in quantity. The final realisation of Pope's idea through the resolution of the acid into optically active components (carried out with the assistance of Dr. John Read, now Professor of Chemistry at St. Andrews) was one of the first-fruits of his scientific work at Cambridge.

Of the results of the work of his later years, the most noteworthy were his contributions to our knowledge of the co-ordination compounds of aliphatic tri- and tetramines (with F. G. Mann) and the stereochemistry of spirocyclic compounds

(with J. B. Whitworth and S. E. Jansen). One of his last achievements, the resolution of *spiro*dihydantoin (V)



(V)

(carried out with Whitworth) provided a particularly elegant demonstration of the molecular dissymmetry of compounds of this class.

As a lecturer Pope had an unusually facile delivery, and his lectures were remarkable for their clearness. Indeed, one felt there was some danger of his making organic chemistry seem so easy that the more able students might underestimate the serious study it required. He directed his department with little apparent effort. Exerting a minimum of interference, but giving constant support and encouragement, he knew how to get the best out of his staff.

Despite an air of apparent gloom he could be a most entertaining companion, for he had a rich fund of delightful stories. They were often based on simple incidents of the laboratory or his earlier experiences in London, but he told them inimitably, and they almost invariably had an exquisitely humorous climax.

To those who did not know him well he might seem not readily approachable, but this apparent reserve concealed deep stores of kindness. His help was ever forthcoming to those who were in any way in real need of assistance, and he did many acts of kindness that were scarcely known except to himself and the recipient.

He was a gracious host, and a visit to his house was always full of interest. He had an unusually fine chemical library, and his collections of pharmaceutical jars and mortars and pestles are probably unique. He had also a fine collection of alchemical paintings and engravings. Although he was no ornithologist, the birds in his garden were a source of much interest to him, and he had the gift of winning their confidence. The sight of a robin flying to his hand to take the morsel it had learnt to expect there revealed a side of his nature that was not generally known. He was musical and had played the violin in his youth, but he had no interest in games, and the only exercise he ever took was the walk between his house and the laboratory.

He bore the trials of his long illness with astonishing fortitude, and his indomitable will-power carried him through his duties until within a few months of his death.

W. H. MILLS

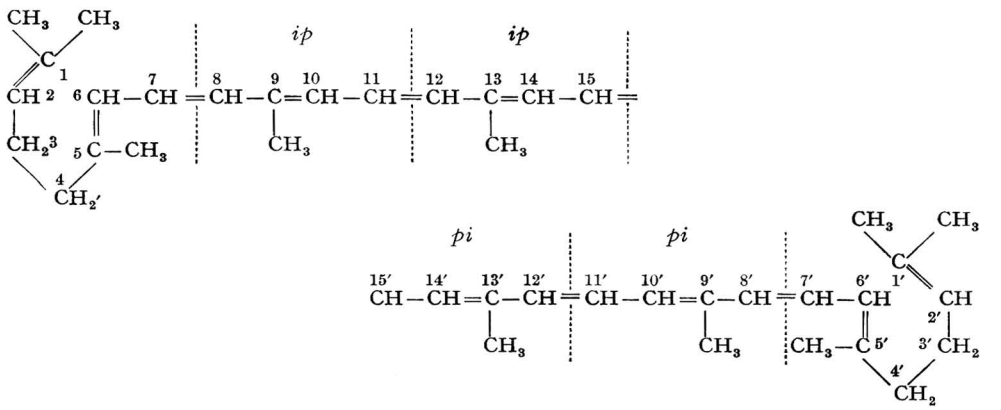
Carotene and Allied Substances in Foods and Feeding Stuffs

(Read at the Joint Meeting of the Society with the Food Group of the Society of Chemical Industry, February 7th, 1940)

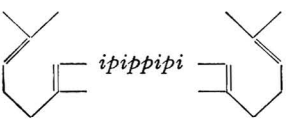
I. THE CONSTITUTION AND PHYSIOLOGICAL SIGNIFICANCE OF CAROTENE AND ALLIED PIGMENTS*

By R. A. MORTON, D.Sc., F.I.C.

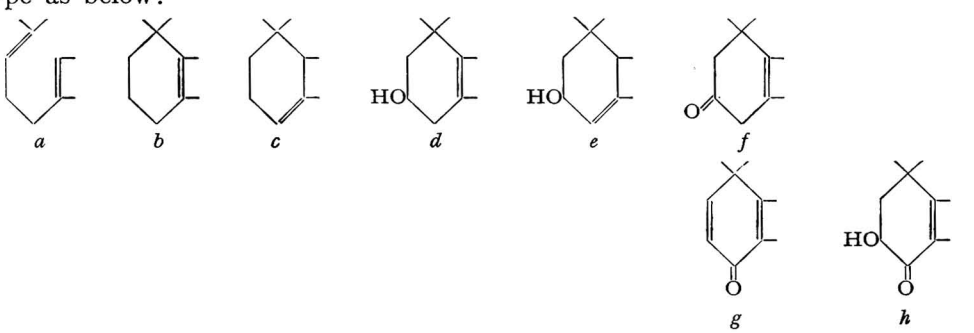
THE simplest carotenoid is lycopene, $C_{40}H_{56}$; on complete hydrogenation to perhydrolycopene, $C_{40}H_{82}$, thirteen bonds disappear. Quantitative degradation by means of ozone, permanganate and chromic acid as oxidising agents leads to the formula:—



The central polyene chain, C_8–$C_{8'}$ inclusive, is made up of four isoprene units arranged in pairs which are united in reverse order at C_{15} and $C_{15'}$, and may be abbreviated as *ipippipi* (*ip* denoting an isoprene unit). Lycopene may thus be written:



The polyene chain is common to a large number of carotenoids, but the terminal groups may consist of substituted or unsubstituted rings of the α- or β-ionone type as below:

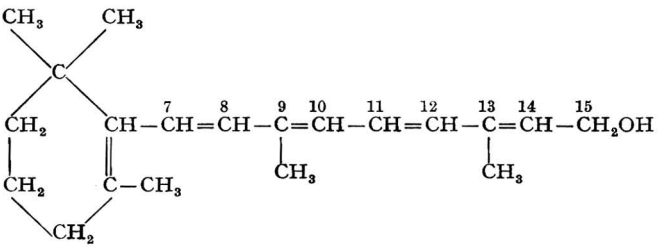


a *b* *c* *d* *e* *f* *g* *h*

* Condensed, by permission, from the summary in *Nature* (1940, **145**, 286). The paper has has been published in *Chemistry and Industry*, 1940, **59**, 301–307.

Green leaves and other vegetable products contain α- and β-carotene and "xanthophyll," a mixture of hydroxylated carotenoids, zeaxanthin and lutein predominating. Their functions in plant physiology are not yet understood, but, owing to the connection between carotene and vitamin A, the position concerning the rôle of carotenoids in the nutrition of animals is much less obscure.

Vitamin A from fish liver oils ($C_{20}H_{29}OH$) possesses a constitution which differs from one half of the symmetrical β-carotene molecule only by the addition of the elements of water:



The fact that green foodstuffs may cure avitaminosis A just as well as fish liver oils was explained when it was found that pure "carotene" undergoes fission *in vivo* with formation of vitamin A.

The only carotenoids which act as precursors or provitamins A are those that possess intact one half of the β-carotene molecule. They include those shown in the accompanying table, echinonene and a few derivatives prepared *in vitro* from natural provitamins. There is no evidence that animals can synthesise either provitamins A or vitamin A *de novo*, or that the conversion of carotene to vitamin A is reversible.

Substance	Constitution	Occurrence
Lycopene	*a-ipippipi-a*	Ripe tomatoes.
* α-Carotene	*b* ,, *c*	Red palm oil, mountain ash berries.
* β-Carotene	*b* ,, *b*	Carrots, leaves, etc.
* γ-Carotene	*b* ,, *a*	Leaves of lily-of-the-valley.
* Kryptoxanthin	*b* ,, *d*	Yellow maize.
Zeaxanthin	*d* ,, *d*	Maize, egg yolk, leaves.
Lutein	*d* ,, *e*	Grass, green leaves.
* Myxoxanthin	*b* ,, *g*	Algæ, especially blue-green algæ.
* Aphanin	*b* ,, *f*	
Rubixanthin	*d* ,, *a*	
Astaxanthin	*h* ,, *h*	Crustacea.

* Provitamins A.

The animal body contains only small quantities of carotenoids, and is not equipped to assimilate large doses. Carotene utilisation is optimal when minimal doses are fed in oil solution, and the transport of carotene through the intestinal wall is conditional on normal fat absorption. The site of the conversion of carotene to vitamin A is generally held to be the liver; certainly the liver is the main storage depot for the vitamin. In most species there is a normal level of concentration of vitamin A—and of carotenoid—in the blood. Carotenoids are also found in the pigmented layer of the eye and in yellow bone marrow.

Milk contains both vitamin A and carotenoids (largely β-carotene) and for a given species the total vitamin A activity of normal milk tends to be fairly constant, but in domesticated animals there are interesting variations with breed. Thus Holstein and Ayrshire cows yield milk with little carotene but more vitamin A,

whereas Guernseys give a cream more deeply coloured by carotene but less rich in vitamin A. The new-born possess very low liver reserves of vitamin A, and it is significant that colostrum may possess vitamin A activity one hundred times that of normal milk. Human colostrum is two or three times as potent as early milk, which in turn, is five or ten times as rich as the later milk.

The earliest sign of shortage of vitamin A or provitamin A is defective low-intensity vision. Visual purple, the photosensitive substance of the rods, may be obtained from retinas; it is a conjugated protein from which vitamin A can be separated. Faulty dark adaptation is due to delayed regeneration of visual purple, and in the majority of subjects can be remedied by supplementing the diet with vitamin A or carotene. In order to prevent night blindness in cattle, sheep, pigs, rats and horses, Guilbert finds that either some 25–30μ g./kg. body weight (1μ g. = 10^{-6} g.) of β-carotene or 6–8μ g./kg. of vitamin A is needed daily.

Vitamin A deficiency is characterised by widespread atrophy of epithelial structures and often by xerophthalmia, but retardation of growth (weight) is the criterion most readily amenable to quantitative interpretation.

The diet of most town dwellers shows inadequate vitamin A activity, especially during the winter months. It is also certain that most winter milk from stall-fed cattle is inferior to summer milk. Artificially dried grass is superior to hay both in respect of protein and provitamin A content, and its value as a feeding stuff has been established in many well-controlled experiments. The addition of vitamins A and D to margarine is a valuable way of alleviating vitamin deficiency, but the problem of utilising the available resources to the best advantage and of increasing the supply to meet the known needs has not been solved. That it is necessary and possible to do so cannot be doubted, nor that the cost of effective action would be a small fraction of the cost of inaction.

This raises the point of the relative efficiency of carotene and vitamin A. The accepted unit of vitamin A activity is that exerted by 0·6μ g. of pure β-carotene, so that the pure substance has a potency of $1{\cdot}66 \times 10^6$ I.U./g. (by definition). Vitamin A, according to the best available data, has a potency near $3{\cdot}0$–$3{\cdot}3 \times 10^6$ I.U./g., whereas all provitamins other than β-carotene have an activity near $0{\cdot}83 \times 10^6$ I.U./g. These figures apply to rats receiving minimal doses.

There is urgent need for more research on the relative efficiencies of provitamins and vitamin A at the level needed to convert a marginal diet into an optimal diet.

The commonly accepted view that the equation

$$\underset{\beta\text{-carotene}}{C_{40}H_{56}} \xrightarrow{2H_2O} 2C_{20}H_{29}OH$$

corresponds with a process occurring *in vivo* would lead to $1{\cdot}56 \times 10^6$ I.U./g. for pure vitamin A. This is not in accord with experience; it rests upon the assumption that fission occurs exclusively at C15—15′ double bond, and its only advantage is that it agrees with the superiority of β-carotene over other provitamins. Unsymmetrical fission is more plausible, and an equation

$$\underset{\beta\text{-carotene}}{C_{40}H_{56}} \rightarrow C_{20}H_{29}OH + \text{decomposition products}$$

is in closer harmony with the observed potency of vitamin A.

Vitamin A is estimated spectroscopically by utilising the absorption maximum at 325$m\mu$. There is at present no reason to justify changing the accepted conversion factor, namely:

$$E^{1\%}_{1cm.}\ 325m\mu,\ 1 = 1{,}600\ \text{I.U./g.}$$

II. THE COMMERCIAL DETERMINATION OF CAROTENE AND ALLIED PIGMENTS, WITH ESPECIAL REFERENCE TO DRIED GRASS AND OTHER LEAFY MATERIALS

By W. M. SEABER, B.Sc., F.I.C.

The work in our laboratory has been mainly concentrated upon the determination of carotene in dried grass, alfalfa and similar materials. In England, from a commercial point of view, α- and β-carotenes are the only substances of this class whose determination is usually required. In fact, it has been usual to include α-, β- and γ-carotenes as one substance under the name of "carotene." For most purposes this is convenient, and in grass and many leafy materials β-carotene is practically the only one present; the determination of total carotenoids is not often called for in commercial work. Xanthophyll is sometimes determined separately, but for approximate purposes can be taken as the difference between total carotenoids and carotene.

In this paper the main lines of the procedure adopted for the determination of the carotenes is outlined, and the process we have worked out in the laboratory of John Hughes is then described in detail. There are broad principles common to most of the classes of substances containing carotenes, and it is usually only the details of extraction that need be varied.

It was not until 1913 that serious attempts were made to distinguish between the various carotenoid pigments. In that year Monteverdi and Lubimenko reported a spectrographic method, and the well-known Willstätter and Stoll process was also put forward.[1] This consisted in extracting the tissue with acetone, saponifying the chlorophyll and removing it in the aqueous layer, and partitioning the xanthophyll and carotene between methanol and petroleum spirit. Most subsequent processes have been based upon Willstätter's procedure.

Extraction of Pigments.—The processes can be divided into two groups: (*a*) those in which the pigments are extracted with a solvent and saponification is carried out subsequently, and (*b*) those in which the original substance is heated with alkali before extraction with a solvent.

In the first group numerous solvents have been proposed, including petroleum spirit, heptane, ethyl ether, acetone, methanol, ethanol (*e.g.* by Bolin and Khalapur,[2] and in the process of the Danish State Laboratory[3]), pyridine (Russell, Taylor and Chichester[4]; Smith and Smith,[5]) and *n*-butyl alcohol (proposed for flour by Binnington, Sibbitt and Geddes,[6] who found that it had better extractive action than most of the other solvents investigated).

The use of mixed solvents (one of which is usually petroleum spirit) has advantages, especially if one of the solvents is removable by water. Examples are the use of acetone followed by ether (Schertz[7]), a mixture of 93 per cent. of light, cleaners' naphtha and 7 per cent. of ethanol (Ferrari[8]), benzine and methanol (Kuhn and Brockmann[9]), petroleum spirit and methanol (Campbell[10]), and our

own process, in which a mixture of 15 parts of acetone and 45 parts of petroleum spirit is used.

Grinding either with sand or in a small ball mill is often recommended. We have found grinding with sharp sand in a mortar satisfactory for dried grass, etc.

After extraction of the pigments it is usual to carry out a saponification process, followed by treatment with water. By this means, chlorophyllines and flavones are carried into the aqueous layer, and xanthophyll esters are split up, so that free xanthophylls are obtained, which can be removed from the petroleum spirit by the usual treatment with methanol. Kuhn and Brockmann[9] apply the treatment with methanol before saponification. A very simple method of saponification is to shake the petroleum spirit solution at ordinary temperature with a 30 per cent. solution of potash in methanol, but sometimes it is preferred to heat under a reflux condenser, in order to make sure of splitting up the xanthophyll esters. So far as dried grasses, etc., are concerned, we can find no evidence that any xanthophyll esters remain undecomposed after the cold treatment. In any process in which acetone is used the acetone must be washed out before the treatment with hot alkali, otherwise brownish substances not removable by methanol are formed.

The main object of the processes in Group (*b*) is to break up tissue and allow the solvent to come into more intimate contact with the pigment. Coward[11] appears to have been the first to employ such a method. She ground with sand and heated with alcoholic or aqueous potash (the latter being preferred on account of less formation of resins) and then extracted with petroleum spirit. In our experience preliminary alkali treatment tends to give low results in some instances.

Probably the best known process in this class is that of Guilbert,[12] which was designed for forage. The sample is boiled for 30 minutes with a saturated solution of potassium hydroxide in ethyl alcohol, which is added in the proportion of 20 ml. per gram of the material, and then extracted with ethyl spirit. The ether is distilled, first at atmospheric pressure and finally under reduced pressure, and the residue is taken up in petroleum spirit for the subsequent methanol treatment.

It seems desirable to avoid the use of ethyl ether, not only to save time, but also because there is a risk of losing carotene. Petersen, Hughes and Freeman[13] omit the ethyl ether and extract direct with petroleum ether (40–60° C.). Munsey, of the U.S. Food and Drug Administration, Washington, has published a collaborative investigation of the Petersen, Hughes and Freeman process, including various methods of measurement of the carotene in the final solution.[14] Moon[15] also discards the use of ether.

Some prefer to heat with aqueous potash, *e.g.* Coward,[11] Fergusson and Bishop,[16] Moon.[15] In all these processes, the main object is to get the yellow pigments finally into petroleum spirit, and remove the chlorophyll into the aqueous layer. The latter is drawn off (and, if necessary, re-extracted with petroleum spirit), and the petroleum spirit layer is washed a little and is then ready for the next step, which is usually a treatment with methanol containing a little water. The object of this process is to remove the so-called xanthophyll.

In the past there has been a little confusion as to the definition of xanthophyll, but it seems now to be accepted that there is a definite substance obtained from

leaves, eggs, and the petals of many flowers, to which the name xanthophyll was given by Karrer and Notthafft[17]; this compound, which was called lutein by Kuhn, Winterstein and Lederer,[18] is a di-hydroxy derivative of α-carotene. The xanthophyll extract from leaves will consist mainly of that substance and zeaxanthin, but sometimes it may contain other derivatives of the so-called "phytoxanthins" of Karrer and Notthafft, and may include zeaxanthin, flavoxanthin, violaxanthin, taraxanthin, fucoxanthin, rhodoxanthin, capsanthin, crocetin, etc. From a commercial point of view, all these are included as xanthophylls (kryptoxanthin, which occurs in yellow corn, sunflower, capsicum, eggs, etc., is also classed as a xanthophyll, but is found mainly in the petroleum spirit layer).

Many workers (*e.g.* Schertz, Pyke[19]) used 85 per cent. methanol, but it seems now to be generally accepted that the best proportions are 10 vols. of water to 90 vols. of methanol, to give a strength of about 92 per cent. by volume (sp.gr. 0·830 at 15°/15° C.).

The petroleum spirit solution is shaken in a separating funnel with the methanol, which is drawn off, and the process is repeated until the last washing is practically colourless. If desired, a colour reading of the petroleum spirit solution can be made before the methanol treatment; this gives a result which is usually called "total carotenoids," the value generally being calculated in terms of β-carotene. This is a partition process, and presumably a small quantity of carotene goes into the methanol layer, and the xanthophylls to a small extent into the petroleum spirit layer. Miller[20] found 6 per cent. of the xanthophyll in the petroleum spirit layer, when using 92 per cent. methanol (by volume). According to our experiments, the quantity of carotene dissolved in methanol of sp.gr. 0·830 under these conditions is very small indeed.

The tendency is for the hydrocarbons (*e.g.* carotene, lycopene) and the less highly oxygenated xanthophylls (*e.g.* kryptoxanthin) to stay mainly in the petroleum spirit phase, while the more highly oxygenated xanthophylls go into the methanol phase (note, however, that some rhodoxanthin may go into the petroleum spirit).

In the ordinary commercial processes the coloured substances in the petroleum spirit layer are counted as carotene, and, if this is all that is required, it is only necessary to make up to a suitable volume and determine the colour value in terms of β-carotene, or use the spectrograph at an agreed wave-length.

The solution thus obtained is not pure carotene, however, and in a strict analysis it is necessary to go further. Sometimes spectrophotometric methods may be used, but often further separation is possible only by chromatographic methods. A few notes on spectroscopic methods may be of interest, although the equipment is not usually found in the average commercial laboratory. Many chemists have worked out methods depending upon absorption spectroscopy, both with visual and ultra-violet spectroscopes. (Gillam, Heilbron, Morton, Bishop and Drummond[21] did a large amount of work in this direction, using chloroform solutions, and taking readings before and after the addition of an antimony trichloride reagent.) Usually the methanol separation is first carried out, and the spectroscope is used to examine the separate portions, but Miller[22,23,24,25,26]

has worked out methods for two, three and even four constituents in presence of each other, and by using his methods it might be possible with some materials to work on solutions that have had no preliminary methanol separation. Miller gives examples of the use of the method for determining mixtures of α- and β-carotenes.

Wave-length 450$m\mu$ is suitable for the determination of β-carotene. In our laboratory we have obtained E values for two separate supplies of β-carotene. One of these was kindly sent by Professor A. C. Chibnall. Our value at 450$m\mu$ for $E^{1\%}_{1\,cm.}$ was 2520 in petroleum spirit. This is very close to that of Gillam,[27] who obtained 2500 in petroleum spirit and 2200 in chloroform, both at 450$m\mu$. Gillam in the same paper gives maxima for various carotenoids.

Working with a sample of S.M.A. β-carotene from Cleveland, Ohio, we obtained $E^{1\%}_{1\,cm.}$ 2440 (petroleum spirit), which agrees very well with the values generally given in the United States.

Petersen, Hughes and Freeman[13] employ three wave-lengths, *viz.* 4550, 4700 and 4800Å and calculate the carotene from the average.

Among recent workers employing spectrography may be mentioned Clark and Gring,[28] who determined β-carotene and kryptoxanthin together in yellow corn. Shrewsbury, Kraybill and Withrow[29] describe the use of a photo-electric photometer and of a Bausch and Lomb spectrophotometer.

Chromatographic Methods.—Chromatographic methods are rather difficult to deal with in any systematic way, as they depend upon somewhat obscure adsorptive properties of inorganic materials. Twsett was one of the earliest workers with this method. For full details, reference should be made to such works as those of Zechmeister and Cholnoky,[30] and of Winterstein,[31] and to articles by Wiseman and Kane, Strain and others in the *Journal of Biological Chemistry* and the *Biochemical Journal*. Briefly, the method consists in dissolving the mixed pigments in a suitable solvent (frequently petroleum spirit), and passing the solution through a well-packed column of a suitable adsorbent. Usually the pigments will distribute themselves in bands according to their adsorption affinities.

Frequently the bands can be made to separate by washing with the solvent, or by pouring a mixed solvent through the tube (for example, a mixture of petroleum spirit and benzene). The bands can then be dealt with by pushing the column up the tube and removing them as they appear at the top, or, with narrow tubes, the tube can be cut off at suitable places. In a very elaborate analysis, the various substances can then be extracted from the adsorbent, and their absorption curves by means of the spectroscope.

The most usual adsorbents are alumina (see Willstaedt and With[32]), lime, calcium hydroxide, calcium carbonate and magnesia. As a general rule, the most oxygenated carotenoids are the most strongly adsorbed, and will be found in the top of the tube, while the pigments with less oxygen and the hydrocarbons, such as lycopene and α- and β-carotenes, will tend to pass to the bottom of the tube. As α-carotene is more loosely held than β-carotene it can often be washed out of the tube with solvents. In obtaining the separations it is sometimes an advantage to mix active and inactive adsorbents, *e.g.* the use of 1 part of Merck's alumina

with 1 or 2 parts of ordinary anhydrous alumina allows α-carotene to be washed down more readily. Frequently it is difficult to get more than roughly quantitative results by this method, but we have worked out a modification which we have found very useful in the examination of dried grass. This is described later.

The following are some examples of the use of this method in the separation of carotenoids. (1) Buxton[33] separated kryptoxanthin from carotene by dissolving the pigments in heptane, and using calcium carbonate activated at 200° to 300° C. (2) Hegsted, Porter and Petersen[34] removed non-carotene pigments from silage by passing the solution in "Skellysolve" through calcium carbonate. (3) Violaxanthin is separated from lutein and zeaxanthin by means of calcium carbonate (Kuhn and Brockmann[9]). (4) Lycopene is separated from β-carotene by dissolving in benzene, and running the solution through a mixture of activated magnesia and Supercel (Strain). (5) Coward[11] passed a petroleum spirit solution from tomatoes through calcium carbonate, and found that carotene washed through first, then lycopene. (6) Lime, or calcium hydroxide, has often been used to separate α- from β-carotene; the latter is said to form a top red layer, and the former a lower yellow layer. Strain separated α- and β-carotenes by the use of a mixture of 1 part of activated magnesia and 1 part of Supercel. (7) Zechmeister[30] separated kryptoxanthin, lycopene, γ-carotene, β-carotene and α-carotene, in that order, with alumina.

My own experience with adsorbents other than Merck's alumina has been disappointing. We have found filtration very slow, and adsorption very much inferior to that obtained with the alumina. Our experiments with alumina are described later, but I will give here the process worked out in our laboratory for the routine determination of carotene in dried grasses.

ROUTINE DETERMINATION OF CRUDE CAROTENE.—From 0·25 to 0·5 g., according to the richness of the sample, is thoroughly ground with about 20 times its weight of sharp silver sand (60-mesh sieve) in a suitable mortar until a soft powder is obtained. This is transferred to an extraction thimble lined with filter-paper, the mortar being rubbed out with a little sand which is added to the ground powder. A plug of cotton-wool is placed in the top of the thimble and the sample is extracted in a continuous-drip type of extractor (this is preferable to the Soxhlet type) with a mixture of 15 ml. of acetone and 45 ml. of petroleum spirit for at least one hour, or until no more colour is extracted. The residue can be re-ground and re-extracted. The extract is cooled and transferred to a separating funnel, and the extraction flask is rinsed with first 3 ml. and then 2 ml. of petroleum spirit. Five ml. of a 30 per cent. solution of potassium hydroxide in pure methyl alcohol (this solution must be quite colourless) are added, and the funnel is shaken very thoroughly for at least two minutes, after which 200 ml. of water are added and the funnel is very gently inverted once or twice. The aqueous layer is run off, and the petroleum spirit layer is shaken vigorously with 200 ml. of water. In this way chlorophyll and flavones are removed.

Xanthophylls are removed from the petroleum spirit solution by shaking successively with 30, 15 and 15 ml. of methyl alcohol of sp.gr. 0·830 at 15°/15° C. (made by mixing 90 volumes of absolute methyl alcohol with 10 volumes of water

separately measured). The petroleum spirit solution is made up to 50 ml. or to such other volume as is convenient for colour measurement. To ensure brightness, about 1 g. of anhydrous sodium sulphate may be added.

There is nothing especially novel about this process, but our aim has been to arrange the manipulation so that it is workable for a large number of routine samples. With slight modification, it has been accepted tentatively as the official process of the Grass Driers' Association, as a result of the investigations of a Committee of Chemists called together by Mr. Davies, a member of the Council of that Association; the report of their findings will be issued very shortly.

In our process, we have followed Pyke[19] by working with small quantities. Extraction is easy, and the whole manipulation is much simpler than when larger quantities are taken. As regards the solvent, there is an advantage in using acetone as part of the mixture, on account of its low b.p. The residue is usually colourless after an hour's extraction, but, if any doubt exists, it is easy to re-grind and continue the extraction for a while.

We have also tried a variation of the process by shaking from 0·5 to 1 g. of the grass (after grinding with sand) with 75 ml. of petroleum spirit and 25 ml. of acetone for 1 hour on a mechanical shaker. An aliquot part is taken, and the remainder of the process is as described. Extraction appears to be complete under these conditions, and the procedure has the advantage that the whole operation is carried out at the ordinary temperature.

There seems to be no need to re-extract the aqueous portion. Whenever this has been tried, the second extract has invariably been practically colourless. It may be desirable with very rich grasses to make a second treatment with 30 per cent. potassium hydroxide solution, but residual traces of chlorophyll do not seem to interfere with the determination of the carotene, provided that the first shaking has been very thorough.

Colorimetric Estimation.—It is very difficult, in England at any rate, to get pure β-carotene for use as a standard, and, if obtainable, it is very expensive; moreover, it is practically impossible to keep a solution without change. On this account it has been proposed to use as standards more stable solutions, which can be compared with pure carotene solutions, and the values so obtained used for all subsequent determinations.

Azobenzene has been used by Kuhn and Brockmann. A solution of 14·5 mg. in 100 ml. of 95 per cent. alcohol was said to be equivalent to one containing 0·235 mg. carotene per 100 ml. in light petroleum. Potassium dichromate is the substance most commonly employed, and has been used in various strengths by different workers. Fraps used a 0·1 per cent. solution, and Russell a 0·036 per cent. solution. Ferguson[35] used the Klett colorimeter, with 0·1 per cent. solution. Under those conditions his figure for a 0·025 per cent. solution works out at 0·150 mg. of carotene per 100 ml.

Lovibond Readings.—Ferguson has also constructed a curve for Lovibond readings plotted against carotene. His reading for a solution of carotene at 0·1 mg. per 100 ml. is 1·6 yellow units.

The Committee of the Grass Driers' Association, referred to above, used a 0·025 per cent. solution of potassium dichromate, and agreed upon the figure of

0·158 mg. of carotene per 100 ml. of petroleum spirit as the equivalent carotene solution. (This agrees well with the figure 0·160 given by Connor.[36]) This comparison was obtained by means of the Lovibond Tintometer, but with glasses that were probably of very different ages. During the course of the investigations, it was found that individual observers obtained widely different results in reading, particularly in the higher ranges. Even with a carotene solution containing 0·1 mg. per 100 ml. values ranging from 1·6 to 2·2 were obtained, with an average of about 2·0. It was found, however, that if observers constructed their own curves, using either a carotene solution or potassium dichromate, much better agreement could be obtained on the actual carotene figure for any sample. In using the Lovibond instrument it is usual to match with yellow and red, and to count only the yellow reading. The red reading is usually very small, but most operators find that it helps them to get a closer match.

We always prefer to read at a point not higher than about 3·0 yellow Lovibond. With very careful work it is possible to get good results with the Lovibond instrument, but we much prefer a photoelectric instrument.

Photoelectric Comparison.—Actually, most of our work has been carried out by means of the Spekker photoelectric absorptiometer. It appears to be generally accepted that the graph showing the relation between carotene and potassium dichromate by various colorimeters is a straight line, provided that the concentrations are not too great, and we have found that this applies to the Spekker instrument. With that instrument we obtained a straight line by plotting dichromate strengths against drum readings, but the relation of carotene to dichromate was a little higher than the figure given above for Lovibond, *viz.* a 0·025 per cent. solution of potassium dichromate was equivalent to a carotene solution containing 0·165 mg. per 100 ml.

TABLE I

	Crude "Carotene" (calculated on dry sample) p.p.m.		Crude "Carotene" (calculated on dry sample) p.p.m.
Carrot	720	Barley shoots	155
Dried carrot meal ..	500	Spinach	600
Wheat flour (bleached) ..	1	Turnip leaves	550
Silage	140	,, stalks	40
,, ...	230	Tomato (green)	55
,, ...	200	,, (pink)	110
,, (red clover) ..	209	,, (red)	220
,, (white clover) ..	523	Hay	90
,, ...	378	Palm oil	920

Our working plan has been to set our instrument by means of β-carotene and to keep a stock (0·025 per cent.) solution of potassium dichromate, which is read from time to time, in order to ensure that no change has taken place.

Other special instruments in common use are the Bolton–Williams Absorptiometer, and the Zeiss–Pulfrich visual absorptiometer; also various types of visual colorimeters, such as the Klett and the Duboscq instruments mentioned

above. Direct comparison in Nessler tubes can, of course, be used, but in our experience it is difficult to get consistent results by this method.

In Table I, some examples of our determination of crude carotene in different substances are given.

TABLE II

EXAMPLES OF TREATMENT BY SPUR'S CHROMATOGRAPHIC METHOD FOR DRIED GRASS AND ALFALFA

Sample	"Carotene" by the John Hughes method Before treatment	Carotene After treatment	Ratio
Grass meal, 1185	190	135	0·70
,, ,, 1186	425	315	0·74
,, ,, 1187	400	325	0·81
,, ,, 1188	275	220	0·80
,, ,, 1189	180	130	0·72
,, ,, 1190	240	175	0·73
,, ,, 1191	250	195	0·78
,, ,, 1192	320	225	0·70
,, ,, 1193	160	125	0·78
,, ,, 1194	175	140	0·80
,, ,, 1195	300	235	0·78
,, ,, 1196	390	275	0·71
,, ,, 1199	255	200	0·78
,, ,, 1200	245	200	0·82
Grass meal and molasses, 1198	145	115	0·79
,, ,, ,, ,, 1203	400	310	0·77
Grass meal, 1210	250	220	0·88
,, ,, 1211	270	220	0·82
,, ,, 1218	535	410	0·76
Lucerne leaf, 1207	310	275	0·89
,, stalk, 1208	205	193	0·94
,, meal, 1230	220	190	0·86
,, ,, 1243	430	320	0·75
Canadian alfalfa, A.663 ..	175	160	0·91
,, ,, A.664 ..	165	145	0·88
Silage, 297	92	50	0·54
,, (red clover), 250 ..	65	45	0·70
,, (white clover), 251 ..	130	102	0·78

Chromatographic Procedure.—Reference has already been made to the chromatographic procedure we have adopted in arriving at some estimate of the purity of the final solutions obtained in our process. Our first investigations along this line were due to Mr. Bernard Spur of Copenhagen, who showed us this method of using Merck's alumina. In his method, the petroleum spirit solution after the usual alkali and methanol treatments is washed free from methanol and drawn through a column of Merck's alumina in a tube about 2 cm. in diameter; β-carotene is adsorbed to form a red layer, and above it can be seen a yellow layer. The adsorbed layers are developed with a mixture of 1 part of petroleum spirit and 1 part of benzene, and then the whole column is pushed upwards, and the yellow layer is removed as it comes up.

The red layer of β-carotene is dissolved from the alumina by means of a solution of 10 per cent. ethanol in petroleum spirit. The solution is made up to definite volume, and the carotene is determined by the usual methods. In Spur's method, we prefer to use a very small tube, about 4 mm. in diameter, and about 5 or 6 cm. long. This serves for the usual quantities obtained from 0·25 or 0·5 g. of dried grass. After passage of the solution and development of the bands, the tube is cut at a point in the yellow band just above the red, and the yellow is scraped off, until it is all removed and the red appears. There is always a little doubt as to the sharpness of this mechanical separation; also, there seems a tendency for the outside portion of the red to change to yellow.

In experiments to discover a more convenient method we found that the addition of 3 per cent. of acetone to the solution before passing through the alumina caused the carotenes to be carried through, while the yellow substance remained in the alumina. The tube can be washed with a small quantity of 3 per cent. solution of acetone in petroleum spirit, and the solution of carotenes that comes through can be dealt with in the usual way.

Table II shows some results obtained by Spur's method, and Table III gives comparative results obtained by Spur's method and by the 3 per cent. acetone method. These agree well as a rule, and it is probable that the yellow bands obtained by the two methods are identical.

It is difficult to ascertain the nature of the yellow bands. In one test an ultra-violet spectrograph was taken, but I was unable to identify them. In any event the substance is definitely non-carotene pigment. With grasses, the unadsorbed portion appears to be homogeneous, as judged by the results of washing it and passing it through a tube packed with one part of Merck's alumina and one part of ordinary alumina, and it is reasonable to assume that it is β-carotene.

TABLE III

EXAMPLES OF THE THREE PER CENT. ACETONE PROCESS APPLIED TO THE "CAROTENE" SOLUTION OBTAINED BY THE JOHN HUGHES METHOD

	Original	3 per cent. acetone process	Modified Spur's method
1.	470	385	370
2.	385	350 (repd. 350)	—
3.	380	320	300
4.	240	200	210
5.	280	190	180 (old sample)
6.	250	170	190 (old sample)

Comparison between 3 per cent. acetone treatment:

(*a*) on original green solution,

(*b*) on yellow solution after removal of chlorophyll and xanthophyll.

	(*a*) p.p.m.	(*b*) p.p.m.	
1.	190 (repeated 180)	190	(Spur 180)
2.	320	300	
3.	325	305	

Note.—All the above figures represent milligrams per kilogram.

It will be seen that the ratio between this pure carotene and the crude carotene varies from 0·7 to 0·8, though apparently for old grasses the proportion may fall still lower than 0·7.

To explore the possibilities of simplification, we applied the 3 per cent. acetone process at an earlier stage, *i.e.* on the original extract of the grass. We washed out the acetone by means of a sodium sulphate solution (made by adding 1 part of saturated sodium sulphate solution to 1 part of water) with gentle shaking, as emulsions sometimes form. We then added 3 per cent. of acetone to the petroleum spirit solution, and passed it through the alumina (a rather longer column than usual is safer, say 7 cm.) and washed with a little of the 3 per cent. solution of acetone in petroleum spirit. The amount of carotene obtained by this method agrees very well with that obtained by passing the solution, after treatment with alkali and methanol, through the alumina, as all the xanthophyll and chlorophyll are removed, as well as the non-carotene substances already mentioned. Occasionally, a trace of chlorophyll may escape, but it can be held back by passing the solution through a fresh tube of alumina.

As a further simplification, 0·5 g. of the grass was added to 100 ml. of 3 per cent. acetone solution and shaken on a mechanical shaker for 2 hours. An aliquot portion (25 ml.) was taken, and passed through the alumina. The carotene-content thus found was practically identical with that obtained by making the usual extraction with a 25 per cent. solution of acetone in petroleum spirit, washing away the acetone, adjusting the solution to contain 3 per cent. of acetone, and passing it through the alumina as usual (Table IV).

TABLE IV

EXAMPLES OF REPETITIONS BY THE THREE PER CENT. ACETONE PROCESS

(1) 0·5 g. of grass meal shaken with 100 ml. of a 3 per cent. solution of acetone in petroleum spirit for 2 hours on a mechanical shaker at ordinary temperature, and aliquot parts of 25 ml. each passed through alumina.

Length of tube cm.	Readings on Spekker instrument	Carotene mg. per kg.
5	0·15, 0·16, 0·16	330—
7·5	0·16, 0·16	330
10	0·165, 0·16	330+

(2) Shaken with 3 per cent. of acetone in petroleum spirit during 2 hours at 40° C.

Length of tube cm.	Readings on Spekker instrument	Carotene mg. per kg.
7·5	(mean) 0·16	330
10	(mean) 0·165	340

(3) Extracted with 25 per cent. of acetone in petroleum spirit in a drip extractor; acetone washed out and petroleum spirit solution made to 3 per cent. acetone strength.

Length of tube cm.	Readings on Spekker instrument	Carotene mg. per kg.
7·5	(mean) 0·17	350

More work is necessary to find out whether this extremely simple process could be generally applied to dried grasses. There would be the difficulty, of course, at the present time, that Merck's alumina is unobtainable, but no doubt an activated alumina of suitable quality could be prepared, or some other adsorbent used.

In this connection reference may be made to an interesting paper recently published by Fraps, Kemmerer and Greenberg[37] upon the adsorbent properties of magnesia and magnesium carbonate. It is very difficult to predict what any particular batch of magnesia is going to do, but the authors give some hints upon control. Since reading that paper, I have used a sample of light magnesia in connection with my 3 per cent. acetone process. Although filtration was much slower than with Merck's alumina, it was workable, and the results appear to be practically identical.* Further work along these lines should be useful.

PROCEDURE FOR SPECIAL MATERIALS.—The determination of carotene in dried grass, alfalfa and similar materials has been fully treated above. As regards wet grass, our process can be applied with the modification that plain acetone is first used to extract the sample to remove the water, after which the material is ground with sand and extracted with petroleum spirit.

We have examined spinach and several other leafy materials by our process; it also seems to work well with carrots and tomatoes. The 3 per cent. acetone process appears to allow β-carotene to pass through the alumina tube, while the lycopene (the chief pigment of tomatoes) is held as a purple-red layer, and can be dissolved out with a mixture of petroleum spirit and alcohol, and estimated by its colour value or by the spectrograph.

Palm Oil.—In this and similar fatty substances, extraction coincides obviously with saponification. Our cold saponification process worked satisfactorily for this, using 30 per cent. methanolic potash at the rate of 15 ml. for each gram of oil, then 50 ml. of petroleum spirit, diluting with water, removing the aqueous layer, and repeating the treatment with 7·5 ml. of potash solution. In the samples we have examined we have found no pigments other than α-carotene and β-carotene.

Butter.—A very simple approximate process has been put forward by Baumann and Steenbock.[38] They separate the fat and determine the extinction coefficients at $485m\mu$ and $460m\mu$ at 30° C. These are compared with the coefficients of β-carotene in refined cottonseed oil at 30° C.

A saponification process is described by Morton.[39] If desired, the xanthophyll can be removed by methanol as usual, but it is frequently assumed that about 6 per cent. is present, and a suitable deduction made. The reading of the final petroleum spirit solution can be made colorimetrically or spectrophotometrically. Many papers on this subject have been published by Gillam, Morton, Heilbron, Drummond and others in the *Biochemical Journal*.[40]

We have applied successfully cold saponification with a 30 per cent. solution of potash in methanol. When using the process with 3 per cent. solution of acetone

* Since writing the above I have tried a sample of heavy magnesia kindly sent me by Mr. D. J. Campbell. This gave a better filtration than the light magnesia, but it was necessary to use a 7 per cent. solution of acetone in petroleum spirit.

It appears that the percentage of acetone in the petroleum spirit must always be adjusted to suit the adsorbent.

in petroleum spirit, only a very small yellow layer was found, and the pigment appeared to be practically all α- and β-carotenes; Gillam and Heilbron,[41] however, found small quantities of kryptoxanthin.

Egg Yolk.—We have not done much work with this substance, but in one experiment we mixed the sample with sand and extracted it first with acetone. The residue was taken from the cartridge, ground and then extracted with a mixture of petroleum spirit and acetone. Hot saponification appeared to be necessary. In one experiment, working with the 3 per cent. acetone process, we found 0·7 p.p.m. of carotene in the yolk of an egg, and approximately the same proportion of kryptoxanthin.

Flour.—Kent-Jones used petrol for extracting the carotenoids. Ferrari used a mixture of 7 per cent. of ethyl alcohol with light, cleaner's naphtha (93 to 160° C.), whilst Binnington, Sibbett and Geddes[6] used *n*-butyl alcohol (saturated with water to avoid turbidity), and claimed that better extraction was obtained than with the usual solvents.

Apparently it has generally been believed that nearly all the yellow pigment in flour is carotene, but Munsey[42] claims that, although the total pigment may amount to from 2 to 3 p.p.m., the carotene is not more than 0·2 p.p.m. He uses a hot saponification process on the lines of that of Petersen and Hughes, and believes that the main portion of the pigment is xanthophyll or something very similar, and that previous high results for carotene were obtained because xanthophyll esters were not properly saponified and were found in the petroleum spirit layer.

Experiments on a few flours with our 3 per cent. acetone process confirm Munsey's statement, that the amount of β-carotene is very small (probably of the order of 0·15 per cent.) but in the particular flours examined I found very little xanthophyll after either hot or cold saponification. In one experiment the evidence seemed to point to the presence of lycopene, and in another to kryptoxanthin.

Yellow Corn.—Considerable attention has been given in the United States to the carotene content of yellow corn. This has been referred to above. Kryptoxanthin forms the major portion of the petroleum spirit phase in the partitioning with methanol. Our process appears to work well for this substance, and the 3 per cent. acetone treatment seems to separate the kryptoxanthin from the carotene.

CONCLUSIONS.—A process such as is used in our ordinary routine gives a useful commercial valuation, in spite of the fact that it does not determine the actual carotene and that the true carotene is sometimes only 80 per cent. of the figure obtained. There is some evidence that this percentage sinks with the age of the stored grass, and this suggests that the yellow substance may be an oxidation product of carotene.

I have shown that the use of Merck's alumina in the 3 per cent. acetone process appears to be a very promising method, capable of giving in an extremely simple way the true carotene percentage in dried grass and perhaps in many other materials. The great disadvantage is that Merck's alumina is now unobtainable;

possibly, however, magnesia, some experiments with which I have mentioned, may eventually take its place.

I wish to thank our staff for their assistance, and in particular Mr. Ward, who worked out most of the details of the process, and Mr. Rabnott for spectrographic and general research work.

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March 9th, 1940

Discussion

Mr. E. B. ANDERSON said that, as Chairman of the Food Group, he would like to convey to the Society the appreciation of the Group for this joint meeting, which had now become an annual event. His knowledge of carotene was concerned with its connection with the colour of the cream of milk. It was well known that

in the winter, owing to the lack of colour in the cream, there were frequent complaints from customers that there was no cream on the milk. A year or two ago experiments were made on the use of dried grass for feeding cattle in winter, to see if a milk-cream with the colour of summer milk could be obtained. This feeding, however, had no effect whatever on the colour. They had found that the colour dropped until about the beginning of December and then remained constant until the cows were put out to grass, when an immediate improvement was shown. In the experiments, one herd was given the ordinary winter feed and two others dried grass, and the results were the same for all three herds. It was well known to most users of the Lovibond Tintometer that the inaccuracies were greatest when measuring colours in the yellow region.

Dr. S. K. KON said that, by a coincidence, he had with him a copy of a paper, prepared by himself and Mr. Thompson, which contained exactly the same findings as those of Mr. Seaber, and he could confirm those results in every detail. They had extracted the pigment and had had it analysed for them by Dr. Gillam, and now Dr. Morton was of opinion that it was an oxidation product of carotene. They were carrying out biological tests to ascertain if it was biologically active. With regard to the feeding of dried grass to cattle, they had carried out some experiments at Shinfield with dried grass, kale and mangolds in the quantities:—dried grass 14 lbs., kale 46 lbs., and mangolds 42 lbs. There was a very marked difference in the results; the milk from the cows fed with the dried grass contained twice as much carotene as the milk from the cows fed on kale and some five times more than the milk of the mangold cows. Of course, this was only an experiment on a small scale.

Mr. H. PRITCHARD said that Mr. Seaber had mentioned the difficulty of obtaining Merck's alumina; this question had also been raised at a meeting in the North. He would suggest that old supplies of alumina should be conserved as much as possible, and to this end his practice was to retain the alumina which had been used for chromatography and burn off all organic matter in a muffle furnace at 450° to 600° C. By this means the alumina was rendered quite white, its activity was unimpaired, and it could be used again many times. He had tried other aluminas. One supplied by a British firm seemed to contain a certain amount of water in combination, which made the rate of percolation of the solvent through the column very slow.

Dr. R. G. DAVIES, referring to Mr. Anderson's remarks on the feeding of grass to cows, said that he thought that results depended largely on the breed of cow. With Holstein cows the colour of the milk was sometimes changed, whereas with Jersey cows, which had not such power of converting carotene into vitamin A, the colour of the milk remained lighter.

Dr. D. KENT-JONES asked what sort of agreement there was among analysts when dealing with alfalfa grasses, as he had found that the variation between results obtained by different analysts was very large. As regards the feeding of carotene, when it was oxidised, as it was in bleached flour, did one destroy the carotene from the point of view of vitamin A? It was generally agreed that this was so, but papers had been published in which the contrary was stated.

Mr. A. L. BACHARACH referred to the shortage of Merck's alumina; the suggested procedure of recovering it after use might involve destruction of its adsorbent properties. The excellence of Merck's product might lie in the presence of a certain amount of the so-called "fibrous" alumina (Wislicenus). There had recently been marked improvement in the adsorbent alumina made in this country, and probably at least two were quite satisfactory. In spite of the importance of the conversion of carotene into vitamin A, little was known about it. "Carotinase" might exist in living tissues, but it had only so far been seen in text-books. The conversion ratios of carotene and vitamin A were singularly constant, according to Guilbert, for different species, but varied very much with intake of carotene,

which might have in mammals one-third the molar activity of vitamin A at low doses and only 1/10th at high doses. If Dr. Morton was right in suggesting that there was no reason why carotene should break down at any one double bond more than another, a very complex series of changes must be involved in the conversion, and it was not surprising that no single conversion factor could be applied.

Dr. R. L. EDWARDS, referring to the yellow colour of the carotene solution, said that in the work of the Committee on carotene determination in dried grass mentioned by Mr. Seaber, readings were taken by about twenty-five observers on solutions of dichromate (0·025 per cent.) and on carotene (0·10, 0·20, 0·25 mg. per 100 ml.). Each observer calculated his own carotene concentration equivalent to 0·025 per cent. dichromate from these readings. If there were no inherent disadvantage in using Lovibond glasses, one would expect a standard deviation from the mean of this carotene equivalent involving two readings with their combined errors, to be greater than that of either of the separate readings. Actually, it was less than either. Expressed as percentage of the mean, Lovibond reading on 0·025 per cent. dichromate, s.d. 11·5 per cent.; Lovibond reading on carotene 0·10 mg. per 100 ml., s.d. 14 per cent.; 0·20–14 per cent.; 0·25–21 per cent. Carotene equivalent to 0·025 per cent. dichromate, s.d. 5·5 per cent. It seemed that one obtained better agreement between laboratories if one used the Lovibond Tintometer simply as a comparator for dichromate and carotene. In connection with the theory of unsymmetrical splitting of carotene, it was interesting to carry the speculation a little further to include α- and γ-carotenes, which were supposed to be only half as active as β-carotene. This could be explained if, whatever the mechanism involved, splitting of the carotene molecule occurred with equal frequency on each side of the centre, regardless of the nature of the end groups. Then 50 per cent. of the α-carotene molecules would give β-ionone rings with chains long enough to form vitamin A, whilst the other 50 per cent., having β-ionone with short chains, would be inactive.

Mr. D. J. CAMPBELL referred to a sample of magnesia, apparently unactivated, which he had found to be quite suitable for the separation of carotenoids, and which could be used with 3 per cent. acetone. It seemed to have been established that carotene was most easily available to the animal when fed in oil, and he would like to know if any work had been done on feeding carotene in oil-cake. The availability of carotene was very important. With regard to the Lovibond Tintometer, he thought that errors were often really due to the faulty colour sense of observers. For carotene estimations a personal calibration was necessary. As regards the concentration of methyl alcohol for xanthophyll removal, he had recently made experiments with authentic carotene, and the exact concentration between 85 and 98·5 per cent. alcohol did not seem to make any difference; the stronger the alcohol the more petrol passed into it, but provided that enough free petrol was present, no carotene whatever was removed, even when the alcohol was 98·5 per cent. in strength.

Dr. MORTON, replying, said that Lovibond glasses of recent manufacture left little to be desired, although those of an old set, dated 1881, which he had examined, were far from satisfactory. In reply to Mr. Bacharach, he pointed out that he had reviewed the present situation more in the hope of making clear the difficulties than of sponsoring any particular theory. There was much to be said for buying and selling on the basis of the chemical content in terms of carotene and vitamin A. For many products, although not perhaps all, the time had gone for biological tests, since even a crude physical assay for vitamin A and carotene was usually more significanct than any but the most ambitious biological tests. The great impetus to vitamin A studies had come from biochemical research, and he wished to see the skill of the biochemist released from routine standardisation and applied to the fundamental problems which could be solved by no other type

of worker. Concerning chromatographic analysis he remarked that his own experience bore out the view that experiments on a very small scale gave the best analytical results.

Mr. SEABER, replying, said that his own experience of the use of the Lovibond Tintometer had been rather unfortunate. He had some difficulty sometimes with the yellow colours. He had found that many people said that they got very good results provided that the Lovibond number was not too large. He was very interested to hear that Dr. Kon confirmed his own findings relating to the non-carotene substances usually included as carotene. With regard to substitutes for Merck's alumina, he had tried magnesia; it had recently been prepared in such a way that it adsorbed the xanthophyll and not the carotene. With regard to methyl alcohol, they had found that when they used it in 85 per cent. strength in ordinary work they obtained lower results than with 90 per cent. Dr. Kent-Jones had asked what sort of variations chemists obtained in estimating carotene. Sometimes they were enormous; for example, from 150 to 350 parts per million. That was why the Grass Driers' Association had formed the Committee to which he had referred. On the other hand, in his own laboratory, they found they could get very close agreement in their results.

The Separation of Molybdenum from Tin and from Sulphur

BY D. A. LAMBIE, B.SC., A.I.C., AND W. R. SCHOELLER, PH.D., F.I.C.

In the course of a revision of the analytical chemistry of molybdenum, we found it advisable to re-investigate the separation of that element from tin and from sulphur (in the form of sulphate ion). The results of our work are recorded below.

I. SEPARATION OF MOLYBDENUM FROM TIN.—This separation does not appear to have been thoroughly studied, although the following methods have been proposed: (1) precipitation of molybdenum by hydrogen sulphide from oxalic acid solution; (2) precipitation of molybdenum by hydrogen sulphide from hydrofluoric acid solution; (3) volatilisation of stannic chloride by the action of dry hydrogen chloride upon a strong sulphuric acid solution at 200° C.; (4) for small amounts of tin, precipitation of hydrated stannic oxide on ferric hydroxide—a method employed for collecting minute quantities of a number of elements (phosphorus, arsenic, antimony, bismuth, vanadium, selenium, tellurium).

At the outset we decided to investigate methods (1) and (4), so as to avoid, if possible, the use of platinum vessels (method 2) or special all-glass apparatus (method 3).

Precipitation of Molybdenum Sulphide from Oxalic Acid Solution is an adaptation of Clarke's method for the separation of tervalent antimony from quadrivalent tin.[1] Since the quantitative course of the separation of molybdenum from tin by this reaction does not appear to have been investigated, we conducted a series of experiments with the following procedure, based upon a modification of Clarke's method.[2]

Known amounts of tin and molybdenum trioxide were dissolved in *aqua regia*, 15 ml. of 20 per cent. tartaric acid solution were added, and the solution was

neutralised with 20 per cent. sodium hydroxide solution; another 20 ml. of the alkali were then added, and the liquid was saturated with hydrogen sulphide. The solution of the thio-salts was boiled and cautiously treated with 15 g. of oxalic acid dissolved in a little hot water, after which hydrogen sulphide was again passed for 15 minutes. The precipitate was collected, washed with hot 1 per cent. oxalic acid solution containing hydrogen sulphide, and dissolved in nitric acid and bromine; the solution was then neutralised with ammonia prior to precipitation of lead molybdate. In all the experiments but one, the neutralisation with ammonia produced a precipitate of stannic acid, proving the separation to have been incomplete. In one test (on 0·1544 g. Sn and 0·0701 g. Mo), the stannic acid thus produced was collected and ignited, yielding 0·0072 g. of impure oxide contaminated with molybdenum. The test separation in which no stannic acid was precipitated on neutralisation gave the following result:

Taken, 0·0300 g. of Sn, 0·1000 g. of Mo; found 0·1008 g. of Mo.

In a second series of tests the hot solution of the thio-salts, obtained as above, was poured into the hot oxalic acid solution in an endeavour to obtain a molybdenum sulphide precipitate free from tin; but even so, a satisfactory separation was not achieved except with small quantities of tin.

It is possible, indeed probable, that a quantitative separation could be brought about by re-treatment of the molybdenum sulphide, the precipitate being dissolved in sodium sulphide and the solution poured into hot oxalic acid solution. The combined filtrates containing the tin are, however, liable to be contaminated with a little molybdenum, the recovery of which would prove troublesome and constitute a serious drawback to the method.

These considerations induced us to suspend further work on the sulphide method, and to investigate the possibilities of the process about to be described.

Precipitation of Hydrated Stannic Oxide on Ferric Hydroxide.—As far as we know, the application of this procedure to the quantitative separation of molybdenum from tin in any proportions has not been investigated. Ferric iron can be separated from molybdenum by double precipitation with ammonia, the hot feebly acid solution of the metals being poured during agitation into almost boiling dilute (1 : 1) ammonia. If stannic salt also is present, the tin is precipitated together with the iron, the molybdenum passing into the filtrate as ammonium molybdate. We succeeded in working out conditions under which a quantitative separation of molybdenum from substantial, as well as from small, quantities of tin is achieved, but not without numerous experiments bearing upon the ammonia concentration, the admissible maximum quantities of tin and molybdenum, and the most suitable ratio of iron to tin.

In the final series of tests the results given below were obtained by the following procedure, which we recommend for the separation of the two elements:—The weighed quantities of tin and molybdic oxide were dissolved in 10 ml. of strong hydrochloric acid, and 3 ml. of strong nitric acid; the quantities taken were adjusted to a maximum amount of 0·3 g. of (tin *plus* molybdenum). The resulting solution was treated with enough ferric chloride solution to provide iron equal in weight to the tin present, and diluted to 100 ml. The liquid was heated nearly to boiling, and poured during vigorous agitation into 100 ml. of dilute ammonia

(20 ml. of ammonia of sp.gr. 0·88, and 80 ml. of water already at the boiling-point), boiled for half-a-minute, and allowed to settle. The precipitate was collected on a close-textured filter, washed with 3 per cent. ammonium nitrate solution, returned to the beaker with a jet of water, and dissolved by heating with 10 ml. of strong hydrochloric acid (total volume 50 to 60 ml.). The resulting solution was then diluted to 100 ml., and the operation described above was repeated, the same filter being employed.

The combined filtrates were concentrated to about 200 ml., and the molybdenum was determined in the usual manner as lead molybdate. The tin was determined in the precipitate by the gravimetric method outlined below. The results were well within the limit of experimental error.

	Taken		Found		Error	
Exp.	Sn	Mo	Sn	Mo	Sn	Mo
	g.	g.	g.	g.	g.	g.
1	0·2930	0·0068	not det.	0·0065	—	—0·0003
2	0·2092	0·0685	0·2095	0·0685	+0·0003	0·0000
3	0·1029	0·1347	0·1033	0·1352	+0·0004	+0·0005
4	0·1515	0·1486	0·1511	0·1484	—0·0004	—0·0002
5	0·0243	0·2676	0·0243	0·2673	0·0000	—0·0003
6	0·0134	0·2813	0·0134	0·2822	0·0000	+0·0009

Gravimetric Determination of Tin.—While the separation of molybdenum from tin is under discussion, a few remarks may be usefully devoted to the determination of tin. The iodimetric titration is subject to interference by atmospheric and dissolved oxygen,[3] and the precautions to overcome this detract from the practical value of the process for occasional determinations. For these, precipitation of the tin as sulphide and ignition of the precipitate to oxide is really less troublesome, provided that other members of the hydrogen-sulphide group are absent, or have been eliminated by the usual methods, *e.g.* by means of sodium sulphide or *ferrum redactum*. In the procedure described below, which has been used for some time in actual practice with very satisfactory results, the stannic sulphide precipitate is dense and usually granular, filters well, especially if mixed with a little filter-fibre, and readily attains constant weight on ignition.

Insoluble material is fused with sodium peroxide or hydroxide as usual. The melt is disintegrated with hot water, and transferred to a 150- to 400-ml. beaker, and the crucible is warmed with dilute sulphuric acid till clean. The acid is transferred to the beaker, the contents of which are treated with 1 : 1 sulphuric acid until an excess of 10 to 30 ml. is present. The liquid is then evaporated until strong white fumes are evolved.

Precipitates (such as the mixed precipitate of hydrated stannic and ferric oxides obtained in the separation procedure under discussion) are placed in a beaker together with the filter-paper, and heated with strong sulphuric acid; nitric acid is cautiously added until all the organic matter is destroyed, after which the liquid is heated more strongly until dense sulphuric acid fumes are given off.

The cold acid is diluted with an equal volume of water, followed by 20 to 50 ml. of 20 per cent. ammonium chloride solution. The liquid is digested at

gentle heat until any anhydrous nickel or ferric sulphate has dissolved, then diluted, treated with creamed filter-fibre to entangle gelatinous silica, and filtered through a pad of filter-pulp into a 250- to 600-ml. beaker. The filter-pad is washed first with hot 0·5 *N* hydrochloric acid, and then with water.

The filtrate is strongly diluted (200 to 400 ml.) and saturated with hydrogen sulphide; addition of a little creamed filter-fibre causes rapid flocculation of small quantities of stannic sulphide. The precipitate is allowed to settle, collected, washed thoroughly with one per cent. sulphuric acid containing hydrogen sulphide, ignited wet in a tared porcelain crucible, first on an asbestos mat until the paper has charred, then a little more strongly until the carbon has been oxidised, finally at a bright red heat, and weighed as SnO_2 (*cf.* A. T. Etheridge, ANALYST, **1924, 49**, 371).

II. SEPARATION OF MOLYBDENUM FROM SULPHUR.—In practice this resolves itself into the separation of molybdate from sulphate, or rather (since the two elements are not usually determined in one and the same solution) into: (*a*) determination of molybdate in presence of sulphate; (*b*) determination of sulphate in presence of molybdate.

(*a*) The determination of molybdate as lead molybdate in presence of sulphate by a single precipitation (dropwise addition of a small excess of lead salt to a solution containing a large excess of ammonium acetate and chloride)[4] offers no special difficulties, re-treatment of the precipitate by solution in hydrochloric acid and re-precipitation by means of a large excess of ammonium acetate being necessary only with greatly preponderating quantities of sulphate.

(*b*) The determination of sulphate as barium sulphate in presence of molybdate, which is of practical interest in the analysis of molybdenum ores, is a subject concerning which very little information is available. We re-investigated a procedure described by Schoeller and Powell,[5] consisting in dropwise addition of 10 per cent. barium chloride reagent to the boiling solution containing 5 per cent. by volume of strong hydrochloric acid. The pronounced solubility of barium sulphate at this acid concentration was found to be more than counteracted by occlusion of molybdenum in the precipitate, with the result that positive errors were incurred. Only one of our test separations gave a seemingly correct result, yet the barium sulphate was obviously contaminated with molybdenum, being yellowish-green in colour; hence the agreement between the calculated and observed values was due to a compensation of errors. The method was rejected as untrustworthy.

Reduction of the molybdic acid to a lower compound prior to precipitation of the solution with barium chloride was tried next, but proved to lead to heavier co-precipitation of molybdenum than the preceding method. We reached the conclusion that, owing to the marked adsorptive power of barium sulphate, elimination of the molybdate prior to precipitation of the sulphate with barium chloride was the correct solution of the problem. In the process described below, the molybdate is precipitated with a slight deficiency of lead acetate in presence of ammonium acetate, and the ammonium salts (which would increase the solubility of barium sulphate) are removed from the filtrate by evaporation with *aqua regia*. The minute amount of molybdate left in the solution is much too small to interfere.

Two reagents are required: (1) a lead solution containing 40 g. of lead acetate and 10 ml. of glacial acetic acid per litre, and (2) a molybdate solution containing 1·86 g. of ammonium molybdate per 100 ml.; the two solutions are approximately equivalent.

The solution, containing not more than 0·3 g. of molybdenum and 0·2 g. of sulphur (the proportions in which the elements occur in molybdenite) as molybdate and sulphate, respectively, is neutralised with ammonia to methyl orange, treated with 5 ml. of glacial acetic acid and 12·5 g. of ammonium acetate, diluted to 200 ml., heated to boiling, and titrated dropwise with the lead solution until a drop fails to give a colour reaction with a freshly-prepared 0·5 per cent. tannin solution on a porcelain tile; the molybdate solution is then added from a 1-ml. pipette until the tannin reaction just re-appears. The suspension is boiled for 15 to 20 minutes and the precipitate is allowed to settle, filtered off, and washed with 2 per cent. ammonium acetate solution. For accurate work the precipitate should be returned to the beaker and dissolved by heating with dilute hydrochloric acid. The boiling solution is treated with 1:1 ammonia until a slight permanent turbidity is produced, then with 10 g. of ammonium acetate, and boiled for 15 to 20 minutes. The precipitate is collected, washed as before, and discarded. The combined filtrates are boiled down and finally evaporated on a water-bath with hydrochloric acid for the expulsion of the acetic acid, after which the beaker is covered and excess of strong nitric acid is added, the heating being continued until all effervescence has ceased. The cover and sides of the beaker are then rinsed down, and the nitric acid is removed in the usual manner by evaporation to dryness and two successive evaporations with hydrochloric acid. The sulphuric acid is then precipitated in the usual manner with barium chloride. A blank test on the reagents used should not be omitted.

The results of four test analyses by this process are given below. Pure molybdic oxide dissolved in dilute ammonia was added to measured amounts of a standard solution of ammonium sulphate (Analar), the sulphate-content of which had been determined gravimetrically. In Exp. 1, the lead molybdate was not re-precipitated, the single treatment resulting in a fairly appreciable negative error. In Exp. 2, the lead molybdate was re-treated, and the two filtrates were worked up separately for sulphur. Exps. 3 and 4 were conducted as described above, the lead molybdate being re-treated and the filtrates combined. The blank was equivalent to 0·0007 g. of sulphur.

	Taken		S found			Error
Exp.	Mo	S	1st pption.	2nd pption.	Total	S
	g.	g.	g.	g.	g.	g.
1	0·300	0·1946	0·1930	—	0·1930	−0·0016
2	0·300	0·1934	0·1926	0·0010	0·1936	+0·0002
3	0·300	0·1934	—	—	0·1927	−0·0007
4	0·300	0·1934	—	—	0·1928	−0·0006

SUMMARY.—Certain methods for the separation of molybdenum from tin and from sulphur are criticised, and improved processes for the two separation cases are presented. A convenient procedure for the gravimetric determination of tin is described.

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The Determination of Thiourea by Oxidation with Selenious Acid

BY A. E. A. WERNER, PH.D., M.Sc., A.I.C.

PREVIOUS methods[1,2] outlined for the determination of thiourea depend mostly on oxidation of the thiourea molecule by iodine, and require to be carried out under carefully prescribed conditions in order to yield consistently accurate results.

The present method is based on the straightforward reaction which takes place in acid solution between thiourea and selenious acid in accordance with the following equation:

$$4CSN_2H_4 + H_2SeO_3 + 2HX \rightarrow 2\left(HN{=}C\begin{matrix}NH_2\\ S{-}\end{matrix}\right)_2 2HX + Se + 3H_2O \qquad (A)$$

i.e. one molecule of selenious acid oxidises four molecules of thiourea with the production of a salt of bis-(amino-imino-methyl)-disulphide. Preliminary experiments have shown that the reaction proceeds quantitatively over a wide range of thiourea concentrations, and it therefore offers the basis for a rapid and accurate method for the determination of thiourea in solution.

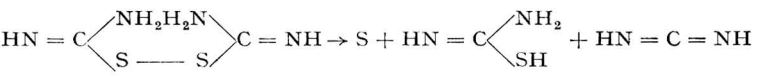
A known excess of a standardised solution of selenious acid is added to the thiourea solution, and the amount of selenious acid left unused is determined either iodimetrically or by direct titration with standard alkali. Since selenious acid reacts with potassium iodide in acid solution with the liberation of iodine according to the following equation:

$$4KI + H_2SeO_3 + 2H_2SO_4 \rightarrow 2K_2SO_4 + Se + 3H_2O + 2I_2 \qquad (B)$$

this reaction affords an admirable method for its determination, the liberated iodine being determined in the usual manner with $N/10$ sodium thiosulphate solution; the end-point is very sharp, and the violet colour of colloidal selenium does not interfere, as the selenium precipitate is readily coagulated, on shaking, to a bulky precipitate, leaving a colourless supernatant liquid (*cf.* Muthman and Schäfer[3]). Selenious acid can also be determined by direct titration with standard alkali, sodium alizarinate being used as indicator; a sharp colour-change from yellow to red occurs at a point corresponding with the formation of sodium hydrogen selenite. However, the iodimetric method is preferable owing to its greater accuracy, as the titration figure is four times as great for the same normality.

REAGENTS REQUIRED.—Solution of $M/5$ selenious acid in $N/5$ sulphuric acid; $N/10$ sodium thiosulphate solution; 5 per cent. potassium iodide solution; $N/20$ sodium hydroxide solution. The solution of selenious acid is made by dissolving 3·22 g. of selenious acid in 200 ml. of water, to which 50 ml. of N sulphuric acid have been added; this solution is then accurately standardised by adding 2 ml. to excess of potassium iodide solution and determining the liberated iodine by titration with $N/10$ sodium thiosulphate solution, or alternatively by direct titration with $N/10$ sodium hydroxide solution.

METHOD.—The following technique has been found to yield consistent and accurate results. Ten ml. or 5 ml. (depending on the concentration) of the unknown thiourea solution are added to 10 ml. of the standard selenious acid solution. After the fine precipitate of selenium has been coagulated by addition of sodium chloride, the solution is filtered through a sintered glass filter, and the clear filtrate is made up to 100 ml. with water. Twenty-five ml. portions of the filtrate are added to excess of potassium iodide solution, a few drops of sulphuric acid are added, and the iodine liberated is titrated with $N/10$ sodium thiosulphate solution. The filtrate should not be left standing before the titration, because in the now dilute acid solution the salt of bis-(amino-imino-methyl)-disulphide slowly decomposes with precipitation of sulphur and generation of more thiourea, which would react further with the selenious acid. This decomposition is represented by the following equation:



To illustrate the degree of accuracy obtainable by this method some typical results are included in the following tables.

TABLE I

RESULTS OF THIOUREA DETERMINATION. IODIMETRIC METHOD

Volume of thiourea soln. added ml.	Volume of selenious acid ml.	Thiosulphate titre per 25 ml. ml.	Thiourea	
			Found Per Cent.	Theoretical Per Cent.
0	10	10·60	—	—
10	10	8·95	0·50	0·50
		9·00	0·48	
10	10	8·00	0·79	0·80
		7·95	0·80	
10	10	7·40	0·97	1·00
		7·35	0·99	
5	10	8·10	1·52	1·50
		8·15	1·49	
5	10	8·00	1·95	2·00
		7·90	2·00	
5	10	3·70	4·19	4·00
		3·75	4·15	

From equations (A) and (B) it follows that 1000 ml. of $N/10$ sodium thiosulphate correspond with 7·6 g. of thiourea. Therefore, according as 10 ml. or 5 ml. of the thiourea solution are used, and when 25 ml. of the filtrate are taken for the titration, the percentage of thiourea is given by the expression:

$$0{\cdot}0076 \times (x - y) \times 40 \quad \text{or} \quad 0{\cdot}0076 \times (x - y) \times 80$$

where x = titre without the addition of thiourea; y = titre after the addition of thiourea.

TABLE II

RESULTS OF THIOUREA DETERMINATIONS. DIRECT ACID TITRATION

Volume of thiourea soln. added ml.	Volume of selenious acid ml.	N/20 NaOH, titre per 25 ml. ml.	Thiourea	
			Found Per Cent.	Theoretical Per Cent.
0	10	14·90	—	—
10	10	13·60	0·79	0·80
		13·70	0·76	
10	10	13·00	1·15	1·10
		12·90	1·20	
5	10	13·70	1·46	1·50
		13·65	1·52	
5	10	13·30	1·95	2·00
		13·35	1·90	

From equation (A) it follows that 1000 ml. of $N/10$ sodium hydroxide are equivalent to 30·4 g. of thiourea. Therefore, according as 10 ml. or 5 ml. of the thiourea solution are taken, and when 25 ml. of the filtrate are used for each titration, the percentage of thiourea is given by the expression:

$$0{\cdot}0304 \times (\mathrm{X} - \mathrm{Y}) \times 20 \quad \text{or} \quad 0{\cdot}0304 \times (\mathrm{X} - \mathrm{Y}) \times 40$$

where X = $N/20$ NaOH titre without addition of thiourea; Y = $N/20$ NaOH titre after addition of thiourea.

I wish to express my thanks to Dr. E. A. Werner for his many valuable suggestions.

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January, 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.

A RAPID STABILITY TEST FOR SEWAGE EFFLUENTS

THE methylene-blue test for relative stability would be of greater value if it took less time to complete. Many workers have attempted to modify the procedure with the object of reducing the time, but most of the attempts have succeeded only at the expense of the simplicity of the original test. Since methylene blue is only one of a large number of oxidation-reduction indicators, there appears to be no particular reason for retaining it in preference to other indicators, especially when its varied toxic effects—as shown, for example, by the work of Yudkin[1]—are taken into account. Remy[2] has compared the sensitivity of dichlorophenol-indophenol with that of methylene blue and has found it to react more easily and with greater certainty in presence of putrescible sulphur compounds.

While investigating the adsorption and reduction of dyestuffs by activated sludge, Dickinson[3] found that methylene green (nitro-methylene blue) is reduced very rapidly and that the reaction is not inhibited by the presence of air. This reaction has been studied in some detail, and the rate of reduction at constant temperature has been found to correspond with the equation $t = aC^n$, where t is the time taken to reduce a quantity C, and a and n are constants. The quantity n appears to be constant for the reaction, while a varies with the sludge. Since all sewage effluents contain particles of sludge in suspension, or the active principles thereof, it was thought profitable to explore the possibilities of methylene green as a substitute for methylene blue. The following method was eventually adopted:—Methylene green solution (0·2 ml. of an aqueous solution containing 2 g. per litre), 0·2 ml. of an aqueous solution of methyl orange (1 g. per litre), and 1 ml. of $M/15$ potassium dihydrogen phosphate solution are measured into a 4-oz. bottle of the type usually used for the methylene-blue test. The bottle is then filled with the sample under examination and incubated at 80° F. The time taken for the solution to change colour from green to yellow is measured. If a "blank" is used for comparison, the end-point is more clearly seen. The methylene green was prepared by the method given by Cumming, Hopper and Wheeler.[4]

The methyl orange was included to render the end-point more distinct; it has no other influence on the test. The phosphate buffer is essential, as in its absence the *p*H of the sample rises, the methylene green changes colour, and the reduction is inhibited. The following table summarises the results obtained:

Methylene green reduced in:	B.O.D.*	No. of successful tests	No. of exceptions
Less than ½ hour ..	>4·0	33†	2
½ to 1 hour	3·0 to 5·0	13	0
1 to 8 hours	2·0 to 4·0	12	3
More than 8 hours ..	Less than 2·0	18	1
	Total:	76	6

* B.O.D.: Biochemical oxygen demand, parts per 100,000.
† Only 4 had a B.O.D. less than 5·0.

The B.O.D.'s were measured variously by the Winkler azide method in 5 days at 65° F., and by the ordinary Winkler method in 3 days at 80° F. Of a number of effluents from a percolating filter, all took at least 30 hours to decolorise the methylene green, and all had a B.O.D. of less than 2·0. Effluents from contact beds reacted with much less certainty than any of the other samples examined.

It is important to point out that the reduction of methylene green is essentially different from the reduction of methylene blue, for whereas the latter is employed as an indicator, the former is used as a reagent, and its reduction does not indicate the exhaustion of dissolved oxygen in the sample.

We have found that the methylene-green test is a useful adjunct to routine analysis, especially when it is desired to form a provisional opinion quickly. It has also proved useful in determining the amount of dilution water to be added to doubtful samples when preparing the B.O.D. tests.

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E. MCGREGOR WEIR

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March 30th, 1940

Department of Scientific and Industrial Research

METHODS FOR THE DETECTION OF TOXIC GASES IN INDUSTRY

PHOSGENE*

OCCURRENCE.—Phosgene (carbonyl chloride) may be encountered in dangerous concentrations in works manufacturing organic chemical and pharmaceutical products, and, in particular, dyestuffs; also as a decomposition product of carbon tetrachloride (used, for example, in fire extinguishers).

POISONOUS EFFECTS.—Phosgene causes severe damage to the alveoli of the lungs, and this is followed by pulmonary oedema, resulting in asphyxiation. The immediate symptoms produced by even a fatal dose may be relatively mild—a little coughing, tightness of the chest and lachrymation—but, after apparently recovering, the patient may become progressively and acutely ill, owing to the injury to the lungs. An atmosphere containing 1 part (by vol.) of the gas in 6000 may cause lung injuries in 2 minutes, 1 part in 30,000 is very dangerous, and 1 part in 200,000 is probably fatal in 30 minutes. The maximum permissible concentration for a prolonged period is about 1 in 1,000,000 (0·004 mg. per litre).

DETECTION.—The yellow or orange stain produced by phosgene on test-paper containing diphenylamine and *p*-dimethylaminobenzaldehyde has been adopted as the standard test for the detection of the gas in industry. The test, which is capable of detecting about 1 part of phosgene in 1,000,000 of air, has been made quantitative by drawing known volumes of the atmosphere through a definite area of the test-paper by means of a hand-pump of specified capacity, and noting the number of strokes required to produce stains of certain intensity; the corresponding concentration is then found by reference to standard stains on a colour chart supplied with the pamphlet. The stains produced by phosgene are only transient, and it should also be noted that the test-papers are sensitive also to chlorine or hydrogen chloride. To remove traces of these gases the atmosphere is passed through a guard-tube containing pumice granules impregnated with sodium thiosulphate before it comes in contact with the test-paper.

* Leaflet No. 8. H.M. Stationery Office, York House, Kingsway, London, W.C.2. 1939. Price 2s. 6d. net. Further copies of the Standard Stains, price 2s.; by post 2s. 3d.

Western Australia

ANNUAL REPORT OF THE CHEMICAL BRANCH, MINES DEPARTMENT, FOR 1938

THE Chemical Branch of the Mines Department, which is under the direction of the Government Analyst (Dr. E. S. Simpson), undertakes the chemical work of all the Government Departments. The total number of samples examined during the year was 6036, and these are classified into three sections: (1) food, drugs and toxicology (893), (2) mineralogy and geo-chemistry (2987) and (3) agriculture and water supply (2156). Of the 120 samples of food, 32 were below standard.

The Food and Drugs Advisory Committee dealt with the following questions among others:

Preservatives in Butter.—The regulation dealing with butter was amended by withdrawing the permission to use any preservative in it other than salt. Previously boric acid to the extent of 0·3 per cent. was allowed.

Labelling of Baking Powder.—A request that the words "acid phosphate" should be removed from labels on baking powder containing this substance was refused. This was on account of the large amount of fluoride that had been found in certain commercial acid phosphates.

n-*Butyl*-p-*hydroxybenzoate as Preservative.*—Pending further information, it was decided not to add this substance (which is sold under several trade names) to the list of permitted preservatives.

Arsenic and Lead Limits for Fresh Vegetables.—"Fresh vegetables" were added to the list of foodstuffs in which the amount of arsenic must not exceed the equivalent of 1–100th grain of As_2O_3 per lb., and that of lead must not exceed 1–7th grain per lb.

Points of interest to which the Report directs attention include the following:

"SWEET MANGO CHUTNEY."—A sample bearing this label was found to contain only traces, if any, of mango, the main constituents being apple, onion and tomato, with raisins, currants, cloves, salt, etc.

VITAMIN C CONTENTS OF WEST AUSTRALIAN FRUITS.—The very high proportions found in a large yellow guava (1·10 mg.) and a papaw (0·98 mg. per g.) are noteworthy. Figures for other fruits agreed closely with those found in other parts of the world, the richest common fruits being orange, mandarin, lemon and "banana passion fruit" (also known as taxonia).

INDUSTRIAL POISONING WITH LEAD AND ARSENIC.—An investigation was made of the liability of men engaged in the Wiluma gold mines to poisoning with lead and arsenic. Eighty-nine specimens of urine and 29 specimens of hair and nails were examined, and the following range of figures was recorded:

	Lead as Pb p.p.m.	Arsenic as As_2O_3 p.p.m.
Urine	nil to 0·75	0·07 to 3·75
Hair	—	nil to 3880
Nails	—	9 to 1250

In most cases in which high results were obtained the patients showed symptoms of poisoning with lead or arsenic or both.

DETERMINATION OF LEAD IN URINE.—The following rapid method has been found to give accurate results. Organic matter is destroyed with the minimum amount of sulphuric and nitric acid plus a little perchloric acid, and the dithizone method of extraction is employed in presence of ammonium citrate and potassium cyanide at *p*H 9·0 to 9·5. The excess of dithizone is removed, and the red colour of the lead complex is compared with the colours obtained under similar conditions with standard lead solutions.

LEAD COMPOUNDS IN CELLULOSE SPRAY PAINTS.—The presence of considerable quantities of lead compounds was proved in two kinds of lacquers used in the spray painting of motor vehicles. Tests of the respirators worn by the workmen showed that they were effective in retaining lead, but constant watching is necessary to ensure that the spray-painting regulations are observed, and that the respirators fit closely and are frequently cleaned and renewed.

POISONOUS FUMES FROM AN ARC LIGHT.—As the result of a complaint by an association of employees an investigation was made of the conditions in the operating room of a cinema where blue flame carbons are used. During the working of the arc, and especially on making contact, white fumes arise from the incandescent zone and form a white deposit in the chamber unless conducted away by an efficient draught. Analysis showed the carbons to be copper-sheathed and to contain a core of cerium fluoride cemented with a silicate composition. The fumes were found to consist of cerium compounds with some fluorine and copper. The symptoms experienced by the operatives were consistent with the ingestion of small amounts of copper; in view also of the known toxicity of fluorine compounds, recommendations were made for improved ventilation and frequent removal of any deposit.

PINE SEEDLINGS DISEASE AND SILVER.—A number of pine seedlings and needles were examined spectrographically to see if any mineral deficiency could be detected. Interesting results were obtained with seedlings from certain plantations where a disease characterised by stunted growth, discoloration and altered pose of the needles was manifest. Spectrographic analysis showed larger amounts of silver in the unhealthy than in the healthy pines.

POISONOUS PRINCIPLE OF ZAMIA FRUIT.—Parts of the fruit of the zamia plant (*Macrozamia Fraseri*, Miq.), common locally, are poisonous to cattle, producing gastro-intestinal irritation. The fruit consists of a hard seed or nut containing a starchy endosperm surrounded, when ripe and fresh, by a thin fleshy layer of mesocarp with an orange-red epicarp. Only the seeds were found to be poisonous—a toxic principle not precipitated by lead acetate, and not extracted by immiscible solvents, being present. The fruit pulp surrounding the seeds contained 14 per cent. of a bright orange-coloured oil, which appears to contain a considerable amount of carotene and closely resembles palm oil in its physical and chemical constants. Further work is in progress.

STANDARD FOR IRRIGATION PURPOSES.—Twenty-four samples of waters have been examined with the object of fixing a standard for irrigation purposes. It was found that waters containing up to 258 grains of total salts per gallon were being used for the irrigation of grape vines, and waters containing up to 276 grains for lucerne, rhubarb, cabbage, and cauliflower cultivation. The sodium chloride content of these waters was usually 75 per cent. or more of the total salts. These figures are much higher than the usually accepted standards for irrigation waters.

COPPER IN APPLE LEAVES.—Samples of leaves from apple trees were examined. In some the soil round the trees had been treated with copper sulphate, and in others copper sulphate had been injected into the trees. The copper found in the leaves ranged from less than 1 p.p.m. to 5·1 p.p.m. in the dried leaves.

Coal Mines Act, 1911

PRECAUTIONS AGAINST COAL DUST*

THIS pamphlet is in three Parts: I The Coal Mines General Regulations (Precautions against Coal Dust), 1939, showing certain modifications made temporarily in view of difficulties created by the war (S.R. & O., 1939, No. 1804). II. The Mine Dust Analysis Order, 1939, prescribing the methods to be followed in analysing samples of mine dust collected in accordance with the Regulations. III. A Memorandum for the information of chemists who are called upon to analyse samples of mine dust for the purpose of the Regulations.

PART I. PRECAUTIONS AGAINST COAL DUST.—The Regulations provide that in every part of the "road" of the mine which is accessible the dust that can be raised into the air shall contain, when tested in the manner prescribed, not less than the percentage of incombustible matter set out in the Schedule to the Regulations (*infra*) according to the volatile matter content of the coal.

The percentage of incombustible matter means the actual percentage of incombustible matter (including moisture) contained in the dust, plus any percentage allowance permitted on account of any of the incombustible matter which is of superior efficacy as compared with ordinary shale dust. The permitted percentage allowance, if any, shall be calculated from the analysis of the dust in the appropriate manner prescribed by the Board of Trade. (*Note.*—For the present no allowances under this provision are to be permitted.)

The volatile matter content of the coal means the average volatile matter content calculated on an ash-free dry basis of the seam of coal worked through the road (or, if more than one seam is worked, of that seam which has the highest average content) and shall be deemed to be more than 35 per cent. unless the contrary has been proved by an analysis made and communicated to the Inspector within the previous 12 months. The analysis shall be made by one of the methods specified by the *British Standards Institution*, and the sample of coal used for such analysis shall be taken either from a representative section of the seam or from a representative quantity of the run-of-mine coal from the seam. (*Note.*—The effect of the temporary amendment to Regulation 10 is to substitute 25 per cent. in this definition.)

The foregoing requirements do not apply to anthracite mines or to certain other mines in which specified conditions (described in detail) obtain. The incombustible dust used for the purpose of these Regulations shall be:—(*a*) of such fineness that of the dry dust which passes through a 60-mesh sieve not less than 50 per cent. by weight and, except with the permission in writing of the Board of Trade, not more than 75 per cent. by weight shall pass through a 240-mesh sieve; (*b*) of such character that it is readily dispersable into the air, when in use in places where it is not directly wetted by water from the strata, and does not cake, but is dispersed into the air when blown upon with the mouth or by a suitable appliance.

No dust shall be used for the purpose of complying with the Regulations of a kind which may be prohibited by the Board of Trade on the ground that it is injurious to the health of persons working in a mine.

Another Regulation (6) prescribes steps to be taken to see that the Regulation (3) relating to the prevention, suppression, collection and removal of coal dust and for treating with incombustible dust are complied with.

Under Regulation 10 these Regulations shall come into force on January 1st, 1940, *except that in so far as any provision requiring the dust to contain more than*

* Regulations, Mine Dust Analysis Order and Memorandum on Methods of Analysis. M. & Q., Form No. 128, December, 1939. London: H.M. Stationery Office. Price 6d. net.

65 *per cent. of incombustible matter shall come into force on January* 1*st*, 1941. (*Note.*—The provision in italics is temporarily suspended.)

SCHEDULE (REGULATION 3)

MINIMUM PERCENTAGE OF INCOMBUSTIBLE MATTER REQUIRED FOR COALS OF VARIOUS VOLATILE MATTER CONTENT

Average volatile matter content of coal Per Cent. Not exceeding	Minimum percentage of incombustible matter required
20	50
22	55
25	60
27	65
30	68*
32	70*
35	72*
Exceeding 35	75*

PART II. ORDER OF THE BOARD OF TRADE PRESCRIBING METHODS OF ANALYSIS OF MINE DUSTS.—The sample shall be sieved in a specified manner through a 60-mesh sieve, or, if too damp, through an 18-mesh sieve, and the fraction passing through shall be allowed to dry for an hour and thereafter sieved through a 60-mesh sieve; a correction is applied for the percentage loss of moisture.

(A) *Dust samples which contain no Carbonates or Gypsum.*—A weighed quantity is dried between 105° and 110° C. and the loss in weight is reckoned as moisture. The residue is heated at red-heat in an open vessel until it ceases to lose in weight. The weight of the incinerated residue added to the weight of moisture gives the amount of incombustible matter; it shall be expressed as a percentage of the total weight of the sieved dust.

(B) *Dust samples which contain Carbonates.*—(1) The moisture is determined at 105–110° C. (ii) The residue is heated at 950° C. (at least) until it no longer loses weight. (iii) The weight of carbon dioxide evolved from the dust is determined directly or calculated from the volume of carbon dioxide (or by other method approved by the Board of Trade). (iv) The sum of the weights of moisture, carbon dioxide and incinerated residue are reckoned as incombustible matter.

(C) *Dust samples which contain Gypsum.*—(i) The sample of sieved dust is dried between 135° and 140° C. and the weight lost is reckoned as moisture. (ii) The residue is heated at red-heat in an open vessel until it ceases to lose weight. The sum of the moisture and incinerated residue is reckoned as incombustible matter.

(D) *Dust samples which require special Methods of Analysis.*—These are to be analysed by such methods as shall be prescribed by the Board of Trade.

PART III. A DESCRIPTION OF APPROPRIATE ANALYTICAL METHODS.—*Moisture-Content.*—The methods of determining the moisture in coals by heating in nitrogen or in a partial vacuum (British Standard Specification, No. 735, of 1937), may be used for mine dusts. If, however, the dust contains gypsum the moisture must be expelled between 135° and 140° C. (or with advantage at 180° C. if air is rigorously excluded). The following method, however, is sufficiently accurate for the purpose:—One g. of the dust is spread over the bottom of a silica dish (2 inches in diameter) provided with a well-fitting cover. The dish without its cover is heated at 105° to 110° C. (or at 135° to 140° C. if the dust contains gypsum) in a suitable oven for 1 hour, after which it is covered, cooled in a desiccator and weighed.

* The effect of the temporary amendment to Regulation 10 is to substitute a figure of 65 per cent.

Ash-Content.—The dish containing the dried dust is heated without its cover in a muffle furnace at 900° to 950° C. (not less than 950° C. if carbonates are present) until constant in weight. Provision should be made for the free circulation of air over the dust. When the volatile matter has been expelled, the final incineration is completed in the hottest part of the muffle.

Carbon Dioxide.—Dusts containing carbonate are decomposed with acid in a calcimeter, and the volume of carbon dioxide is measured and corrected to normal temperature and pressure. A suitable form of calcimeter is described and illustrated, and a separate nomogram for converting the volume of carbon dioxide into its percentage in the dust is provided in the pamphlet.

The Mines Department will welcome any suggestions for additions or further explanations that would make the memorandum on analysis more useful.

British Non-Ferrous Metals Research Association

QUANTITATIVE SPECTROGRAPHIC ANALYSIS WITH THE MICROPHOTOMETER*

THE Association has published a booklet that is well worth studying by anyone using or contemplating the use of spectrographic methods for quantitative analysis. The use of a microphotometer is a refinement of spectrographic technique which is only justified when the many factors that may affect accuracy and reproducibility have been studied and are under control.

The subject matter of the report falls under three main headings. First, a review of published information is given in some detail, this being classified in short sections, each dealing with an aspect of spectrographic technique of importance in quantitative work. Attention cannot be drawn to all the fourteen sub-headings, but such matters as "size and shape of electrodes," "pre-sparking period," "calibration of the photographic plate," and "effects due to alloying constituents" will give some indication of the scope of the discussion.

Secondly, a summary of published procedures is given for a large number of alloys listed under the following principal constituents:—aluminium, copper, lead, magnesium, nickel, tin and zinc. The accuracy claimed for the method and a reference to the original work are given in each instance.

The third part consists of a bibliography of recent papers dealing with applications of the microphotometer. It is of considerable value to have references dealing with a specialised branch of one subject collected in this manner.

B. S. C.

* Part I. A Review of Published Work, by D. M. Smith, B.Sc., D.I.C., F.Inst.P. (British Non-Ferrous Metals Research Association; Research Report, Association Series, No. 524.)

Olive Oil Specifications for the Norwegian Canning Industry*

CANNING tests have shown that types of oil that are suitable for French and Portuguese sardines are not necessarily suitable for Norwegian brisling and sild. The Norwegian trade associations concerned with the canning of brisling and sild require all fish to be packed in olive oil answering to the following specifications:

(1) The oil must be clear, free from water, pulp, or other impurities; *i.e.* it must be carefully filtered.
(2) The colour must be pale yellow with only a tinge of green or brown.
(3) Flavour and odour must be pure and good.
(4) The acid-content (calculated as percentage of free fatty acid) must not exceed 1·7 per cent., assuming that the oil consists exclusively of expressed (not chemically refined) oil.
(5) The content of chemically refined expressed oil used for blending must not exceed 30 per cent. of the mixture. The chemically refined oil must be of good quality.
(6) The oil must not contain refined extracted oil (sulphur oil).
(7) The oil must be resistant to cold; *i.e.* there must be no appreciable deposit of solid fat when the oil is cooled to between 4° and 8° C.
(8) The iodine value must not exceed 88.
(9) The rancidity must not exceed 10 red Lovibond units in the quantitative Kreis test.
(10) The ash-content must not exceed 30 mg. per litre.

With regard to Specification 5, which, it is stated, is the one most disputed, it is pointed out that if chemically refined oil is used as the major part of the mixture, it may, unless it is of absolutely first-class quality, cause deterioration of the oil on storage. The limit of 30 per cent. as the maximum proportion in the mixture is in accordance with the views of well-known olive oil experts in Tortosa, Reus and Borjas Blancas. Moreover, systematic canning tests with brisling and sild have shown that, even if mixtures containing 80 per cent., or more, of refined expressed oil are used by Spanish and French canners, large quantities of the refined oil are not so suitable for the Norwegian fish.

The quantity of refined expressed oil in a mixture is estimated by an optical method (due to Lunde and Stiebel), and the results are expressed in terms of maximum blue fluorescence. If the figure is less than 130 the oil will be accepted as pure expressed oil. The figure increases with the amount of refined oil, up to about 600 for pure chemically refined expressed oil (*cf. Z. angew. Chem.*, **1933**, **46**, 243, 796).

With regard to Specification 7, experience has shown that Spanish oils, whether from the north or south, usually show no tendency to deposit stearine under the conditions given. Oils from Tunis and Algeria (French oils) do not remain so clear as Spanish oils, and the quantity of stearine deposited during the test increases with the locality of the oil from north to south in those countries.

* Laid down by the Research Laboratory of the Norwegian Canning Industry. E. Mathiesen. *Tidsskrift for Hermetikindustri*, Jan., 1940, 10–12.

British Standards Institution

WE have been asked to give prominence to the particulars of the following Standard Specification:

BS/ARP 27. TESTING INCOMBUSTIBLE MATERIAL RESISTANT TO INCENDIARY BOMBS*

With a view to minimising the dangers resulting from possible incendiary bomb attacks of the enemy, active consideration has been given by the A.R.P. Department of the Ministry of Home Security to the development of materials which could be used in the attics and roof spaces of buildings so as to confine and afford protection against the incendiary effect of the bomb.

In order to assist in the development of such materials a test has been devised by means of which the performance of materials under incendiary bomb effect may be ascertained.

This method of test, together with the results which should be expected from a suitable material, has been made the subject of a standard in the BS/ARP series, which has just been issued by the British Standards Institution as BS/ARP 27.

It is stated in a note to the Standard that the method of test is one that has been in use for some time at the testing station of the Fire Offices' Committee, and it is indicated that the tests on materials will be made at the Elstree Testing Station on behalf of manufacturers who would like to have their materials examined.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Milk Fat from the Turkish Buffalo. A. Heiduschka and F. Cicekdagi. (*Z. Unters. Lebensm.*, 1940, **79**, 150–153.)—Buffalo milk constitutes 8·3 per cent. of the total milk production of Turkey. In comparison with cows' milk it has a higher and more variable fat-content, ranging from 4 to 10 per cent. with an average of about 7·6 per cent. It is rarely used in the fresh state because its high fat-content renders it somewhat indigestible. The butter is usually prepared from Yoghurt and the butter-milk is converted into cheese. The milk is also used for the preparation of a very thick cream (Kaymak). The butter is pale, sometimes almost white, with a pleasant cream-like taste and a good aroma. The butter-fat gave the following constants:—sp.gr., 0·9273; m.p., 47·6° C.; solidifying p., 41·3° C.; n_D^{20} (Zeiss–Wollny), 41·3; saponification equiv., 318·6; iodine value (Hübl), 37·78; acetyl value, 32·33; Hehner value, 87; Reichert value, 31·5; Polenske value, 0·6; acid value, 3·8; total fatty acids, 94·05 per cent.; unsaponifiable matter, 0·165 per cent. The total fatty acids had the following composition (per cent.):—butyric acid, 4·24; caproic acid, 1·28; caprylic acid, 0·42; capric acid, trace; lauric acid, 2·99; myristic acid, 7·22; palmitic acid, 25·63; stearic acid, 16·18; arachidic acid, 3·24; oleic acid, 35·20; linolic acid, 1·98. This composition does not differ materially from that of the milk-fat of buffaloes in other districts. A. O. J.

Ultra-violet Absorption of Yeast Extracts and Meat Extracts. J. Schormüller. (*Z. Unters. Lebensm.*, 1940, **79**, 46–57.)—Numerous measurements of the ultra-violet absorption spectra of solutions of 5 yeast extracts from different sources gave curves with a maximum value at 260 to 262$m\mu$ and a

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minimum value at 233 to 236$m\mu$. According to Heyroth and Loofbourow (*Nature*, **1934**, **134**, 461; *Biochem. J.*, 1936, **30**, 65), vitamin B_1 exhibits 2 maxima, at 235 and 268$m\mu$ respectively, and other authors have reported similar results. Quantitative considerations indicate that in the concentrations used (1 g. of extract in 1000 ml. of water) the amounts of the vitamins B_1 and B_2 are too small to account for the measured absorption. According to Williams (*J. Amer. Chem. Soc.*, 1935, **57**, 1093) and Smakula (*Z. physiol. Chem.*, 1935, **230**, 231) the maximum value at 260$m\mu$ is characteristic of the pyrimidine ring of aneurin, and it is now suggested that since yeast extracts exhibit only one maximum value, pyrimidine bodies are principally responsible for the form of the curve. Such bodies are formed by decomposition of the nucleic acids of yeast during preparation of the concentrate. The ultra-violet spectrum of yeast nucleic acid showed remarkable resemblance to that of the extract. Aneurin alone is not therefore responsible for the absorption, and measurement of the maximum absorption is no indication of the vitamin B_1 content of the extract. A comparison of the curves given by yeast nucleic acid with those of the concentrates indicates that the extracts are much richer in pyrimidine bodies. Yeast gum was also investigated, but showed no characteristic absorption. The extract was separated by ultra-filtration into constituents of high and low molecular weight, and it was found that the characteristic portion of the curve is due to the low molecular constituents which include the decomposition products of the nucleic acids. Similar experiments with fractions separated by dialysis were less successful, but tended to confirm this conclusion. Heating in a boiling water-bath for 5 hours caused no change in the absorption curve of yeast extract. The absorption curves of meat extracts were compared with those of yeast extracts. Although the general course of the curves is the same, they do not exhibit distinct maximum or minimum values, but an inflexion-like bending of the curves suggests that the selective absorption of such decomposition products as tyrosine, tryptophane and phenyl-alanine is masked by other substances. By a study of the absorption curves of different mixtures of yeast extract and meat extract it was found that the characteristic form of the yeast extract curve was perceptible when 33 per cent. was present in meat extract, and as this percentage was increased the curve conformed more and more closely to that of pure yeast extract. Examination of the absorption curves of lactoflavin concentrates prepared from meat extracts showed that the absorption is masked by that of other substances, and spectrographic identification of vitamin B_2 in meat extract is thus not directly possible. The spectrographic method cannot therefore replace chemical and biological methods of determining vitamin contents, but can be used to distinguish the two types of extract and to detect admixture of yeast extract with meat extract. A. O. J.

Presence of *l*-Tyrosine in the Alcoholic Extract of Egg Yolk. L. Bracaloni. (*J. Pharm. Chim.*, 1940, **132**, 140–142.)—Serum decanted from the paste of lecithin and lutein left after distillation *in vacuo* of an alcoholic extract of egg yolk contains a small amount of a nitrogenous substance which separates in white flakes (0·10 to 0·16 g. per 200 g. of egg yolk). Its micro-crystalline form, colour reactions (xanthoproteic reaction and reaction with

Millon's reagent), m.p., and percentage of nitrogen (by Kjeldahl's method) are those of *l*-tyrosine and with formic acid it forms a derivative resembling Fischer's formyl-*l*-tyrosine (*Ber.*, 1907, **40**, 3716). Its elementary percentage composition also agrees with that of *l*-tyrosine. E. B. D.

Iodine Absorption of Honey and Artificial Honey. C. Griebel and G. Hess. (*Z. Unters. Lebensm.*, 1940, **79**, 171–177.)—Only a few kinds of honey are able to decolorise considerable amounts of *N*/100 iodine solution. Thyme honey and mint honey show pronounced absorption and a weaker absorption is shown by honey dew, heather honey and buckwheat honey. If the iodine absorption is compared with the titration with dichlorophenol-indophenol it is found that with mint and thyme honeys the ascorbic acid titre is only slightly lower than the iodine absorption, but with honey dew, buckwheat and heather honeys it is considerably lower. Iodine titration yields results agreeing closely with the dichlorophenol-indophenol titration if a relatively large amount of potassium iodide is added to the honey solution before titration. The iodine absorption figure obtained in the absence of potassium iodide is not independent of the amount of sample used, and it is therefore necessary to work with the same amount of sample, *e.g.* 5 g., except with mint and thyme honeys where 1 to 2 g. is sufficient. The difference between the iodine absorption with and without the addition of potassium iodide is considerable for most honeys, but the cause of the difference is not yet known. An investigation was made in the following manner:—The honey (5 g.) was dissolved in 25 g. of cold 2 per cent. metaphosphoric acid, which served to precipitate albuminous substances and to stabilise the ascorbic acid. The solution was titrated with *N*/100 iodine solution in presence of starch, and when the end-point was reached 1 ml. of iodine solution was added in excess and, after the lapse of 30 sec., the mixture was titrated back with *N*/100 sodium thiosulphate solution. The result of this titration calculated for 100 g. of honey was denoted by *a*. The experiment was then repeated with the difference that 1 g. of potassium iodide was added before titration. The corresponding value found by this titration was denoted by *b*. With buckwheat honey the metaphosphoric acid precipitated a considerable amount of albuminous matter. This was separated by centrifuging, and weighed aliquot portions of the centrifugate were taken for titration. With artificial honey the value found for *b* was nil and the value of *a*–*b* rarely exceeded 5. With native honey (excluding heather, buckwheat and the labiate honeys and honey dew) the value of *b* varied from 1 to 4·8, and the value of *a*–*b* was, as a rule, greater than 5, but rarely exceeded 10. Among foreign honeys two samples (Canada White Clover 1939 and Salvador 1938) gave no absorption after the addition of potassium iodide. The value of *b* varied from 0 to 4, and the highest value of *a*–*b* was 9. With heather honey *b* always exceeded 5, and *a*–*b* ranged from 4 to 9. With honey dew (excluding some samples with an odour of floral honey) the value of *b* varied from 5 to 16, and that of *a*–*b* from 9 to 20. With buckwheat honey *b* varied with the source from 5·8 to 16, and *a*–*b* from 8 to 48. The value of *a*–*b* was exceptionally high for Canadian and Hungarian buckwheat honey. Since such large values do not occur with other honeys, it may be assumed that a value for *a*–*b* of over 30 indicates buckwheat honey. With buckwheat

honey it was found that the value of *b* rose after 4 months' storage, and that the value of *a* diminished, so that the value of *a–b* also diminished considerably. The following conclusions may be drawn:—Iodine absorption after the addition of potassium iodide is not influenced by invert sugar because most artificial honeys give no absorption. Although the value of *b* for artificial honey is either nil or very low, it is possible to conclude that artificial honey is present in a sample only if the value of *a–b* exceeds 5, because low absorption and difference-values up to 5 occur with artificial honey owing to the presence of added natural honey. Since only a few kinds of honey (heather and buckwheat honeys and honey dew) show a greater iodine absorption in presence of potassium iodide than the floral honeys, and since buckwheat and honey dew give difference-values that generally exceed 10, these may be recognised by their behaviour towards iodine. Verification by pollen analysis is necessary, however, because raising of the absorption value may be due to the addition of labiate honey. A. O. J.

Detection of Artificial Dyes in Tomato Pulp. H. Thaler and K. E. Schulte. (*Z. Unters. Lebensm.*, 1940, **79**, 74–77.)—Since tomato pulp is manufactured from unripe or imperfect fruit, the colour is frequently intensified by the addition of water-soluble artificial dyes. In order to determine whether such addition can be detected by adsorption analysis, preliminary experiments were made with the natural pigment of the tomato. Aluminium oxide (Merck's technically pure hydroxide heated in a shallow dish at about 400° C. until no more steam was evolved) in a column, 20 cm. high and 1 cm. broad, was used as the adsorbing substance, and the solvents employed were pyridine, benzene, carbon disulphide, ether, petroleum spirit, methyl alcohol, 70 per cent. ethyl alcohol and acetone. The artificial dyes used in the investigation were Tomato Red (3242) and Lobster Red (*Krebsrot* 2872). These were insoluble in benzene, ether, carbon disulphide and petroleum spirit, but soluble in pyridine, methyl alcohol, 70 per cent. ethyl alcohol and acetone. It was found that methyl alcohol, ethyl alcohol and acetone gave the best results, because the natural pigment when dissolved in these is not characteristically adsorbed by the aluminium oxide, whilst the artificial dyes give characteristic chromatographs. For the colouring of the pulp a little of the dye was dissolved in water and incorporated with the pulp. After an hour a portion of the pulp was warmed with the solvent at 30° C. for half-an-hour and the extract was filtered. As a rule the artificial dyes imparted a strong red colour to the surface of the aluminium oxide and to the layers immediately beneath the surface. When Tomato Red was adsorbed from solutions in ethyl alcohol or acetone a yellow zone appeared beneath the red zones. If it is desired to verify that a preparation has been manufactured from undyed tomato pulp the solvents recommended are benzene and ether. From solution in benzene the natural pigment is adsorbed by the aluminium oxide and colours its surface yellow, and below are formed in order white, rose-coloured and red zones. With an ethereal solution of the natural pigment the order of colours is yellow, white, brownish-yellow, rose. The method was used successfully to prove that the colouring matter in a preparation of herrings in tomato was the natural pigment of the fruit. A. O. J.

Thermo-stability of Fats. E. Glimm, H. Wittmeyer and W. Jahn-Held. (*Z. Unters. Lebensm.*, 1939, **78**, 285–293.)—The thermal decomposition of beef-fat, lard, coconut oil, palm oil, sesame oil, arachis oil, olive oil and triolein was studied at temperatures from 60° to 120° C., during periods of 7 days. It was found that fat-splitting by heat is independent of the percentage of free acids initially present. At temperatures below 60° C., no fat-splitting takes place, except in beef-fat and lard. The vegetable fats each show a definite decomposition temperature, at which after 3 days a pronounced increase in the acid value occurs. These temperatures are as follows:—for coconut oil, 100° C.; palm oil, 90° C.; olive oil, 90° C.; arachis oil, 80° C.; soya-bean oil, 80° C.; sesame oil, 75° C. Beef fat and triolein are remarkable in that the isotherms of acid-splitting in relation to time for beef-fat coincide for 60°, 75° and 90° C., and for triolein for 75°, 90° and 105° C. It seems that the splitting at certain temperature intervals starts with a jump. The results of the Kreis test indicate that at the beginning of the decomposition the proportion of aldehyde increases, but decreases on further decomposition. The amount of volatile fatty acids increases with rise of temperature, whilst that of unsaturated fatty acids decreases. Carbon dioxide has no influence on the thermal decomposition of lard, but has a retarding effect on the decomposition of vegetable fats. D. A.

Action of a Cuprous Iodide Reagent on Alkaloids: Precipitation and Colour Reactions. M. Péronnet and J. Guénin. (*J. Pharm. Chim.*, 1940, **132**, 142–147.)—A cuprous iodide reagent for the detection of "yperite" ($\beta\beta'$ dichloro-diethyl sulphide) has been prepared as follows (*cf.* Grignard, Rivat and Scatchard, *Ann. Chim.*, 1921, **15**, 14): To 50 g. of a 30 per cent. solution of crystallised sodium iodide, 30 drops (about 1·23 g.) of a 7·5 per cent. solution of crystalline copper sulphate are added, with shaking. If the resulting solution becomes turbid after a few hours it is filtered; it will then keep indefinitely in the dark. With yperite this gives a yellowish-white precipitate of $\beta\beta'$ diiodo-diethyl sulphide. It has been found that the reaction is characteristic of yperite only if the diiodo compound is identified (small colourless prisms, m.p. 62° C.); the reagent forms insoluble compounds with most alkaloids, the precipitates being yellowish-white, yellowish or brown. The sensitivity values for various alkaloids are given. They vary from 1 in 500 (morphine hydrochloride) to 1 in 200,000 (sparteine sulphate). The principal glycosides and barbiturates do not form precipitates either in aqueous or in hydrochloric acid solution. Characteristic colour reactions are given by: (*a*) eserine and (*b*) ephedrine. If the precipitate that the cuprous iodide reagent forms with eserine hydrochloride solutions is dissolved in ammonia, there is produced a violet-red colour which changes slowly to brown. Sensitivity: 1 in 10,000. If the cuprous iodide reagent is added, drop by drop, to an aqueous solution of ephedrine, the reagent is first decolorised and then assumes a characteristic violet colour, which is very stable in neutral solutions but disappears on acidifying. Sensitivity: 1 in 10,000. Under the same conditions adrenaline gives a reddish colour which disappears on acidifying. For all the tests, 1 ml. of reagent and 4 ml. of the alkaloid solution (1 per cent., or more dilute according to solubility) were used. E. B. D.

Study of *Centaurea scabiosa*. C. Charaux and J. Rabaté. (*J. Pharm. Chim.*, 1940, **132**, 155–162.)—The fresh leaves of *Centaurea scabiosa* L. contain about 2 per cent. of a glycuronoside, $C_{21}H_{18}O_{12}.2H_2O$ (present as a water-soluble salt of an alkali or alkaline earth metal). Hydrolysis of this glycuronoside yields glycuronic acid and a flavonol identical with the scutellareol obtained by hydrolysis of scutellaroside, the glycuronoside of *Scutellaria altissima*. The physical properties of scutellaroside given by Goldschmiedt and Zerner (*Monatsh. Chem.*, 1910, **31**, 439) differ from those of the centaury glycuronoside, but a scutellaroside prepared by the authors by the Goldschmiedt method and purified by crystallisation from 50 parts of boiling absolute alcohol was identical with the new product. The physical properties now found are:—rotation, $[\alpha]_D^{15}$, −128°; $[\alpha]_D^{17}$, −129°5 (anhydrous, $[\alpha]_D^{15}$, −138°); m.p. 230° C. (Maquenne block). E. B. D.

Biochemical

Excretion of Volatile Selenium Compounds after Administration of Sodium Selenite to White Rats. J. Schultz and H. B. Lewis. (*J. Biol. Chem.*, 1940, **133**, 199–207.)—It has frequently been asserted that, after administration of selenium salts, volatile methyl compounds are excreted through the lungs. This has now been confirmed experimentally on rats injected subcutaneously with a solution of sodium selenite at a level of 2·5 to 3·5 mg. per kg. of body-weight. After the injections the animals were placed in cages inside a respiration chamber, the gases from which were passed successively through two tubes containing hydrochloric acid (1:1), a tube containing 20 per cent. sodium hydroxide solution, and a large absorption tube containing glass wool and calcium chloride. The dry gases then entered a specially designed all-glass absorption tube containing 45 ml. of conc. sulphuric acid, and passed through a second tube containing sulphuric acid, which was connected with a vacuum water-pump. With the current of air properly regulated, all the selenium was found in the first absorption tube containing sulphuric acid. At the end of each experiment the acid was transferred to a Kjeldahl flask, 1 ml. of 20 per cent. hydrogen peroxide was added, and the mixture was gently heated for 20 to 30 minutes. After cooling, the volume of the digest was measured, and 10-ml. portions were transferred to test-tubes. Two drops of an aqueous 4 per cent. solution of codeine phosphate were added, and the solution was cooled under the tap. The tubes were stoppered and placed in the dark for 20 to 30 minutes. The colours were compared in a colorimeter with that developed by 10 ml. of a standard solution of sodium selenite in conc. sulphuric acid. When known concentrations of sodium selenite were tested, discrepancies up to 15 per cent. were found between the observed and the theoretical values, but the method was sufficiently accurate for the investigation in hand. The results showed that 17 to 52 per cent. of the selenium injected was excreted within 8 hours in the form of a volatile selenium compound that could be absorbed by conc. sulphuric acid. With two possible exceptions, the simultaneous administration of either methionine or choline chloride failed to influence the excretion. The nature of the volatile selenium compounds remains to be elucidated. F. A. R.

Basic Amino Acid Content of Human Serum Proteins. Influence of the Ingestion of Arginine on the Composition of the Serum Proteins. R. J. Block. (*J. Biol. Chem.*, 1940, **133**, 71–74.)—Dirr (*Z. physiol. Chem.*, 1939, **260**, 65) reported that the intravenous or oral administration of arginine hydrochloride resulted in a considerable increase in the arginine content of the serum proteins. This observation could not be confirmed by the author. Samples of human blood were drawn before and after administration of arginine hydrochloride, and the serum was removed by centrifuging after the blood had clotted. The sera were acidified with 5 *N* acetic acid to *p*H 4·5 and, after the addition of 3 volumes of dilute sodium chloride solution, the proteins were coagulated by heat. The precipitate was washed 3 times with hot water, and the lipids were removed by extraction with acetone, hot alcohol, hot benzene, and ether. The basic amino acids were determined by the author's silver precipitation method ("*The Determination of the Amino Acids*," Minneapolis, 1938). Arginine was isolated as the flavianate, histidine as the nitranilate, and lysine as the picrate. The molecular ratios of histidine to arginine to lysine were unaffected by the feeding of arginine. The same result was obtained when the proteins were isolated by precipitation with alcohol, instead of by heat coagulation. F. A. R.

Activation of Papain. J. S. Fruton and M. Bergmann. (*J. Biol. Chem.*, 1940, **133**, 153–156.)—The experiments described throw considerable doubt on the oxidation-reduction theory of papain activation. An inactive papain preparation (Papain A) was activated with hydrogen cyanide (HCN—Papain A) and subsequently precipitated with isopropyl alcohol (Papain B). If the activation of papain with hydrogen cyanide consisted merely in the reduction of disulphide groups to sulphydryl groups, then Papain B should have been as active as HCN—Papain A. It scarcely showed any activity at all, however, but regained nearly all the activity of the original HCN—Papain A on addition of hydrogen cyanide. This activated Papain B was precipitated by isopropyl alcohol, and the precipitate (Papain C) was again inactive, but capable of being activated with hydrogen cyanide to a product with almost the same activity as the original HCN—Papain A. Cysteine was also used as activator, with similar results. The authors suggest that the precipitation with isopropyl alcohol brings about dissociation of the active HCN—enzyme compound with regeneration of the original inactive enzyme. It seems probable that the specificities of the various activator-enzyme compounds differ, depending on the nature of the activator. The results of these experiments support the theory of Mendel and Blood (*J. Biol. Chem.*, 1910–11, **8**, 177), that hydrogen cyanide and other activators serve as co-enzymes for papain. F. A. R.

Estimation of Vitamin A and Carotene with the Photoelectric Colorimeter. C. J. Koehn and W. C. Sherman. (*J. Biol. Chem.*, 1940, **132**, 527–538.)—Dann and Evelyn (*Biochem. J.*, 1938, **32**, 1008; *cf.* ANALYST, 1938, **63**, 611) first used the photoelectric colorimeter to measure the intensity of the blue colour obtained by the action of antimony trichloride solution on vitamin A, obtaining a factor of 0·41 ± 0·05 for the conversion of $L^{1\%}_{1\,cm.}$ (620$m\mu$) into $E^{1\%}_{1\,cm.}$ (328$m\mu$). In view of the controversy over the value of the factor required to convert $E^{1\%}_{1\,cm.}$

(328$m\mu$) into biological units, it was decided to determine directly the relationship between $L^{1\%}_{1\,cm.}$ (620$m\mu$) and biological activity, and for this purpose $L^{1\%}_{1\,cm.}$ (620$m\mu$) of the U.S.P. reference cod-liver oil was determined and its potency checked by biological assay against β-carotene. At the same time the $L^{1\%}_{1\,cm.}$ (440$m\mu$) of β-carotene was determined in chloroform and "Skellysolve" solutions, and a correction factor was also determined for the amount of light absorbed by the blue colour produced by the reaction of carotene with antimony trichloride solution. The U.S.P. reference oil was found to have a biological potency of 3000 I.U. of vitamin per g., and its $L^{1\%}_{1\,cm.}$ (620$m\mu$) was 3·45, whence, using the factor 0·41 for converting $L^{1\%}_{1\,cm.}$ (620$m\mu$) into $E^{1\%}_{1\,cm.}$ (328$m\mu$), a factor of 2120 is obtained for converting $E^{1\%}_{1\,cm.}$ (328$m\mu$) into I.U. of vitamin A per g. This is in close agreement with the value of 2150 found by Mead, Underhill and Coward (*Biochem. J.*, 1939, **33**, 589) as a result of work with vitamin A-2-naphthoate. β-Carotene was found to have an average $L^{1\%}_{1\,cm.}$ (440$m\mu$) of 1645 and 1980 in chloroform and "Skellysolve" respectively. It was found that 2—log G (G is the corrected galvanometer reading) was not a strictly linear function of the concentration of vitamin A or carotene, but constants were found for use within a certain range of galvanometer readings, which do not incur an error of more than 4 per cent. For more accurate work the use of a calibration curve is recommended. F. A. R.

Ascorbic Acid Content of Rose Hips. W. Goldberg and E. O'F. Walsh. (*Pharm. J.*, 1938, 551.)—Ascorbic acid estimations were carried out on three different samples of rose hips by the 2:6-dichlorophenol-indophenol method, the procedure described by M. Olliver (ANALYST, 1938, **63**, 2) being followed. The first sample, consisting of fruits that were not quite ripe, was collected early in September and was not limited to the fruit of any particular species of rose. The second sample was collected a fortnight later and consisted of ripe fruits of a similar character. The third sample was purchased, and on subsequent enquiry proved to consist of kiln-dried hips from Germany. The unripe and ripe freshly-collected hips contained respectively 385 and 364 mg. of ascorbic acid per 100 g., whereas the kiln-dried fruits contained only 64 mg. per 100 g. The fresh material lost 80 to 90 per cent. of its vitamin content on drying at 40° C., but only about 50 per cent. on drying at 60° C. The seeds contained no ascorbic acid. F. A. R.

Estimation of Adermin (Vitamin B_6) in Urine. J. V. Scudi, H. F. Koones and J. C. Keresztesy. (*Proc. Soc. Exp. Biol. Med.*, 1940, **43**, 118–122.)—In an investigation designed to study the excretion of adermin in the rat, it was necessary to find a method of assaying the vitamin. This was successfully achieved by treatment with 2:6-dichloro-quinone chlorimide reagent, and measurement of the resulting colour in a photoelectric colorimeter. The urine samples were made strongly alkaline to thymol blue (pH above 9·6) with 30 per cent. sodium hydroxide solution. After being allowed to stand overnight, 1-ml. aliquot portions of the urine were neutralised to pH 7 to 7·5, and the volume was adjusted to 25, 50 or 100 ml. as necessary. For the adjustment of the pH, bromothymol blue was used as external indicator. To 5 ml. of the diluted urine were added 5 ml. of veronal buffer (prepared by dissolving 18 g. of sodium diethyl barbiturate in 700 ml.

of water and titrating to *p*H 7·6 with dilute hydrochloric acid using the glass electrode, the solution being filtered from the precipitated barbituric acid) and 20 ml. of a butyl alcoholic solution of the chlorimide reagent (100 mg. of 2:6-dichloroquinone chlorimide dissolved in 1600 ml. of acid-free butyl alcohol). The tubes were briefly but vigorously shaken, and after 5 minutes were shaken again. After an additional 10 minutes the two layers were separated by centrifuging and the supernatant butyl alcohol layer was pipetted into 10 ml. of a fresh veronal solution. After shaking, the two layers were again separated by centrifuging, and the washing process was repeated. Fifteen ml. of the washed butyl alcohol layer were pipetted into a colorimeter tube containing 5 ml. of absolute ethyl alcohol, the contents were thoroughly mixed and the colours were measured 40 minutes after the addition of the reagent in an Evelyn colorimeter, filter No. 660 being used. Under these conditions 100 per cent. recoveries were obtained when the pure vitamin was added to urine. F. A. R.

Vitamin K Activity of 4-Amino-2-Methyl-1-Naphthol and 4-Amino-3-Methyl-1-Naphthol. A. D. Emmett, O. Kamm and E. A. Sharp. (*J. Biol. Chem.*, 1940, **133**, 285–286.)—The hydrochlorides of 4-amino-2-methyl-1-naphthol and 4-amino-3-methyl-1-naphthol are readily soluble in water or saline solution, and can be administered either orally or parenterally. The former was found to have a potency of 1200 units per mg., expressed in terms of 2-methyl-1,4-naphthoquinone as standard (1000 units per mg.), and the latter a potency of 780 units per mg. Thus the former is about 3 times as active as vitamin K_1; it has given excellent results clinically, both in obstructive jaundice and in neonatal haemorrhage. [Dam, Glavind and Karrer (*Helv. Chim. Acta*, 1940, **23**, 224), on the other hand, report methyl-naphthoquinone to be $2\frac{1}{2}$ times as active as the amino-compound.—*Abstractor.*] F. A. R.

Agricultural

Determination of Iodine in Soils, Plant Material and Waters. G. S. Fraps and J. F. Fudge. (*J. Assoc. Off. Agric. Chem.*, 1940, **23**, 164–171.)—Objections to the A.O.A.C. tentative methods for the determination of iodine in soils, plant materials and brine (*Methods of Analysis, A.O.A.C.*, 1935, 8, 133, 528) arise mainly from losses of iodine or from interference by other substances with the liberation of iodine (*cf.* ANALYST, 1935, **60**, 631). The following method has therefore been adopted by the Texas Agricultural Experimental Station, and has the additional advantage of ease of manipulation. The organic matter is removed by burning and wet oxidation, and the iodine, which is thereby oxidised to iodic acid, is subsequently reduced with phosphorous acid and separated by distillation; it may then be determined colorimetrically. Plant materials are first burned (*cf.* Von Kolnitz and Remington, *Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 38), and the solution, containing the ash and washings, is evaporated with sodium hydroxide to about 30 ml. Waters (500 ml.) are evaporated directly with the alkali, and soils are diluted with 30 ml. of water. To the resulting mixture in each instance are added 6 g. of potassium chromate, 30 ml. of a solution of 3 g. of potassium chromate in a mixture of 95 ml. of conc. sulphuric acid and 5 ml. of water, and

10 mg. of cerous sulphate which has been washed 3 times with purified alcohol. The mixture is maintained at 195° C., with occasional shaking, and when all the organic matter has been destroyed (after about 1 hour), 50 ml. of water are added and the precipitate is removed by filtration through asbestos which has undergone the same digestion process. The filtrate is cooled to below 50° C., and transferred to an all-glass distillation apparatus (see below), and sufficient phosphorous acid (about 10 ml.) is added to destroy the yellow colour. The solution is heated, air being drawn through it by suction, slowly at first and then, as distillation proceeds, at the rate of 50 to 75 litres per hour, so that the solution is violently agitated. The receiver contains 10 ml. of 0·02 *N* sodium hydroxide solution, and distillation should be stopped after 30 minutes, when the temperature of the solution should have reached 150° C. The distillate is neutralised with 50 per cent. sulphuric acid, one drop being added in excess, followed by sufficient bromine to produce a brown colour. The solution is then evaporated to 5 ml., and 5 ml. of water, 4 ml. of the dilute sulphuric acid and 1 ml. of 0·1 per cent. potassium iodide solution are added. This mixture is shaken vigorously with 1 ml. of carbon tetrachloride for 2 minutes, and the extract is separated and centrifuged for 1 minute. The colour may then be matched against a series of standards which are prepared in exactly the same way from known amounts of iodine. It is advisable to purify the carbon tetrachloride by adding 50 ml. of bromine water to 500 ml., and allowing the mixture to stand for several hours in sunlight; it is then made alkaline with sodium hydroxide, and on the following day is washed 3 times with water and dried by shaking with plaster of Paris, and the decanted solvent is distilled, the first and last 25 ml. being rejected. The distillation apparatus (*cf.* Trevorrow and Fashena, ANALYST, 1935, **60**, 628; *J. Biol. Chem.*, 1936, 114, 351) consists of a 150-ml. round-bottomed flask, which is connected with a train consisting of (1) an inverted 250-ml. Erlenmeyer flask, into the base of which is sealed (2) a bulb, into which is sealed a Kjeldahl connecting tube, and (3) a condenser, which is connected by means of a ground-glass joint with (4), the cylindrical receiver (capacity, 100 ml.). The technique of the individual steps of the reaction is fully discussed. Comparison with the McHargue method (in which the iodine is removed from the soil by volatilisation in an electric furnace; *cf. J. Assoc. Off. Agric. Chem.*, 1937, **20**, 222) for 3 sandy loams containing 1·1 to 4·5 p.p.m. and one black clay containing 13·4 p.p.m. of iodine, showed that the present method gave results that were higher by 0·1 to 0·6 and 0·7 p.p.m., respectively. In another test different quantities of the same samples of water were used, *viz.* aliquot portions of 500 and 2000 ml. The average iodine contents of 8 samples containing 4 to 25 parts per 10^9 were 15·6 and 16·4 p.p.10^9 for the respective aliquot portions; the greatest difference (4 p.p.10^9) was obtained with a sample containing 20 p.p.10^9 of iodine. J. G.

Organic

Qualitative Test for Organic Compounds containing Oxygen. D. Davidson. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 40–41.)—Ferric thiocyanate dissolves in oxygen compounds, yielding a red solution, but is insoluble in hydrocarbons and organic halogen compounds. *Method.*—Filter-paper is saturated

with a solution of 1 g. of ferric chloride and 1 g. of potassium thiocyanate in 10 ml. of methanol (which has been filtered to remove precipitated potassium chloride) and air-dried. More than one dipping may be necessary to produce a paper having a greenish hue resembling fuchsine crystals. The test-paper is kept in a stoppered bottle away from sunlight. A small piece of the paper is stirred with a few drops of the liquid to be tested. Solids are tested as saturated solutions in a hydrocarbon or halogenated derivative. The production of a dark red solution indicates the presence of an oxygen compound, provided that nitrogen and sulphur compounds are absent. Nitrogen compounds, such as the amines, also dissolve the reagent. Few sulphur compounds have been tested, but among these carbon disulphide reacted negatively, whilst benzyl sulphide gave a positive reaction. The name "ferrox" is proposed for the method. S. G. C

Detection of Elements in Organic Compounds. L. Rosenthaler. (*Pharm. Acta Helv.*, 1939, **14**, 215–216; *J. Pharm. Belg.*, 1940, **22**, 220.)—*Bromine and Chlorine.*—The test substance is mixed with several drops of a saturated solution of potassium permanganate and 1 ml. of conc. sulphuric acid; chlorine or bromine is liberated. The bromine may be identified by means of fluorescein paper (formation of eosin and subsequent bleaching) which is merely bleached by chlorine. Chlorine gives characteristic crystals when it acts upon a 1 per cent. solution of 1, 2, 5-toluylene diamine in dilute sulphuric acid on a microscope slide; bromine does not give this reaction. The bromine test is successful with most bromine compounds except tertiary butyl bromide; exceptions for chlorine include: carbon tetrachloride, *p*-chlorophenol, chloro-*m*-cresol and *p*-chlorobenzoic acid. *Sulphur.*—The action of nascent hydrogen, with formation of hydrogen sulphide (detected by its action on lead acetate paper), is not a characteristic reaction for sulphur in organic compounds. The method of conversion into thiocyanate is being investigated. (*Abstractor's note.*—For satisfactory test for sulphur see Feigl, "*Spot Tests*," 2nd Eng. Edn., 1939, p. 262.) *Carbon.*—The usual test is charring with sulphuric acid. The following substances do not give this reaction:—urea, thiourea, hydrocyanic acid, dimethylglyoxime, diphenylurea, benzene, sulphobenzoic acid, phenol, salicylic acid, sulphosalicylic acid, *p*-hydroxybenzoic acid, anisic acid, coumarin, phthalic anhydride, hydroxyquinoline and acridine. Ethyl alcohol is more readily attacked (150° C.) than methyl alcohol (230° C.). J. W. M.

Preparation, Properties and Thiocyanogen Absorption of Triolein and Trilinolin. D. H. Wheeler, R. W. Riemenschneider and C. E. Sando. (*J. Biol. Chem.*, 1940, **132**, 687–699.)—The triglyceride of oleic acid was prepared by direct esterification of oleic acid and glycerol in an atmosphere of nitrogen with *p*-toluene sulphonic acid as catalyst. It was purified by molecular distillation, the fraction with the theoretical iodine number (86·1) having the following properties: saponification equiv. 295·8 (theory 294·9); n_D^{40}, 1·4621; n_D^{50}, 1·4586; density at 40° C., 0·8988. From a study of the melting- and freezing-points of triolein, it was observed that polymorphism existed, there being apparently three crystalline or solid forms: I, the stable modification, m.p. 4·7 to 5·0° C.; II, m.p. about −12° C.; III, m.p. about −32° C.

The triglyceride of linolic acid was also synthesised and purified in the same way. It had the following properties: saponification equiv. 292·4 (theory 292·9), iodine value 173·9 (theory 173·4), n_D^{40} 1·4719, n_D^{50} 1·4683, density at 40° C. 0·9184. Two solid forms were observed: I, m.p. −13·1 to −12·8° C., and II, m.p. about −43° C. Thiocyanogen values were determined by the modified Kaufmann method (*J. Assoc. Off. Agric. Chem.*, 1938, **21**, 87). It was found that a 4-hour reaction time is most suitable for both glycerides, the value obtained for triolein (85·8) being slightly low (theory 86·1) and that for trilinolein (87·1) slightly high (theory 86·7). Bromination of trilinolin in ethereal solution produced a number of bromine addition products, a 9·1 per cent. yield of crystalline bromides being obtained. These had m.p. 81·0 to 81·7° C. F. A. R.

Separation and Determination of 4-Aminodiphenylamine. I. S. Shupe. (*J. Assoc. Off. Agric. Chem.*, 1940, **23**, 161–164.)—The monohydrochloride of 4-aminodiphenylamine, which is a common constituent of certain hair dyes, forms a dark blue solution on oxidation. The free base is relatively insoluble in water, very soluble in chloroform, ether or benzene, and slightly soluble in petroleum spirit, which can therefore be used for its isolation, as follows:—A solution of a quantity of the sample (equivalent to not more than 0·1 g. of the free base) in warm water or dilute hydrochloric acid is made alkaline with an excess of powdered sodium bicarbonate, and extracted with one 50-ml. and four 20-ml. portions of petroleum spirit. The combined extracts are washed with 20 ml. of water, and the aqueous-extract is washed once with 20 ml. of petroleum spirit, which is added to the other petroleum spirit extracts. The united extracts are then filtered through cotton-wool into a tared dish and evaporated on a water-bath in a stream of carbon dioxide, and the residual 4-aminodiphenylamine is dried at 100° C. for 30 minutes and weighed. It has a characteristic needle-shaped structure, and its m.p. is 66° to 67° C. if it is not dried at 100° C., and 73° to 74° C. if dried in this way and then recrystallised, or if it is distilled in a vacuum at a low temperature. These differences correspond with isomeric modifications of the base, the modification of higher m.p. being also obtained if an extract of the base in chloroform is sublimed in a vacuum. Both isomers give the same acetyl and benzenesulphonyl derivatives, which are obtained as follows:—To a solution of a weighed portion of the extracted base in 5 ml. of 1 per cent. hydrochloric acid are added 0·5 ml. of acetic anhydride for every 0·1 g. of base, and then 1 g. of sodium bicarbonate. The excess of acetic anhydride is removed on the water-bath, 5 ml. of water are added to the residue, and after 1 hour in the cold the mixture is filtered on a tared Gooch crucible, 25 ml. of water being used for transferring and washing the acetyl derivative, which is then dried at 100° C. for 1 hour and weighed. It is slightly soluble in water, and, if recrystallised from a mixture of alcohol and water, has m.p. 162° to 163° C. (*cf.* Deshusses, below). If 0·7 mg. is added to the weight of derivative, the factor 0·8142 may be used to calculate the equivalent of free base. The benzene-sulphonyl derivative is obtained by adding to a solution of the base in 5 ml. of alcohol, 0·5 ml. of benzene sulphonyl chloride for every 0·1 g. of base, and 5 ml. of 25 per cent. sodium acetate solution. The mixture is heated on the water-bath for 30 minutes; 30 ml. of water are then added, and the

mixture is stirred at intervals during 1 hour. The precipitated derivative (m.p. 138°–139° C.) is separated, weighed and recrystallised as described above; the factor 0·5679 gives the free base (no correction required). The recovery obtained from 99·2 mg. of the pure salt was 99·3 per cent.; with a mixture of 49·6 mg. of the salt with 300 mg. each of *m*- and *p*-phenylene diamines and 2.5-diaminotoluene, 60 mg. each of *o*- and *p*-aminophenols, 30 mg. of 2.4-diaminoanisole and 100 mg. of *p*-methylaminophenol the recovery was 99·8, or 103·8 per cent. if sodium hydroxide was used to make the solution alkaline before extraction. With chloroform as solvent the recovery from 116·0 mg. of the pure salt was 100·0 per cent. Recoveries based on the weights of the acetyl and benzenesulphonyl derivatives were 99·3 to 100·0, and 99·5 to 99·8 per cent., respectively (*cf.* Griebel and Weisz, *Z. Unters. Lebensm.*, 1935, **70**, 61; Deshusses, *Mitt. Lebensm. Hyg.*, 1939, **30**, 10). J. G.

Colouring Matters of Flowers of *Tormentilla potentilla*. L. Schmid and A. Polaczek-Wittek. (*Mikrochem.*, 1939, **27**, 42–46.)—The flowers of *Tormentilla potentilla* contain a number of polyene colouring matters and two flavones and flavonols. In the tests described 5 g. of dried petals were used. The polyenes were identified by optical measurement of the positions of maximum absorption; β-carotene and luteine were recognised with certainty; two other spectra were probably due to zeaxanthine and flavoxanthine. In the saponifiable portion myristic acid was identified by micro-combustion, and an unsaturated acid was also present. The unsaponifiable matter contained paraffin hydrocarbons. J. W. M.

New Colour Reaction of Phenarsazine Chloride. J. Delga. (*J. Pharm. Chim.*, 1940, **132**, 73–76.)—Phenarsazine chloride (Adamsite) gives in acetic acid solution and in presence of silver nitrate a yellow colour, and it is possible thus to detect 0·02 to 0·04 mg. To a small quantity of oxide or chloride of phenarsazine in a test-tube are added 5 ml. of the reagent (25 ml. of a 10 per cent. solution of silver nitrate and 25 ml. of glacial acetic acid), and after being kept for 10 minutes in a boiling water-bath the colour is noted. With 0·05 mg. of Adamsite this is a clear yellow becoming intense with 0·2 mg. Twenty other arsenic derivatives, including mono- and di-phenylchloroarsine, acridarsines and sodium cacodylate, gave no colour reactions. With chloride derivatives a curdled white precipitate of silver chloride was formed. Paranitrophenarsazine chloride alone dissolved to a yellow solution in acetic acid, but the colour was not intensified on warming, even in presence of silver nitrate. Diphenylamine, a constant impurity in industrial Adamsite, gave with the reagent a dirty green colour changing to black. To detect Adamsite in water, 0·25 g. of silver nitrate is added to 5 ml. of the suspected water and dissolved by shaking, after which 5 ml. of pure acetic acid are added and the mixture is left for 10 minutes in a boiling water-bath; if Adamsite is present the characteristic colours will be produced; one part of Adamsite in 125,000 of water may thus be detected. D. G. H.

Inorganic

Separation of Cadmium from Zinc by Precipitation with Aluminium. F. E. Townsend and G. N. Cade. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 163–164.)—The acid sulphate solution (200 ml.), containing zinc and cadmium, is neutralised with sodium hydroxide and acidified with 1 ml. of conc. sulphuric acid Granular aluminium (< 40 mesh, 0·3 to 1 g. according to the amount of cadmium present) is added, and the liquid is boiled for 5 minutes. The precipitate is filtered off on a wad of cotton-wool and washed with cold water, and the filter and precipitate are returned to the precipitation-beaker. One-tenth g. of aluminium is added to the filtrate, which is boiled for 5 minutes, and filtered through a filter-paper. The residue is washed with cold water, and the filter is added to the first one. The filters are macerated and treated with 5 ml. of sodium hydroxide solution (50 per cent.) to dissolve out metallic aluminium. After addition of water, the spongy cadmium which remains is filtered off and determined by any convenient method. Practically quantitative results were obtained in the separation of 0·1 g. of cadmium from quantities of zinc up to 2·0 g. Analysis of various metallurgical products with the aid of the method gave good results for cadmium in comparison with the hydrogen sulphide separation method. S. G. C.

Detection of Gold by Means of Morpholin. L. S. Malowan. (*Z. anal. Chem.*, 1939, **118**, 100–102.)—Morpholin, C_4H_9NO, is a cyclic secondary amine, prepared from diethanolamine. It is a colourless liquid with an odour of ammonia and is miscible in all proportions with water (Wilson, *Ind. Eng. Chem.*, 1935, **27**, 867, 870; Malowan, *Mikrochemie*, 1939, **26**, 319; Abst., ANALYST, 1939, **64**, 765). Owing to its alkalinity it precipitates hydroxides of various metals; only cadmium forms an organo-compound. Morpholin can easily be oxidised and reduces metallic salts. Gold and silver are not precipitated in the cold, but only on boiling the solution. Silver gives a mirror, and gold first a yellow colour and then red-violet flakes. The reaction is obtained with as little as 1 ml. of a solution containing 1 part of gold in 50,000. For the test, 2 to 3 ml. of the solution are mixed with 0·3 ml. of pure morpholin, or such amount that the solution becomes distinctly alkaline. If copper or iron is present it is precipitated; the filtrate from the precipitate is boiled, and if gold is present the solution first shows a yellow colour, which changes gradually to bluish-violet, and finally bluish-violet flakes are precipitated. The reaction is very reliable and enables an approximate estimation of the amount of gold to be made. D. A.

Assay for Platinum Metals in Ore Concentrates. J. Seath and F. E. Beamish. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 169–171.)—Details of the dry assay of platinum metals in concentrates from nickel ores are discussed. S. G. C.

Determination of Chromic Oxide in Leather. I. Comparison of Wet and Dry Oxidation Methods. J. C. Mertz. (*J. Amer. Leather Chem. Assoc.*, 1940, **35**, 36–43.)—Six commercial chrome-tanned leathers in strip form (including blue-, brown- and black-dyed, and undyed one- and two-bath calf, suede-calf and

kid leathers) were ground in a Wiley mill and analysed by the following methods:—(1) *Smith and Sullivan Method* (see ANALYST, 1935, **60**, 779).—It was found preferable to use 2-g. samples and 0·1 *N* solutions. (2) *Modified American Leather Chemists' Association Method* (*cf.* Wilson and Merrill, "*Analysis of Leather*," 1931, p. 22).—The sample (3 g.) was ignited in a platinum dish, the ash was fused for at least one hour with 4 g. of a mixture containing equal weights of sodium carbonate, potassium carbonate and powdered borax glass, and the melt was extracted with an excess of dilute hydrochloric acid. A few drops of sulphuric acid were added to the acid solution, which was then boiled for 2 minutes. As no barium sulphate was found, the clear solution was diluted to 250 ml., and a mixture of 100 ml. of this solution with 5 ml. of conc. hydrochloric acid and 10 ml. of a 10 per cent. solution of potassium iodide was placed in a stoppered flask in the dark for 2 minutes. The iodine liberated was then titrated with 0·1 *N* sodium thiosulphate solution, 2 ml. of a 0·5 per cent. solution of starch being used as indicator. The iron and aluminium were removed from another 100 ml. of the solution in the usual way, and the chromium in the resulting combined filtrate and washings was determined as described above. (3) *Combination Method.*—The Smith and Sullivan method (*loc. cit.*) was followed up to, and including, the operations of diluting to 200 ml. and cooling. The solution was then diluted to 250 ml., and 100-ml. portions were taken for the iodimetric determination of chromium by Method (2), both with and without removal of iron and aluminium.

The results varied from 2·65 to 6·69 per cent. of Cr_2O_3 (dry basis), and those obtained by Methods (1) and (2) were in excellent agreement. Method (1), however, is preferred, as the wet oxidation eliminates the long fusion period (which must not be shortened), the appreciable losses of platinum from the crucible, and the interfering effect of contamination by platinum on the colour of the starch-iodine complex, which is changed to an amber-red shade. It is important, however, that in Method (1), 80 per cent. sulphuric acid should be used in the oxidising solution, since use of 95 to 98 per cent. acid without dilution will dehydrate the perchloric acid to an extent incompatible with safety. The iron present in the samples examined was sufficient to influence the results, and if it was not removed the results were high by 0·1 to 0·2 (in one test, 5) per cent. of Cr_2O_3. Method (3) gave results that were consistently low, *viz.* by 3 to 10 per cent. of the chromium-content. This difference is attributed to the effect, on the iodimetric titration, of the anions introduced by the oxidising agents, and not to incomplete oxidation. J. G.

Determination of Molybdenum in Cast Iron and Steel. C. Sterling and W. P. Spuhr. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 33–34.)—Knowles's α-benzoinmonoxime method is employed (*cf.* ANALYST, 1932, **57**, 799–801) with the modification that, instead of the precipitate being ignited to molybdic oxide, an operation found to be troublesome and necessitating corrections for impurities, the α-benzoinmonoxime-molybdenum precipitate is dissolved in ammonia and the molybdenum is precipitated as lead molybdate. The treatment is as follows: The α-benzoinmonoxime-molybdenum precipitate after filtration is washed back into the precipitation-beaker, the filter being reserved; 10 ml. of ammonia and 10 ml. of 30 per cent. hydrogen peroxide are added, and the liquid is diluted to

75 ml., and boiled until oxygen evolution ceases. The liquid is filtered through the original filter, which is then washed with hot dilute ammonia (1:50). The lead molybdate precipitation is effected by pouring the filtrate into 100 ml. of boiling lead acetate buffer mixture (4 g. of lead acetate crystals dissolved in a mixture of 550 ml. of ammonia (sp.gr. 0·90), 900 ml. of 50 per cent. acetic acid, 275 ml. of conc. hydrochloric acid and 275 ml. of water). The liquid is boiled, the precipitate is allowed to settle for 30 minutes (a longer period is advised for small precipitates), filtered off on a close-textured paper, and washed with hot 2 per cent. ammonium acetate solution containing a little acetic acid. The filter is ashed, and the precipitate is finally ignited at a dull red heat. The method is applicable to tungsten-free molybdenum-bearing steels and irons. Tungsten, tantalum and niobium interfere with the α-benzoinmonoxime precipitation method. With tungsten present, preliminary separation of the molybdenum as sulphide is advised (*cf.* Arrington and Rice, *Bur. Mines Rept. Investigations*, 3441, pp. 39–59, 1939).

S. G. C.

Determination of Cerium by Means of 8-Hydroxyquinoline. R. Berg and E. Becker. (*Z. anal. Chem.*, 1940, **119**, 1–4.)—The neutral solution, containing 0·01 to 0·05 g. of cerium, is warmed with 1 g. of hydroxylamine hydrochloride until colourless, treated with excess of sodium tartrate and 20 ml. of 2 *N* ammonia, diluted to 100 ml., and treated at 60° C. with a 2 per cent. alcoholic solution of the reagent until the supernatant liquid is orange-yellow. It is then heated to boiling and left for 30 minutes over a small luminous flame. The precipitate is collected in a porous glass crucible (G 4) or on an ashless filter, and washed with warm feebly ammoniacal water until the washings are colourless, and weighed after having been either dried at 110° C. or ignited to CeO_2 under a layer of sublimed oxalic acid (Ce factor for $Ce(C_9H_6ON)_3$: 0·2447). The precipitate may also be treated bromometrically (ANALYST, 1934, **59**, 325). For the separation of thorium from cerium, Hecht and Ehrmann's hydroxyquinoline method for the determination of thorium is applicable (*ibid.*, 1935, **60**, 272), 1 g. of hydroxylamine hydrochloride being added (*supra*). The filtrate from the thorium precipitate is treated with sodium tartrate, etc., as described above. W. R. S.

Volumetric Determination of Tungsten. M. L. Holt and A. G. Gray. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 144–146.)—The method is based on the reduction of sexavalent to tervalent tungsten by liquid lead amalgam in a specially designed reductor. *Apparatus.*—The reductor consists of a 400-ml. tap funnel with a stopper carrying a leading tube for admission of carbon dioxide and a smaller tap-funnel for admitting liquid. The reductor-funnel is provided with an exterior winding of nichrome wire covered with asbestos cement for electrical heating of the contents. The stem of the reductor-funnel has a two-way tap with two leading tubes, one to act as a run-off and the other communicating with the titration bottle. The titration bottle is a three-necked Woulff bottle; the three necks are stoppered and carry respectively (1) the tube from the reductor, (2) burette, (3) tap-funnel for admission of liquid; a side-tube entering near the bottom of the Woulff bottle carries a tube for admission of carbon dioxide for the purpose of agitating the liquid during titration. *Lead amalgam.*—A mixture of 40 g. of

granulated lead and 600 g. of mercury is heated at 100° C. for 1 hour with constant stirring. The amalgam is cooled and filtered through a fine cloth filter to remove the solid portion, which is rejected. The liquid amalgam is washed with water and dried with filter-paper. *Method.*—The test solution, containing the tungsten as sodium tungstate, is evaporated almost to dryness in a separate vessel; 75 ml. of conc. hydrochloric acid are added; the liquid is heated to produce a clear solution, and introduced into the reductor, from which air has previously been swept by carbon dioxide. Lead amalgam is run in, and the reductor is gently shaken at 60° until the solution has passed through various colour changes and reached a deep yellow colour; reduction is continued for a further 5 minutes (about 15 minutes in all) to ensure completeness. The amalgam is then run off through the two-way tap. A slight excess of ferric iron solution (10 per cent. ferric ammonium sulphate solution in 25 per cent. phosphoric acid) is added to the reduced tungsten solution, and the mixture is run off into the titration bottle together with 10 to 20 ml. of water used for rinsing out the reductor; 75 ml. of water, 40 ml. of syrupy phosphoric acid and a few drops of diphenylamine sulphonic acid indicator solution are added to the contents of the titration bottle, and the solution is titrated with $N/10$ dichromate solution. The calculation of the result is based on the change of valence of tungsten from 3 to 6. Results close to the theoretical were obtained in tests with 0·003 to 0·16 g. of tungsten. The method was applied to the analysis of electroplated tungsten-nickel alloys and to ferro-tungsten, the tungsten being first obtained in solution as sodium tungstate by normal methods.

S. G. C.

Determination of Uranium and Copper with the aid of the Silver Reductor. N. Birnbaum and S. M. Edmonds. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 155–157.)—*Uranium.*—The solution (50 ml.), containing 0·1 to 0·4 g. of uranyl salt in 4 *M* hydrochloric acid, is heated to 60° C., and passed at the rate of 20 ml. per minute through a silver reductor which has been pre-heated by the previous passage of hot 4 *M* hydrochloric acid. (The silver reductor is described by Walden, Hammett and Edmonds, *J. Amer. Chem. Soc.*, 1934, **56**, 350; *cf.* Fryling and Tooley, *id.*, 1936, **58**, 826, Abstr., ANALYST, 1936, **61**, 722). The reductor column is washed through with 150 ml. of hot 4 *M* hydrochloric acid. Uranium is reduced to the quadrivalent state. The reduced solution is cooled, 3 ml. of 85 per cent. phosphoric acid and 1 drop of *o*-phenanthroline ferrous-complex indicator solution are added, and the solution is titrated with 0·1 *M* ceric sulphate solution. Acetic acid does not interfere. Nitric acid, iron, molybdenum, vanadium and copper, which are also capable of reduction, must be absent. *Copper.*—The solution (50 ml.) containing 0·1 to 0·4 g. of copper in 2 *M* hydrochloric acid is passed through the reductor at 25 ml. per minute (temperature of solution not stated). The reduced solution, containing cuprous chloride, is collected under 20 ml. of 0·5 *M* ferric alum solution. The reductor column is washed through with 150 ml. of 2 *M* hydrochloric acid, and the solution is titrated with 0·1 *M* ceric sulphate solution, 1 drop of *o*-phenanthroline complex being added as indicator. Nitric acid (up to about 5 per cent. strength) is without effect on the accuracy.

S. G. C.

Detection of Magnesium by means of *p*-Nitrobenzeneazoresorcinol. J. P. Mehlig and K. R. Johnson. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 30–31.)—The effect of forty-four of the common ions upon the *p*-nitrobenzeneazoresorcinol test for magnesium has been studied. To the solution, containing 10 mg. of magnesium and up to 1000 mg. of the ion under test, 1 drop of reagent solution (0·5 g. of *p*-nitrobenzeneazoresorcinol dissolved in 100 ml. of 1 per cent. sodium hydroxide solution) is added, and the solution is rendered alkaline with sodium hydroxide. Magnesium yields a sky-blue precipitate; sensitiveness 0·002 mg. in 10 ml. Interference is caused by the ions forming coloured precipitates masking the magnesium blue. Black precipitates are given by mercurous and mercuric ions; brown by silver, manganous, ferric and arsenate ions; green by chromic, nickelous, and ferrous ions; yellow by chromate and blue by cupric ions. Only arsenate, chromate and permanganate, out of the 20 anions tested, interfered. The white precipitates formed by aluminium, antimonious, bismuth, cadmium, stannic, stannous and zinc ions do not interfere, as no masking of the blue is produced. Ammonium salts cause no trouble unless present in considerable excess. The method is considered advantageous for use in the "group" detection of magnesium; interfering ions are removed in the course of the prior separations. Aluminium, barium, calcium and strontium, while tending to interfere with the traditional magnesium ammonium phosphate test, are without effect on the present method. S. G. C.

Determination of Bromides in Presence of Chlorides. A. Denoël. (*J. Pharm. Belg.*, 1940, **22**, 179–184.)—The principle of the method (which has the advantages of accuracy and rapidity) is the liberation of bromine from the bromides by the action of potassium bromate under specified conditions of acidity, the chlorides being unattacked. The bromine is then extracted with carbon tetrachloride, and allowed to liberate iodine from potassium iodide; the former may be titrated. To a mixture of 100 ml. of the sample (which should not contain more than 0·1 g. of bromide ion), with 8 ml. of 10 per cent. sulphuric acid and 10 ml. of 5 per cent. potassium bromate solution, are added 50 ml. of carbon tetrachloride. The mixture is shaken well, and after 10 minutes the carbon tetrachloride layer is transferred to a stoppered 500-ml. flask containing 50 ml. of a 4 per cent. potassium iodide solution. Four further extractions, each with 40 to 50 ml. of carbon tetrachloride, are made; the last should remain colourless. The combined extracts are then shaken in the flask, and the liberated iodine is titrated with 0·1 *N* sodium thiosulphate solution, the end-point being the simultaneous disappearance of the violet colour of the carbon tetrachloride and of the yellow colour of the aqueous layer. If the chloride-content is required, a preliminary determination of the total halogens must be made by the Volhard–Charpentier method and the bromide-content deducted; it is thus possible to determine 1 per cent. of potassium chloride in potassium bromide, or *vice versa*. The method is specific for bromides in absence of iodides, and is unaffected by the presence of up to 0·3 g. of sodium chloride. It is shown that no acid or bromate is carried over with the carbon tetrachloride, but a blank test on the reagents is advised. Tests with solutions of pure potassium bromide containing 3 to 100 mg. of bromide ion gave results that

were 0·7 per cent. low, but agreed well with those obtained by Charpentier's method and by gravimetric analysis. In presence of up to 0·3 g. of sodium chloride the recorded error varied from −0·69 to +1·03 per cent., but higher chloride-contents produced greater errors. When the quantity of bromide is very small the best results are obtained by the use of 100 ml. of water, 5 ml. of 10 per cent. sulphuric acid and 5 ml. of 5 per cent. potassium bromate solution. With organic compounds the possibility exists that nitrites formed by ignition in presence of nitrates will reduce some of the bromate to bromide and so give high results. It is not advisable to destroy the nitrites by means of potassium permanganate, and it is preferable to destroy the organic matter by ignition in presence of sodium carbonate.

J. G.

Argentometric Determination of Cyanide with Diphenylcarbazone as Indicator. R. Ripan-Tilici. (*Z. anal. Chem.*, 1940, **118**, 305–307.)—The cyanide solution (0·01 to 0·05 *M*) is treated with 4 or 5 drops of an alcoholic 0·3 per cent. solution of the indicator, which colours it reddish-brown, and titrated during agitation with 0·01 to 0·05 *M* silver nitrate solution. When all of the cyanide has been converted into the complex $[Ag(CN)_2]''$, a further drop of silver solution turns the liquid violet. If now the titration is continued, the intensity of the violet colour increases, until one drop of silver solution colours the precipitate blue and causes it to flocculate. The total volume of silver solution thus consumed is twice that required for the production of the violet end-point, where the diphenylcarbazone acts as a colour indicator for the ratio 2CN : 1Ag; at the blue end-point, it acts as an adsorption indicator for the ratio CN : Ag. Both end-points are accurate and sharp (*cf.* ANALYST, 1935, **60**, 428). W. R. S.

Determination of Free Cyanide in Brass Electroplating Baths. W. M. McNabb and S. Heiman. (*Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 161–163.)—The authors' work indicates that a brass-plating solution contains $NaCu(CN)_2$, $Na_2Cu(CN)_3$, $Na_2Zn(CN)_4$, Na_2ZnO_2, free NaCN, with small amounts of ammines and alkali. The equilibrium among these constituents, and consequently the value of the free cyanide, is shifted by changes in the concentration of the constituents, the temperature and the *p*H value. Nevertheless it is possible to determine the quantity of "free cyanide" required to yield results which, though empirical, are useful for the purpose of controlling the operation of the bath. The most satisfactory method would appear to be that of Pan (*Metal Industry*, New York, 1932, **30**, 402–404), which is as follows:—To 10 ml. of the brass-plating solution are added 4·3 g. of potassium iodide and sufficient water to produce a volume of 70 ml. at the end of the titration The solution is titrated with *N*/10 silver nitrate solution to the appearance of a bluish opalescence. The free sodium cyanide, in g. per litre, is given by multiplying the number of ml. of *N*/10 silver nitrate by 0·980.

S. G. C.

Determination of Silica in Cryolite and Materials containing Fluorine. H. Spielhaczek. (*Z. anal. Chem.*, 1940, **119**, 4–16.)—A new method is described in which the ore is fused with bisulphate, volatilisation of silicon being prevented

by addition of borax, with the result that the fluorine escapes as boron trifluoride. One g. of ore is mixed with 3 g. of fused (or 6 g. of crystallised) borax and 14 g. of powdered potassium bisulphate in a platinum crucible. The covered crucible is cautiously heated round the sides until the reaction is subsiding, then more strongly until quiet fusion is attained. The cooled crucible is heated with 100 ml. of water and 3 ml. of hydrochloric acid in a porcelain basin, carefully cleaned, and the liquid is evaporated to the consistency of a syrup. After cooling, the crystalline mass is crushed with a flattened glass rod, the basin is heated in an oven at 110° C., and after renewed cooling the dry mass is thoroughly pulverised. It is then returned to the oven and heated until hydrochloric acid can no longer be detected by its odour, cooled, moistened with hydrochloric acid, and taken up with hot water after a few minutes' standing. After digestion on a water-bath the solution is filtered through close-textured paper [a pad of filter-pulp in the filter would be advisable—*Abstractor*], the precipitate is washed free from sulphate, heated in a tared platinum crucible, and ignited to SiO_2. This is tested for purity with hydrofluoric acid as usual. A blank test is advisable. The method is simple, more rapid and reliable than other procedures, and specially suitable for serial determinations. The results obtained on synthetic mixtures are very satisfactory.

W. R. S.

Microchemical

New Method for Washing and Isolation of Barium Sulphate in the Micro-Determination of Sulphur. R. Grangaud. (*Mikrochem.*, 1939, **27**, 52–56.)—After decomposition, by the micro-Carius or Pregl method, of the organic substance containing sulphur, the precipitation is carried out in a Jena glass tube in the usual way, the liquid is evaporated to dryness, and the residue is stirred up with 1 per cent. hydrochloric acid and transferred to a weighed quartz test-tube by rinsing alternately with alcohol and 1 per cent. hydrochloric acid. After centrifuging, the supernatant liquid is drawn off by suction, a tube bent round to turn upwards being used to avoid disturbing the precipitate. The precipitate is washed 3 times with 1 per cent. hydrochloric acid and centrifuged after each washing. Finally the test-tube is dried, heated to redness, cooled and weighed.

J. W. M.

Colorimetric Micro-method for the Estimation of Sodium with Manganous Uranyl Acetate. E. Leva. (*J. Biol. Chem.*, 1940, **132**, 487–499.)—The whole blood, serum or urine is first deproteinised by introducing 0·10 or 0·20 ml. into a 5-ml. flask containing 0·5 ml. of 20 per cent. trichloroacetic acid. The solution is diluted to the mark with water and thoroughly shaken, the foam being broken by touching the surface with a wire dipped in capryl alcohol. With protein-free materials, the trichloroacetic acid treatment may be omitted. One-ml. aliquot portions of the filtered solution are transferred to 15-ml. centrifuge tubes, 9 ml. of 25 per cent. alcoholic manganous uranyl acetate reagent are added to each, and the solutions are mixed with a clean glass rod. (This reagent is prepared as follows: To 160·0 g. of $UO_2(CH_3COO)_2.2H_2O$, 490·0 g.

of $Mn(CH_3COO)_2.4H_2O$ and 138·0 ml. of 30 per cent. acetic acid, 1292·0 ml. of water are added. The manganous acetate should be in the form of pink lustrous crystals, free from greyish powder. The salts dissolve if the flask is shaken occasionally, and a precipitate of the sodium salt separates out and settles after a while, leaving a clear solution. To prepare the reagent from this stock solution, 9 ml. are mixed with 3 ml. of 95 per cent. alcohol, and the mixture is allowed to stand in the dark for at least 4 hours and then filtered. The reagent keeps for about 3 weeks if stored in the dark.) A precipitate is formed in about a minute and the rod is then rinsed with a further 1 ml. of the reagent and removed. The centrifuge tubes are covered with rubber caps and allowed to stand in the dark for at least 4 hours, after which they are centrifuged and the supernatant liquid is discarded. The precipitate and the walls of the tubes are washed free from manganese by adding 4 ml. of washing solution from a pipette, and suspending the precipitate in this solution by stirring with a fine glass rod, which is then rinsed with 1 ml. of washing solution and removed. (The washing solution is made as follows: To 160·0 g. of $UO_2(CH_3COO)_2.2H_2O$, 440·0 g. of $Zn(CH_3COO)_2.4H_2O$, and 138·0 ml. of 30 per cent. acetic acid, 1342·0 ml. of water are added, the salts being dissolved in the same way as in the preparation of the manganous uranyl reagent. If insufficient sodium is present to saturate the solution, 0·1 *N* sodium chloride solution should be added until a precipitate persists. Twelve ml. of this solution are mixed with 4 ml. of 95 per cent. alcohol, and the solution is saturated with manganous triple salt by adding 20 mg. of the solid salt to each 100 ml. of solution. The solution is allowed to stand for 1 hour or longer and is then filtered; it keeps for 3 weeks in the dark. The triple salt is prepared by adding 125 ml. of the stock solution of manganous uranyl acetate to 2 ml. of a 5 per cent. sodium chloride solution. The mixture is stirred and allowed to stand for half-an-hour, when the supernatant liquid is decanted. The precipitate is transferred to a 50 ml. centrifuge tube and centrifuged, and the supernatant liquid drawn off. The precipitate is washed by suspending and centrifuging three times with 95 per cent. alcohol and twice with ether. The triple salt is dried and stored in a brown tube.) The tubes are again centrifuged and the washing is repeated twice. For the third washing it is unnecessary to re-suspend the precipitate, 4 to 5 ml. of the washing solution being simply added to the tubes. These are then placed in water, the temperature of which is raised slowly to the boiling-point, and most of the remaining supernatant liquid is evaporated off. Ten ml. of potassium periodate solution (2·5 g. dissolved in about 400 ml. of water, and the solution treated with 100 ml. of 85 per cent. phosphoric acid, shaken and made up to 1 litre with water) are introduced into each of the tubes, the contents of which are stirred with a glass rod, and they are then immersed in boiling water for 10 to 15 minutes. After cooling, the solutions are transferred quantitatively to graduated flasks, 25 or 50 ml. for 0·10- or 0·20-ml. samples, respectively. The solutions are diluted to the mark and mixed. The sodium concentrations are estimated either by comparing the colours with standard potassium permanganate solution (0·2200 g. of dry potassium permanganate is dissolved in water, 200 ml. of the potassium periodate reagent are added, and the solution is diluted to 1 litre with water; 4 ml. of this solution are then diluted to 50 ml. with water), or by measuring the extinction value in an

Evelyn photoelectric colorimeter, filter 520 being used. Using the former method, the concentration of sodium is calculated from the equation

$$\frac{20 \times \text{m}M.\text{KMnO}_4 \text{ in standard} \times 1000}{\text{Reading} \times \text{ml. of sample precipitated}} = \text{m}M^* \text{ sodium per litre}$$

the standard being set at 20 mm. When the photoelectric colorimeter is employed, the calculation is made by the use of the equation

$$\frac{\text{Density (from the galvanometer reading)} \times 1000}{39{\cdot}27 \times (100/\text{volume of oxidised solution}) \times \text{ml. of sample analysed}} = \text{m}M^* \text{ sodium per litre}$$

39·27 being the value of the density of 1 mM potassium permanganate solution per 100 ml. for the particular instrument used in the investigation; it should be re-determined for each laboratory. The presence of phosphate, even in equi-molecular proportions, does not affect the accuracy of the sodium estimations, the values for sodium varying by not more than ±1·5 per cent. from the theoretical. Potassium causes high results if the molar ratio K : Na in the sample exceeds 1·5. Excess of potassium may be removed by precipitation as the perchlorate. The results obtained by the method (with either method of measurement) were in close agreement with the results obtained by the gravimetric method of Butler and Tuthill (*J. Biol. Chem.*, 1931, **93**, 171; *cf.* ANALYST, 1931, **56**, 764).

F. A. R.

* Milli-moles.

Reviews

SULPHATED OILS AND ALLIED PRODUCTS.—THEIR CHEMISTRY AND ANALYSIS. By DONALD BURTON, M.B.E., D.Sc., F.I.C., and GEORGE F. ROBERTSHAW, A.M.S.T., A.I.C. With a Foreword by Prof. T. P. HILDITCH, D.Sc., F.I.C. Pp. iv + 163. London: A. Harvey. 1939. Price 12s. 6d. net.

Many different oils react with sulphuric acid, and each yields a wide variety of products whose nature depends on the conditions employed. The reaction, first studied over a hundred years ago, found early technical application in the use, by Runge in 1834, of sulphated olive oil as a mordant in dyeing calico red. Since then it has been extensively developed and many sulphated products are now used in leather manufacture, in the textile industry, as detergents, wetting agents and machine oils, and even in food manufacture.

As Professor Hilditch points out, our knowledge of the chemistry of the process of sulphation is still very incomplete, partly because the reactions are of great complexity and partly because the analytical chemistry, perforce restricted to determinations of general "characteristics," is not always easy to interpret.

Varying technique in analysis formerly led to much confusion, but the position has been considerably clarified by international collaboration, in which the

authors of this monograph have taken a prominent part. A scheme of analysis was put forward in 1931 and 1933, and the present work is the result of its cordial reception and requests for a revised edition.

The book includes a brief historical survey of the subject and descriptions of the raw materials used and of the chemistry and methods of sulphation. Most of it is devoted to three chapters dealing with the analysis of sulphated oils, sulphated fatty alcohols and petroleum sulphonic acids. In these three chapters there are excellent descriptions of methods drawn from widespread sources, and the practical notes appended to each description are of the greatest value as a guide to the interpretation of results and as a warning of the limitations and difficulties yet to be overcome.

The survey of the present position of the analytical chemistry of the subject is complete and critical and will provide an immediate stimulus to further progress. The authors have rendered a great service in collecting and presenting in so clear a manner all the analytical information available about this complex subject.

K. A. WILLIAMS

THE CHEMICAL CONSTITUTION OF NATURAL FATS. By T. P. HILDITCH, D.Sc., F.I.C. Pp. x + 438. London: Chapman & Hall, Ltd. 1940. Price 35s. net.

In the year 1823 Chevreul announced his discovery that natural fats consist of the glycerol esters of certain acids, among which palmitic, stearic and oleic are prominent; this book is the logical outcome of this discovery, but it would appear desirable to consider the reasons which have caused so long a time to elapse between the discovery of the raw material and the issue of the "finished product."

For years the discovery of Chevreul failed to attract the attention of a research school, and the little progress that was made was due to the work of isolated observers, who considered the matter as a hobby rather than as the serious work of a lifetime. The apparent difficulty of the subject, together with the existence of what seemed to be more attractive fields for investigation, did not add to the favour with which the subject was regarded. It is now, of course, well known that the presence of simple triglycerides in a fat is the exception rather than the rule, and that they are only present when the formation of mixed triglycerides is impossible owing to the presence of a predominating proportion of one acid; yet nearly eighty years after Chevreul's discovery, the presence of a mixed triglyceride in a fat was described as "unusual."

During the last fifteen to twenty years, however, our knowledge of the chemistry of natural fats has been revolutionised. A number of institutions have taken up the work in a thorough and convincing manner. In the forefront we have the Liverpool School, ably led by Professor Hilditch, which has been responsible for so many of the new devices, and improvements of the older devices, and which has played so large a part in the progress made.

In eleven chapters Professor Hilditch unfolds the mechanism of attack and the vast number of results obtained. After an introductory chapter of 21 pages, three chapters (occupying 159 pages) deal with the constituent acids found in many

hundreds of natural fats obtained from the simplest to the highest organisms. Professor Hilditch is careful to point out that our knowledge is by no means complete, but even a superficial comparison of these pages with books published less than twenty years ago will show the tremendous advances made during this period.

The next three chapters (81 pages) deal with the constituent glycerides of natural fats, this being the first time that a comprehensive account of this division of the subject has been attempted. Chapter VIII deals with some aspects of the biochemistry of fats, Chapter IX with the constitution of individual naturally occurring fatty acids, and Chapter X with synthetic glycerides. The final Chapter, XI, gives an account of the experimental methods now used in investigating the constitution of fats. Most of these methods have either been devised or elaborated at Liverpool, so that the working details here given can be regarded as authentic.

It is not often that a worker who has made wide and important advances in a particular subject can be persuaded to write a book giving the results obtained by himself and by others, and the task has perforce to be undertaken by those less qualified to do so. Our debt to Professor Hilditch is therefore the greater because, not only has he successfully completed a labour of great magnitude, but readers can be satisfied that the whole array of facts has been presented by one who has personal knowledge of the details of the subject on which he writes.

This work will be literally indispensable to all those who require a knowledge of the composition of natural fats. Paper, printing and binding are of satisfactory quality, and help to produce a book of which the author may well be proud.

G. D. ELSDON

LAVOISIER. By J. A. COCHRANE. Pp. xii + 264, 9 illustrations. London: Constable & Co. 1939. Price 3s. 6d. net.

This is a cheap edition of a work first published in 1931. The author mentions the fact that Lavoisier, at his death, was, and had been for fifteen years previously, one of the most eminent men in France; yet the general historian does not usually think it worth while even to mention him. Among chemists he has been honoured from the day of his death as a chief founder of modern chemistry; yet even they are less acquainted with his career as a whole than might have been expected. We are thoroughly well informed about his discovery of oxygen and his routing of the phlogistians, as well as of the work he did in connection with the devising of a new nomenclature of chemistry, but we are not nearly so familiar with other aspects of his life and work in relation to the times and conditions in which his lot was cast. We have imbibed something of his scientific spirit from his own *Traité élémentaire de Chimie*, wherein he stated: "Il n'est jamais permis, en physique et en chimie, de *supposer* ce qu'on peut *determiner* par des expériences directes"; we know, too, with what contempt he was treated by Coffinhal at his trial, when he was told that "The Republic has no need of savants."

Mr. Cochrane not only discusses fully the main facts of Lavoisier's chemical work and writings familiar to all interested in the history of the science, but also

deals at length with his very varied work in connection with the numerous investigations he undertook on behalf of the Academy of Sciences. We have here provided for us a picture of this versatile man of science seen in the perspective of events and personalities which exhibit him as a truly great man suffering obloquy and death because of an eminence begotten of his disinterested labours on behalf of his fellow citizens. The book contains chapters on Domestic Affairs, Politics, Personal Attacks, National Education, Arrest, In Prison, and The Guillotine. The illustrations are especially satisfactory. It is altogether a very cheap and desirable volume. W. KIRKBY

DISCOVERY OF THE ELEMENTS. By MARY ELVIRA WEEKS. Fourth Edition; enlarged and revised. Pp. viii + 470, with many illustrations. Published by *Journal of Chemical Education*, Easton, Pennsylvania, U.S.A. 1939. Price $4.00 post free.

The fact that a fourth edition of this book has been called for so soon is, perhaps, its best recommendation to the reader, as few books dealing with the history of science can claim such distinction. Its popularity is not difficult to understand, since the author makes an interesting story out of what others might record in a dry-as-dust manner, yet achieves this without any departure from scholarship. Not only are the researches leading to the first recognition and isolation of the various elements described with a wealth of detail, quotation from original papers and ample bibliographical references, but short biographical accounts are also given of the persons most closely connected with these discoveries. In addition, the many illustrations, portraits and reproductions of MSS., collected from many sources by Professor Dains, make the book attractive to the chemist interested in the human side of his subject.

The least satisfactory portions of the book are the first two chapters, on the elements known to the ancient world and elements known to the alchemists, which, with the exception of a good account of the discovery of phosphorus, are little more than fragmentary. In this section, too, the author does not show the judgment exercised in the latter portion of the book; for example, quotations from the Authorised Version of the Bible cannot be used as evidence of the scientific knowledge of the ancients. Perhaps this is best borne out by the passage cited in connection with allusions to iron in the Bible: "Oh that my words were now written! Oh, that they were printed in a book. That they were graven with an iron pen. . . ." (*Job*, xix. 23 and 24). If one accepts this as evidence of the use of iron for making a writing or engraving implement, one must assume that the printing press was in use in that era.

The additions to the book are becoming essays in historical chemistry rather than accounts of the discovery of the elements, so, perhaps, in future editions the sections devoted to classical and mediaeval knowledge of the elements will be elaborated. Although for the taste of some scientific readers the author perhaps shows rather too great a tendency to sentimentalise, the book is a mine of information on its subject and can be thoroughly recommended. O. L. BRADY

RECORDS AND RESEARCH IN ENGINEERING AND INDUSTRIAL SCIENCE. By J. EDWIN HOLMSTROM. Pp. 302. London: Chapman & Hall. 1940. Price 15s.

The scope of this work is described by the sub-title—"A Guide to the Production, Extraction, Integrating, Storekeeping, Circulation and Translation of Technical Knowledge." In these days of systematic record of the results of research and experiment, the whole of knowledge should be available to everyone, but it is not always easily accessible. In addition to about 14,000 books on technical and scientific subjects published annually, upwards of 750,000 articles and papers appear in about 14,000 technical and scientific journals. The systematic indexing and recording of all this material is a complicated and difficult business, but one of supreme importance if the accumulated knowledge is to be made readily available.

Dr. Holmstrom deals with the "Nature and Methods of Technical Science" and "Phases in the Application of Science to Practice," and then proceeds to discuss the work of Experimental and Collative Organisations. He describes in detail various methods of indexing, including the Universal Decimal Classification and the Kaiser system, as well as the facilities afforded by Bibliographical Bureau Services—not so well known as they deserve to be. No one particular system will suit every need, but the reader will find much useful and stimulating information in the details of the author's own system evolved to suit his particular problems. On a large scale the indexing and filing of technical information requires trained staff, especially in dealing with the problem of overlapping fields. This particularly thorny problem is fully discussed.

A chapter headed "The Expression and Transmission of Ideals" contains practical details of the mechanical processes involved in writing, dictating, typewriting and duplicating, as well as photographic reproduction and micro-photography, and on dealings with printers and publishers. The application of micro-photographic methods to collative research in reducing the bulk (by 99 per cent.) of literary material, both for more convenient circulation and storage, probably constitutes the most revolutionary advance in documentation since the invention of printing. America is ahead of Britain in the technique, but it is a satisfaction to record that a micro-film service is about to be initiated in this country at the small cost of about ½d. per quarto document. A reduction of 60 diameters would enable a 500-page book to be reproduced on a single 5 × 3 inch film for filing like a card index.

Another chapter contains much practical information on the Translation of Foreign Languages and Technical Dictionaries, especially as to the pitfalls that lie in the path of the unwary translator, usually more expert in his subject matter than in the language translated. Finally, the author touches on the possibilities offered by careers in occupations dependent on technical knowledge, with notes on specialisation and the employment of leisure time.

Essentially a practical work for practical men, Dr. Holmstrom's book fills a gap. It will be found particularly useful to the research worker and technician, who wishes to keep abreast of his subject, to know where to look for information, to obtain quick reference to original records and thus to avail himself to the full of the technical and scientific knowledge available. R. W. SLOLEY