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Isabel B. Elliott

THE ANALYST

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Obituary

MABEL BEATRICE ELLIOTT

HONORARY MEMBER OF THE SOCIETY

THE death of Miss Elliott will be felt as a personal loss by many, for she had the great gift of friendship. Born in 1885, she was educated, first at a private school in the north of London, and then at a German convent school in Holland, where she acquired a fluent knowledge of German and Dutch. Afterwards she studied in Brussels and became equally proficient in French. On returning to England she took a course in business training at Pitman's Commercial College, where, incidentally, she was awarded the prize for French and German. She also won in open outside competition the All-England gold medal for speed in shorthand and typing in foreign languages.

When the war of 1914 broke out she was working as foreign correspondent with an engineering firm in the City. Immediately she offered her services to the Postal Censorship, then newly established as a branch of the War Office. Beginning at the bottom as an unknown Examiner, it was not long before she was promoted to be a Deputy Assistant Censor. While she was in charge of a room of Examiners her keen observation led her to suspect that an apparently innocent business letter contained invisible writing, and, on applying heat to the paper, she brought to light a secret message of the German spy, Kuepferle; at the subsequent trial in the Tower and at the Old Bailey this evidence was an essential link in the case for the prosecution. That night Kuepferle hanged himself in Brixton prison, leaving a confession, written on a slate, that he was a German officer. Shortly afterwards Miss Elliott again discovered secret messages in the letters of two more German agents, Müller and Hahn, and her evidence helped to convict them both. A few months later she was promoted to be Assistant Censor, a post demanding organising ability and tact, and so successful was she that in the following year she was chosen to be Censor in control of the whole of the women (over 3000) employed in the Postal Censorship. For these services to the country she was made an M.B.E., and was also awarded the Palme d'un Officier d'Académie by the French Government.

After the war Miss Elliott opened a secretarial and translation bureau in Westminster, and this led, in 1921, to her association with the Society, when she was appointed indexer and business manager of THE ANALYST. In the following year she became an unofficial assistant to the Secretary, and for the next 15 years she gave efficient and ungrudging service to the Society, being affectionately known as the "O.G." When she retired at the end of 1937 from the secretarial side of the Society's work, the Council showed their appreciation of all that she had done by presenting her with a diamond wrist watch and electing her an Honorary Member—the only woman on whom the Society has conferred this honour during the whole of its 70 years. This retirement did not entirely sever her connection with the Society, for she continued to be the official indexer of THE ANALYST to the end of her life.

At the outbreak of the present war Miss Elliott threw herself with her usual energy into the war work of her district in London. She took a prominent part in all the activities of the W.V.S., such as acting as interpreter to Belgian, French and Dutch refugees after Dunkirk, and escorting train loads of women en route to an internment camp. Then, having passed the examinations of the British Red Cross Society, she gave all the time she could spare to nursing. It was soon recognised that she had the qualities of a leader, and she was invited to become Commandant of the 78th Middlesex Detachment. In a very short

while she had won the affectionate loyalty of all who served under her. Many of them will recall the fearless devotion with which, during the autumn of 1940, she tended the old people at a Sick Bay, returning home night after night during the incessant air raids and having to take shelter in doorways from the falling shell splinters.

The gradual failing in her health forced her to take up work less arduous than Red Cross nursing, although her enthusiasm and her will power remained unweakened Thus, she helped to organise a local branch of the Red Cross Penny-a-Week Fund, becoming its Hon. Treasurer, controlled a local depôt of the National War Savings campaign, and worked with the Red Cross Hospital Supply Association to the very last; even when she was too ill to go out she had the meetings of the working party in her own home and supervised them from her bed.

The end came peacefully in her sleep on January 9th. At her funeral at Golder's Green crematorium she was received by a guard of honour of detachments of the British Red Cross Society and the W.V.S., and within the chapel representatives of the other organisations with which she had been so closely associated were among the large gathering, which included her brother, Mr. A. H. Elliott, Mrs. Elliott and other relatives and intimate friends. At his special request the President (who was unable to be present) and Mrs. Melling were represented by the Editor, and the Society was represented by Dr. H. E. Cox (*Vice-President*), Mr. G. Taylor (*Hon. Treasurer*) and Mr. J. H. Lane (*Secretary*). Miss Elliott had asked her old friend, the Rev. W. I. Bulman, Vicar of St. Gabriel's, Cricklewood, to conduct a simple service, and had herself chosen the music for it. Her choice of Mendelssohn's *Spring Song* for the dismissal was a final gesture typical of her whole attitude towards the ills of life; she wanted no mourning.

Mabel Elliott's sympathetic nature and thought for others endeared her to everyone with whom she had anything to do, and so the number of her friends was constantly increasing. Her deep interest in the affairs of others was sincere—not merely a polite convention—and those (and they were many) who came to her with their troubles knew instinctively that she would not spare herself to help them. But she also loved to share in the pleasures of others. Many of us who attended the enjoyable meetings of the North of England Section at Scarborough and Llandudno will recall with what zest she joined in every amusement that was suggested. She had a dry, rather subtle humour, but it was coupled with a sense of fun; one never heard her make an unkind remark or impute unworthy motives. Unquestionably, she was the Good Companion.

Still more outstanding than these traits was her courage. She would not let herself be cast down by troubles that would have made many despond. Even when it came to the last great trial, she faced an operation and a painful lingering illness, which she knew would probably prove fatal, with the same unflinching spirit that she had shown towards the German blitz. A favourite quotation of her's was Hugh Walpole's

"It isn't life that matters! It's the courage we bring to it.

She could have no more fitting epitaph.

C. AINSWORTH MITCHELL

The Separation of β-Carotene, Neo-β-Carotene and Xanthophyll of Dried Grass, Pasture Grass and Silage

BY T. BARTON MANN

(*Read at the Meeting, October* 6, 1943)

INTRODUCTION—It has long been known that farm stock responds to an intake of green food, whether fresh or as dried grass or silage, by laying up a stock of β-carotene and vitamin A in the liver, or depositing one or both of these substances in the milk colostrum or the egg, and it is generally accepted that the animal derives vitamin A from the β-carotene occurring in the green food.

It would not be true to say, however, that the quantity of β-carotene in the foodstuff is the only factor influencing the quantity of this pigment, or of vitamin A, laid down by

the animal in the liver, milk or egg. For example, I and probably others have observed that, for a given soil, poultry deposit a greater proportion of β-carotene and vitamin A in their livers and eggs when grazing on new pastures than on old. This can only be explained on the assumption that for some reason the β-carotene is more available in new pastures.

It is none the less important, particularly in view of the fact that dried grass is bought largely for its β-carotene content, that any method of estimating this pigment should give a true figure for the amount present. The present paper describes a rapid and convenient chromatographic method, using bone meal as adsorbent, for separating β-carotene from other pigments that occur with it in dried grass and, with modifications, the method may be extended to the separation of β-carotene from other pigments in fresh or preserved green leaf material.

The view that partition of the yellow pigments of grass on phase separation between light petroleum and 90% methanol allows only β-carotene to pass into the petroleum phase is erroneous, for it has been shown that this petroleum fraction is not necessarily a soln. of β-carotene alone.

Moore,[1] using dicalcium phosphate as an adsorbent, showed that β-carotene was not the only petrol-soluble yellow pigment in green leaves, and demonstrated that with prolonged storage of alfalfa leaf meal the β-carotene content, as shown by the adsorption method of analysis, diminished at a greater rate than was indicated by the Peterson–Hughes[2] phase separation method. Further, Moore has stated that preliminary biological tests on the petrol-sol. non-β-carotene pigments indicate an activity of only 1 to 2% of the total carotene of the original sample.

Seaber[3] also showed that by dissolving the "carotene" fraction in light petroleum containing 3% of acetone, and passing the soln. through an adsorption column of Merck's alumina, it was possible to remove up to 30% of the pigments. The more highly adsorbed pigment appeared to be definitely non-carotene in character, but Seaber was unable to identify the compound.

Beadle and Zscheile,[4] in the examination of fresh vegetables, asparagus, broccoli leaves, tips and stems, spinach, peas and green beans, found the β-carotene of hexane extracts to fluctuate from 79·2 to 89·6%, the remaining pigment being mainly neo-β-carotene, a term used by these workers in preference to the pseudo-α-carotene of Zechmeister. Beadle and Zscheile consider β-carotene to be the parent substance of neo-β-carotene, and evaluate $E^{1\%}_{1cm}$ 442·5mμ hexane 2060 for the latter pigment. In my opinion the term "neo-β-carotene" appears suitable for describing this pigment, which seems to be very closely related to β-carotene, and it is used throughout this paper.

The tentative official method for estimating β-carotene proposed by the Carotene Committee of the Crop Driers' Association[5] regards the petroleum fraction of grass extract (after suitable treatment to exclude chlorophylls and partition with 90% methanol to remove xanthophyll) as essentially the pro-vitamin A carotene of dried grass. It is unable to distinguish between high and low pro-vitamin A pigments, and may often result in an analytical and monetary value being placed on dried grass beyond its biological worth.

The following rapid and accurate technique which I have developed will be found suitable for the routine analysis of dried grasses for β-carotene; it also includes the separation and spectroscopic identification of neo-β-carotene and xanthophyllic pigments.

Extraction of Pigments from Dried Grass, Silage, etc.—Weigh a suitable quantity of dried grass (3 g of a sample of poor quality or 1 g of one of good quality) and grind with fine, hard sand with an agate pestle in a porcelain or an agate mortar, until the grass is smooth and flourlike. If the sample consists of fresh leaf material and silage, grind it with anhydrous sodium sulphate together with the sand and, if it is silage, neutralise the acidity by further addition of 1 g of anhydrous sodium carbonate for each 5 g of silage. Next transfer the sample and sand to a Soxhlet thimble in a straight-through drip-type extraction tube, fitted with a 100-ml round flask and a reflux condenser, rinse the mortar until free from colour with a few ml of ether into the flask, and extract on a water-bath with a mixture (1 : 3) of acetone and peroxide-free ether, freshly distilled from sodium. Maintain the water-bath at a temp. just sufficient to boil the acetone-ether mixture. (If β-carotene determination only is required, use a mixture of light petroleum and acetone (3 : 1) for extraction and for rinsing the mortar.) After 30 min. remove

the thimble, re-pound the grass and re-extract for 15 min. Again re-pound and re-extract for a further 15 min. Any colour then remaining in the grass will be due to chlorophylls.

Transfer the acetone and ether extract of the grass (or petroleum-acetone extract if only β-carotene is being determined) to a 300-ml round-bottomed flask and distil on a water-bath until only *ca.* 5 ml remain. Complete the evaporation by blowing nitrogen or carbon dioxide into the flask, which is meanwhile rotated on the bath. Then add 10 ml of light petroleum and blow into the flask as before until the extract has a viscous consistence. Next add 30 ml of light petroleum, mix the contents of the flask by shaking, and immediately cool under running water.

PREPARATION OF ADSORPTION COLUMN—Bone meal (commercial steamed bone flour, feeding bone flour) previously extracted for 24 hr. in a straight-through drip-type extraction tube with a mixture of light petroleum (b.p. 40°–60° C.), acetone and ether, 3 : 1 : 1 (to remove fat and sterols) until no more colour can be extracted, is used as adsorbent for chlorophylls, neo-β-carotene and xanthophyll as follows.

Mount a glass tube, 6 in. long by 1 in. internal diam. and having a narrower open stem 3 in. long at the base, on a filter-flask, put a small plug of cotton wool in the bottom of the tube and press it firmly, so that *ca.* ⅜-in. of the stem is filled with wool, leaving ¼-in. in depth in the tube proper. Next place the extracted bone meal in the tube to a depth of 2 in., then another layer of cotton wool, and finally a ¼-in. layer of anhydrous sodium sulphate. Lightly tap the tube during filling, and press the contents gently, but not too tightly, with a flat-ended rod. To obtain bright solns. of β-carotene pass *ca.* 30 ml of acetone through the column of bone meal, and then remove the acetone from the column with about 50 ml of light petroleum.

SEPARATION OF β-CAROTENE FROM OTHER PIGMENTS—Apply gentle suction to the column, and pass some light petroleum through it. Before the column is dry add the grass extract in light petroleum and rinse the flask with light petroleum until no petrol-sol. pigment remains, the successive washings being added to the column. If xanthophyll and neo-β-carotene determinations are required (*cf.* next paragraph) add a few ml of ether to the flask to dissolve any xanthophyllic pigment adhering to the flask wall. Then close the flask with a cork and set it aside until needed. Elute the column with light petroleum until no further colour can be removed, and throughout the separation maintain it in a flooded condition. Chlorophylls, neo-β-carotene and xanthophyll will remain adsorbed, and the eluate will contain the total β-carotene; 100 ml of light petroleum are usually sufficient to elute the whole of the β-carotene from any of the suggested samples. Finally, estimate the β-carotene in the yellow soln. by the usual spectroscopic or colorimetric methods.

SEPARATION OF CHLOROPHYLLS, NEO-β-CAROTENE AND XANTHOPHYLL—For the separation of chlorophylls, neo-β-carotene and xanthophyll, the following procedure has been adopted. Elute these pigments, adsorbed on the bone meal, by passing ether through the column; it is impossible to elute the whole of the chlorophyll with ether, but in practice 100 or 150 ml are sufficient to remove the yellow pigments and most of the chlorophylls. Return the eluate to the *original* round-bottomed flask and reduce to *ca.* 5 ml by distillation from a water-bath at as low a temp. as possible. Add 5 ml of colour-free 30% methyl alcoholic potash (freshly prepared) and heat the extract under reflux with an air condenser for 10 min. with periodical shaking.

Cool the flask and transfer the contents to a 500-ml separating funnel, using *ca.* 50 ml of peroxide-free ether in all to rinse the inside of the flask, and adding the rinsings to the funnel. Also use 60 ml of water, in portions, to rinse the inside of the flask, add these to the contents of the funnel, and shake the whole *gently*. After standing, the yellow pigments will be found dissolved in the upper ether phase, while the potassium chlorophyllins remain with the lower aqueous phase. Drain the latter phase into another separating funnel, and re-extract it with further 50-ml portions of ether, until no more colour is extracted. After 2 or 3 extractions the shaking may be more vigorous. The extraction of a small quantity of chlorophyllin along with the yellow pigments will not vitiate subsequent operations.

Combine the ethereal extracts, wash with water, until the washings give no colour with phenolphthalein, evaporate by distillation, and blow nitrogen or carbon dioxide into the flask, exactly as described for the preparation of the original extract; take up the

residue in *ca.* 30 ml of light petroleum, pass the soln. over a fresh column of bone meal, prepared as previously described, and then pass *ca.* 150 ml of light petroleum through the column in order that neo-β-carotene and xanthophyll may be adsorbed (Note, *vide infra*). Add a few ml of acetone to dissolve any adherent xanthophyll, cork the flask and set it aside. After adsorption of these pigments, the column, while still damp with light petroleum, is transferred immediately to another filter-flask and flooded with benzene (thiophene-free), and gentle suction is applied.

Next elute the column with benzene until the eluate runs colourless. The eluate contains the whole of the neo-β-carotene $E^{1\%}_{1cm}$ 454*mμ* benzene 1900 (provisional). Remove the benzene remaining on the column with light petroleum, elute the xanthophyll with acetone, and add the eluate to any xanthophyll in the flask which has been corked and set aside; $E^{1\%}_{1cm}$ 447*mμ* acetone 2400 (provisional).

Lastly, remove the acetone with light petroleum, leaving the column ready for many further similar separations.

Note—At this point, especially with dried grasses which are aged, a slight amount of colour may be washed through the column. This pigment gives two absorption bands in the visual spectrum, 445*mμ* and 475*mμ* (in light petroleum). It has not yet been possible to obtain sufficient of this pigment to investigate and identify it.

SUMMARY—A method is described for the separation of β-carotene of dried grass and leaf material from all other yellow pigments and chlorophylls, by the use of extracted bone meal as an adsorbent.

A method is also described for separating neo-β-carotene from xanthophyll after removal of β-carotene and chlorophylls, extracted bone meal being used as an adsorbent.

A combination of both methods is suitable for the analysis of leaf material, whether fresh, dried or preserved, for the estimation of β-carotene, neo-β-carotene and xanthophyll.

Extracted bone meal is easy to prepare, cheap and readily obtainable. Its filtering properties make it especially suitable for use in chromatography. Its use for the determination of β-carotene in green leaf material makes the customary treatment with alkali for the removal of chlorophylls unnecessary.

ADDENDUM: THE QUESTION OF PSEUDO-α-CAROTENE

Beadle and Zscheile[4] state that the purpose of their paper is "to show that the carotene fraction from certain fresh plant material consists of β-carotene plus a pigment which is distinguishable chromatographically from β-carotene." They consider this pigment to be neo-β-carotene and identify it with pseudo-α-carotene recorded in the literature.

I am unable to accept this identification and consider that Beadle and Zscheile, by turning from the manipulation of pigments found in plant extracts to pure β-carotene in an endeavour to discover the part played by this new plant pigment in the β-carotene isomerisation complex, led themselves to confuse neo-β-carotene with pseudo-α-carotene.

Gillam and El Ridi,[6] dealing with crystalline β-carotene, have shown that pseudo-α-carotene having an E max. at 446*mμ* in light petroleum, b.p. 70°–80° C., is an isomer of β-carotene and is capable of reversion to β-carotene on further chromatography. Neo-β-carotene, the second pigment of fresh plant extracts, has an E max. at 442·5*mμ* in hexane, which is only slightly displaced in light petroleum, b.p. 70°–80° C., and under the conditions of my examination, in light petroleum, has not been greater than E max. 444*mμ*.

The order of adsorption on alumina of the carotene pigments of fresh plant extracts, beginning with the more lightly adsorbed, is β-carotene (sometimes another pigment, probably β-carotene monoxide) and neo-β-carotene.

As β-carotene only of all these pigments is not retained on a column of bone meal from light petroleum (b.p. 40°–60° C.) containing fresh grass extracts, it is interesting to follow the isomerisation of β-carotene with the formation of pseudo-α-carotene from freshly prepared extracts of fresh grass.

For the purpose of the expts. a bone meal column was used, as described above, and 4 columns (10 × 2 cm) of alumina, which was in such a critical state of activation that pseudo-α-carotene readily separated from β-carotene. Such selective adsorption is difficult to secure, but I used Brockmann's standardised alumina *ex* Merck, which from repeated use had lost its adsorptive properties, and effected the necessary critical re-activation by heating at 380° C. for 30 min.

Young lawn grass from a recent sowing was ground with sand and sodium sulphate, and extracted for 30 min. with light petroleum and acetone, the operations being carried out as soon as possible after collection of the grass. The extract was evaporated by distillation, and carbon dioxide was blown into the flask as described above, and the residue was dissolved in light petroleum, which was passed through the column of bone meal to obtain a soln. of β-carotene. This β-carotene soln. was immediately passed through the first column of alumina, and the chromatogram was developed with light petroleum until two distinct zones formed. The upper, β-carotene, was of a salmon-pink colour, and the lower, pseudo-α-carotene, was brownish-yellow. It was impossible to effect complete separation of the zones, but further development of the column with light petroleum resulted in the elution of the base of the lower zone. This eluate,

containing pseudo-α-carotene free from β-carotene, was immediately passed through the second column of alumina. Here, again, two zones formed, the upper salmon-pink, and the lower brownish-yellow. The expt. was continued until pseudo-α-carotene soln., free from β-carotene, had been passed from the second through the third, and from the third through the fourth column in sequence, in each instance with identical results. The pseudo-α-carotene reverted immediately to β-carotene, the proportion reverting being about 9/10 on each chromatogram, as judged by the depth of the zones and the intensity of the colour.

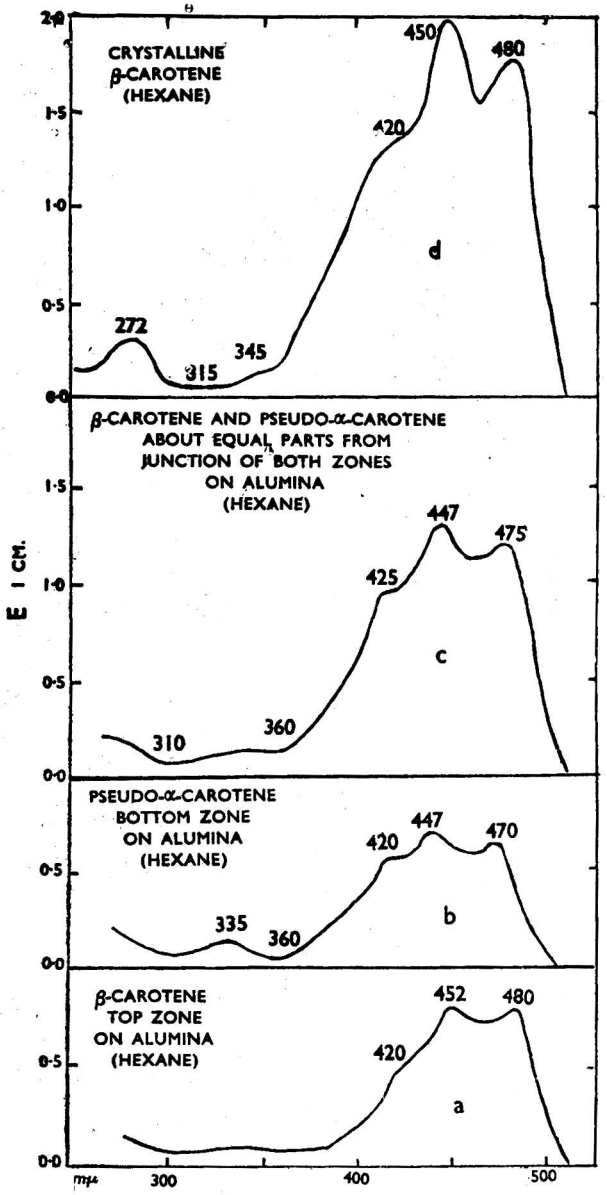

Fig. 1

A spectrogram, made immediately, of part of the original soln. from the bone meal column showed the soln. to consist mainly of β-carotene almost devoid of pseudo-α-carotene (Fig. 2 *g*). When the expt. was repeated on the following day, the lawn grass having meanwhile stood in a paper bag for 24 hr. at room temp., the reversion of pseudo-α-carotene to β-carotene took place much more slowly on this alumina, the proportion reverting being about 1/10, as judged by the depth of zones and intensity of colour.

Attention was then paid to the chromatographic behaviour of crystalline β-carotene. Dr. R. A. Morton kindly supplied me with a sample of crystalline β-carotene conforming to accepted criteria of purity. A soln. of this in light petroleum spirit (b.p. 40°–60° C.) was passed through a column of this alumina, 50 × 2 cm, and it was found that, although two well-defined zones formed, no complete separation of pseudo-α-carotene from β-carotene occurred. Apparently pseudo-α-carotene will continue to form from β-carotene as long as the soln. has contact with fresh alumina capable of exerting such discriminating adsorption. There can be no doubt that β-carotene in soln. undergoes isomerisation, and critical chromatography on alumina hastens the isomerisation. This isomerisation may be studied by spectrographic methods, but it seems unlikely that quantitative estimations can be made by these methods on such dynamic solns., which tend to revert to equilibrium during manipulation. Under these conditions spectroscopic and spectrographic data are apt to be uncertain, but it is interesting to examine such data in the light of the foregoing facts.

In the visual spectrum, in hexane β-carotene E max. occurs at 452*mμ*, and pseudo-α-carotene E max. at 446*mμ*. A hexane soln. of a mixture of these pigments will give an E max. lower than 452*mμ* and higher than 446*mμ*. These are very slight differences, but are nevertheless discernible. The differences of the secondary absorption bands at *ca.* 470*mμ* to 480*mμ* are not readily ascertainable because of lowered dispersion in this region of the spectrum, and for this reason will not be dealt with.

When the spectrum is enlarged to include part of the ultra-violet region, a hexane soln. of crystalline β-carotene gives evidence of isomerisation (Fig. 1, *d*), showing a picture of the superposing of some of the spectral characteristics of freshly prepared pseuco-α-carotene solution (Fig. 1, *b*) upon the spectrum of freshly prepared β-carotene soln. (Fig. 1, a), both obtained by chromatography from alumina, from a solution of the original crystalline β-carotene.

If these two chromatographically produced isomerides are compared, it will be seen that pseudo-α-carotene gives a marked inflexion at 415*mμ*–425*mμ*; the β-carotene inflexion in this region is not well marked. Pseudo-α-carotene gives inflexions at 330*mμ*–340*mμ*, and at 355*mμ*–365*mμ*; β-carotene at these wavelengths shows no appreciable absorption differentiation. In addition, extinction values of the solns. of the

individual isomerides do not agree with the extinction value of the original β-carotene soln. which has attained equilibrium.

Unfortunately, ultra-violet spectroscopy of petroleum soluble carotenoids obtained with concomitant grass oil and sterols from leaf material is handicapped by the fact that these and other ultra-violet absorbing substances accompany the plant pigments during chromatographic separation. Apparently these substances occupy a position on the column very near to, and are eluted with, the respective pigments, so that their presence is an indication of the sequence of adsorption.

In the final identification of the carotene fraction of grass, these light-absorbing substances will therefore have some positive value, for whereas interference with the photography of the pseudo-α-carotene absorption spectrum begins at *ca.* 300mμ and continues to shorter wavelengths, interference with the photography of neo-β-carotene begins at 370mμ and also continues to shorter wavelengths (Fig. 2, *f* and *e*).

The contention of Beadle and Zscheile, that 2 carotene fractions exist in fresh leaf material must be accepted; they are β-carotene and neo-β-carotene. Neo-β-carotene is not to be identified with pseudo-α-carotene, for I must also accept the fact that pseudo-α-carotene is an integral part of any β-carotene soln. from any source, these two latter pigments being isomers tending to be held in a physical state of equilibrium.

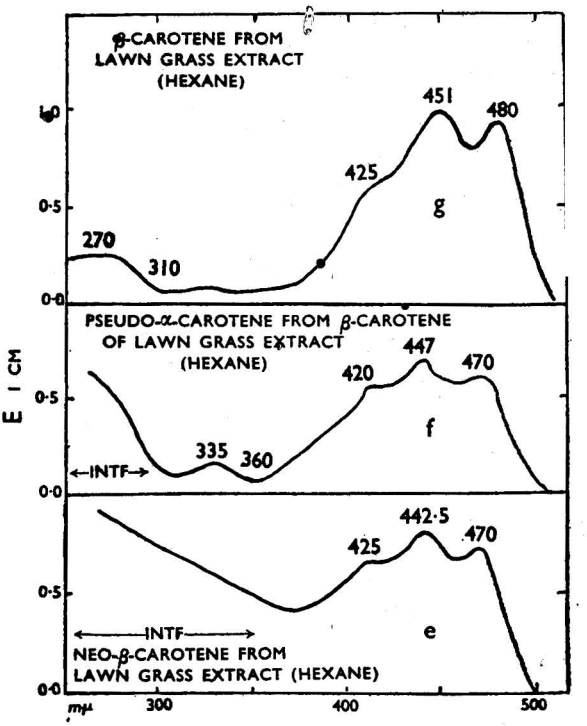

Fig. 2

On the other hand, neo-β-carotene, identified by its absorption E max. at 442·5mμ in hexane, forms a second pigment in the carotene fraction of leaf material and may be regarded as a natural congener of β-carotene.

Fortunately the presence of pseudo-α-carotene in β-carotene soln. does not lessen the biological activity of the soln.; for a feeding test (kindly performed by Dr. K. H. Coward) on the β-carotene fraction, dissolved in vegetable fats, of a sample of dried grass 12 months old, containing pseudo-α-carotene, shows that the carotene fraction passing through bone meal has the full biological activity of β-carotene. The fraction adsorbed on bone meal appears to have no biological value, notwithstanding the fact that in this expt. it contained about 20% of petrol-soluble carotenoids, mainly neo-β-carotene, in addition to xanthophylls.

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August, 1943

The Analysis of Commercial Lecithin

BY H. H. HUTT, A.I.C., AND H. WEATHERALL, F.I.C.

THE analytical examination of the commercial lecithin used in the food and other industries has not received much attention in the literature, apart from the description by Rewald[1] of a method for the determination of phosphatides in the organic raw materials from which they are obtained.

Before the war, the bulk of the lecithin used was produced from the soya bean, either on the Continent or in the United States. Since 1940, however, the Empire-grown groundnut (*Arachis hypogaea*) has been the main source of phosphatides for industrial use in this country and the production of groundnut lecithin has been developed.

NATURE OF THE PHOSPHATIDES—Commercial lecithin is a mixture of lecithin and kephalin which occurs in plant tissue. As prepared industrially it contains *ca.* 30–40% of free oil, which may have varying proportions of neutral glycerides and free fatty acids. It also contains minor amounts of carbohydrates and proteins, either free or as prosthetic groups, and small quantities of phytin may be present in some samples.

The formula of lecithin has been recorded by McLean and McLean[2] as

$$(R.COO)_2C_3H_5O.P{:}O(OH)O.C_2H_4.N(CH_3)_3OH,$$

where (*R*.COO) represents a fatty acid radicle.

It should be noted that Fischgold and Chain[3] consider lecithin to be a zwitterion. The formula for this may be written as follows:

$$(R.COO)_2C_3H_5.O{-}\overset{\displaystyle O-}{\underset{\displaystyle O}{\overset{|}{\underset{\|}{P}}}}{-}OC_2H_4N(CH_3)_3{+}$$

The constitution of kephalin is not regarded as established beyond doubt,[4] but for present purposes the following formula may be ascribed to it:

$$(R.COO)_2.C_3H_5O.P{:}O(OH).OC_2H_4.NH_2.$$

The most important characteristics of lecithin and kephalin from the analytical point of view are their phosphorus contents and their behaviour with organic solvents. Theoretically, dioleyl-lecithin contains 3·86%, the corresponding kephalin 4·17% of phosphorus.

Both kephalin and lecithin are soluble in light petroleum, diethyl ether, benzene, chloroform and carbon tetrachloride but insoluble in acetone and are pptd. by acetone from solution in other organic solvents. Kephalin is distinguished from lecithin by its insolubility in ethyl alcohol.[5]

The total phosphatide content of a commercial sample may be estimated by either of two methods: (1) by determining the total phosphorus and multiplying by 26·31* or, (2), by determining the % of acetone-insoluble matter. Owing to the presence of small quantities of petroleum-insol. matter and other substances associated with the phosphatides, the % of acetone-insoluble matter is usually higher than the phosphatide content as estimated from the phosphorus determination. The matter insol. in light petroleum consists of finely divided meal, and the presence of protein renders the nitrogen content a less reliable guide to the amount of total phosphatides than the two methods mentioned above.

In assessing the quality of the product, the proportions of free fatty acid, moisture and petroleum-insol. matter should also be taken into account. It should be noticed that, since kephalin behaves as an acid in titration with alkalis,[3] the free fatty acid should be determined in the acetone-sol. fraction, *i.e.*, in the oil after separation from the phosphatides. Crude lecithin is sometimes refined by washing out the glycerides and free fatty acids with acetone and replacing them with a high grade edible fat. These products should be reasonably free from acetone. Methods of analysis are described below.

(1) TOTAL PHOSPHORUS—The organic matter is destroyed by wet ashing, and the phosphorus is pptd. as ammonium phosphomolybdate, re-pptd. as magnesium ammonium phosphate, ignited and weighed as magnesium pyrophosphate. Weigh 3 g of the sample on an 11-cm ashless filter and warm it in a 1-litre Kjeldahl flask with 20 ml of conc. nitric acid for 2–3 min. Cautiously add 20 ml of conc. sulphuric acid in successive small portions and heat strongly for *ca.* 1½ hr., with occasional shaking. Cool, add 10 ml of conc. nitric acid and boil until brown fumes are no longer evolved; then add a further 10 ml of nitric acid and boil off again. Repeat this process until no darkening occurs after evaporation of the nitric acid. Cool, add 100 ml of water, transfer the liquid to a 1-litre squat beaker with a further 100 ml of water, boil for 10 min., make the soln. alkaline with ammonia soln. (0·880), adding 30 ml in excess, re-acidify with nitric acid and ppt. the phosphoric acid with 10 g of ammonium molybdate A.R. in 300 ml of water, in the usual way.

* 26·31 is an arbitrary factor in general use.

There may be a slight colour, due to organic matter, in the supernatant liquid, but if the colour after pptn. is no greater than before, it may be ignored. Dissolve the washed ppt. in ammonia soln. and ppt. the phosphorus with magnesia mixture by the ordinary method.

The above method, checked against pure anhydrous sodium glycerophosphate B.P. gave the following results—(a) 14·27; (b) 14·29; (c) 14·43; (d) 14·25% of phosphorus (theory, 14·35%). In (a) the soln. was not heated after addition of magnesia. This made little or no difference to the result, but there is a greater tendency for the magnesium ammonium phosphate to adhere to the beaker in cold pptn. In (d) 2 g of edible groundnut oil were added to the glycerophosphate.

An alternative method for the destruction of organic matter, using hydrogen peroxide, has been published.[6]

(2) ACETONE-INSOLUBLE MATTER AND FREE FATTY ACIDS—In method (*a*), used in this laboratory, the acetone-insol. matter is determined by direct elutriation of the soluble fraction. The determination can also be made by pptng. the phosphatides with acetone from another solvent as described in method (*b*). The pptn. method (*b*), which was communicated to us by Mr. A. C. Francis, probably gives more accurate and concordant results than (*a*), but is somewhat lengthy. We have adopted the elutriation process, as it is quite reliable when used in conjunction with the phosphorus figure and is more suitable for works control analysis where many samples are examined in the course of a week.

(*a*) *Elutriation Method*—Weigh 5–10 g of the sample into a 150-ml CO_2 flask provided with a glass stirring rod, the lower end of which is widened out to facilitate kneading of the lecithin. Add about 100 ml of pure acetone and transfer the mixture to a steam-heated plate. When the solvent has reached b.p. knead the lecithin thoroughly, stirring the solvent into it. Cool to room temp. (20° C.) and allow to settle. Pour off the acetone-oil soln. into a 250-ml beaker. Re-extract the lecithin with two further 100-ml portions of acetone. Transfer *ca.* 50 ml of the acetone-oil soln. to a weighed 150-ml CO_2 flask, evaporate the solvent and re-weigh. Determine the free fatty acid by titration in boiling neutral alcohol (phenolphthalein as indicator). Calculate as oleic acid % in the acetone-sol. matter and convert to free acid % in the original sample by means of the formula at the end of this section.

Remove the solvent from the insol. residue in a current of air on a steam-heated plate and weigh. Express as acetone-insol. matter %.

(*b*) *Precipitation Method*—Weigh 5 g of the sample into a 250-ml beaker. Pour on to it 6 ml of ether and leave with occasional stirring until solution is complete. Add 100 ml of pure acetone gradually, stirring continually. Leave for 10 min. or until the supernatant liquid is clear or nearly clear. Decant the soln. through a No. 4 Whatman filter paper into a 400 ml flask. Evaporate the residual acetone from the insol. matter. Re-dissolve the latter in 6 ml of ether and reppt. with 100 ml of acetone as before. Decant the supernatant soln. through the same filter into the same flask as before. Treat for a third time by repptn. and decantation. Distil the acetone-ether mixture from the soluble matter, removing the last traces in a steam-oven. Dissolve the residual oil in 50 ml of acetone and filter through a No. 4 Whatman filter-paper into a weighed flask. Wash the filter with acetone until free from oil. Evaporate the solvent from the oil and dry to constant weight at 100° C.

$$\text{Acetone-insol. matter, \%} = 100 - \text{moisture \%} - \text{sol. in acetone, \%}.$$

Determine the moisture of the sample as described under (3). Calculate the free fatty acid content of the original lecithin from the above data as follows.

F.F.A. % (as oleic acid) in the original sample =

$$\frac{\text{F.F.A. \% in the acetone-sol. matter} \times (100 - \text{acetone insol. matter \%} - \text{moisture \%})}{100}$$

(3) MOISTURE—Weigh 3 g of the sample into a glass dish (60 mm in diam.), preferably provided with a ground-in stopper and dry in an oven at 100° C. for 3 hr. Take the loss of weight % as the moisture content.

The loss of volatile matter under these conditions is mainly water but a small increase in weight of the actual phosphatides, due to oxidation, takes place concurrently. This, of course, can be avoided by conducting the heating in a stream of nitrogen, but, as the moisture determination is normally only used to ensure that no excessive amount such

as would lead to mould formation, is present, small inaccuracies may be overlooked. The upper limit for permissible moisture content is *ca.* 2·5%. In samples which have been dried to much less than 1% a small fraction of the loss at 100° C. may be due to alkaline decomposition products.

We have examined several specimens of commercial lecithin by the common methods for the determination of water and have found that in distillation methods using heptane, toluene and xylene decomposition occurs, and an alkaline substance (probably cholamine), appears in the aqueous distillate. This leads to emulsification of the aqueous and solvent layers. Constant figures were obtained in some expts. by heating *in vacuo*, but fatty acids were observed condensing on the upper parts fo the oven.

The following instances exemplify these points.

Specimen A—Loss in 3 hr. at 100° C. 0·78%.

Moisture determined by distillation with heptane								
No. of hr. under reflux.. ..	18	30	42	52	60	72	80
Moisture, %	0·85	0·89	0·94	1·05	1·10	1·12	—
Moisture determined by distillation with toluene							
No. of hr. under reflux.. ..	18	30	42	54	60		
Moisture, %	0·78	0·89	0·94	1·06	1·06		
Moisture determined by distillation with xylene							
No. of hr. under reflux.. ..	18	30	42	54	60	72	80
Moisture, %	1·25	1·50	1·90	2·00	2·10	2·12	2·35
Moisture determined at 100° C. in 28 in. vacuum*							
Time, hr.	2	4	6	8	10		
Moisture, %	1·18	1·48	1·97	1·97	1·97		

* Fatty acids were observed on the upper parts of the vacuum chamber.

Loss of weight by the vacuum method on three further specimens failed to reach constancy in twelve hours.

Specimen B.

Moisture determined by heating in nitrogen at 100° C.							
Time, hr.	1	2	3	4	5	6	7
Moisture, %	4·25	4·60	4·80	4·84	4·84	4·97	—
Moisture determined by heating in air at 100° C.							
Time, hr.	1	2	3	4	5	6	7
Moisture, %	2·88	3·72	4·30	4·52	4·71	4·66	4·73

A 3-g portion of B was placed in a flask on a boiling water-bath and a stream of warm dry nitrogen was passed over it for 3 hr. The effluent gas was led through 25 ml of *N*/10 sulphuric acid. Back titration showed that no alkaline decomposition products were removed.

Although the data given above lend support to the use of oven drying for the moisture determination, the possibility of oxidation of lecithin from more unsaturated oils should not be overlooked, and with such samples nitrogen should be used.

(4) Determination of Petrol-insoluble Matter—Weigh, accurately, about 5 g of the sample and dissolve it in 30 ml of petroleum spirit (B.P. 40–60° C.) in a weighed centrifuge tube by warming on a water-bath. Centrifuge at 3,500 r.p.m. for 10–15 min. Pour off the supernatant liquid and repeat the washing twice with 30-ml portions of petroleum spirit. Dry on a water-bath. Re-weigh and express the results as petrol-insoluble matter %.

(5) Determination of Residual Acetone in Refined Lecithin—The following method has been adapted from that described by D. D. Van Slyke.[7]

Solutions required—10% mercuric sulphate: 73 g of pure red mercuric oxide dissolved in 1 litre of 4 *N* sulphuric acid; 50% (by vol.) sulphuric acid adjusted to 17·0 *N* H_2SO_4; 5% sulphuric acid.

Method—Weigh, accurately, *ca.* 25 g of the sample and dissolve it in 150 ml of light petroleum (b.p. 40–60° C.) and extract with four 50-ml portions of 5% sulphuric acid. Run the first two extracts into a second separating funnel containing about 75 ml of light petroleum and shake lightly. Filter the combined aqueous layer through a fluted Whatman No. 4 filter paper into a 600-ml conical flask. Use the third and fourth extracts from the lecithin-petrol solution to wash the petrol in the second separator and the filter. Add 20 ml of 50% sulphuric acid and 70 ml of 10% mercuric sulphate soln. to the combined

extracts and boil under a reflux condenser for 45 min. Cool and filter off the ppt. of mercuric sulphate and acetone on a weighed Gooch crucible, wash with 200 ml of cold water, dry for 1 hr. at 110° C. and re-weigh (20 mg of ppt. ≡ 1 mg of acetone).

Care is needed in shaking the petrol-lecithin soln. with the sulphuric acid, as too much shaking causes emulsions and too little leads to incomplete extraction.

ACKNOWLEDGMENTS—We wish to thank Dr. Bruno Rewald for communications on which some of the methods are based, Mr. A. C. Francis for permission to include his method for the determination of acetone insol. matter, Mr. C. L. Bibby for his help in the preparation of this paper, and the Directors of Messrs. J. Bibby & Sons, Ltd., for permission to publish.

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DISCUSSION

Mr. K. A. WILLIAMS congratulated the authors on having prepared the first comprehensive account of the analysis of commercial lecithins. Referring to the determination of phosphorus, he asked whether the authors had any comparative figures for the simpler and quicker modifications of the method in which partial combustion of the product was carried out in presence of carbonates in place of wet oxidation, and in which the Pemberton method was used for the actual determination of the phosphorus. The latter, described by E. R. Bolton (*Fatty Foods*, 1st Ed., p. 341), which involved the solution of the phosphomolybdate precipitate in a known excess of standard alkali and titration of the excess by means of standard acid, was in his experience very satisfactory.

He confirmed the difference noted by the authors between the figures calculated for lecithin content and matter insoluble in acetone, and mentioned that the difference had at times been even greater in Japanese and Manchurian lecithins than in those now being produced in this country. He agreed that most of the difference could rightly be ascribed to the presence of sugary matter. It was possible to obtain a good check on the amount of oil present in a sample by determining the total fatty acids yielded by saponification, acidification and extraction with ether, subtracting from this figure 70·39 % of the calculated percentage of lecithin and dividing the result by 0·957; the factor, 70·39%, was based on the composition used for lecithin by Lewkowitsch and corresponded to a factor of 26·02 for the conversion of phosphorus to lecithin.

He asked whether the authors had had any success in attempting to determine acetone-insoluble matter in crude oils known to contain lecithin, as his experience was that no satisfactory precipitation could be obtained; he ascribed this chiefly to the solvent effect of the accompanying oil—an effect that was well-known in determinations of impurities in sulphur olive oils and black greases. For crude oils it appeared necessary to rely on the phosphorus determination as a guide to the lecithin content.

Dr. H. C. LOCKWOOD asked if the authors had considered the determination of free choline, as this substance was stated by some authorities to be the cause of bitterness in lecithin which had been stored for long periods. The method he had in mind was that of Beattie, who used ammonium reineckate.

Dr. B. REWALD said that lecithin, especially plant lecithin, was used in increasing quantities, not only in the food industry but in many technical processes. He asked how plant lecithin could be estimated if other sources of lecithin were present, such as whole egg or egg lecithin. Acetone would dissolve a certain proportion of phosphatides, especially if the amount of free fatty acids was high. This had to be taken into consideration.

Mr. HUTT, replying to Dr. Lockwood, said that he had examined a number of samples (both fresh and stored) for free choline, using an adaptation of Beattie's method (*Biochem. J.*, 1936, **30**, 1554; ANALYST, 1937, **62**, 57). Very low figures were obtained, and these were possibly due to lecithin passing through the filter during the extraction. Free choline did not appear to be present.

Mr. Bacharach had asked if possible variation in the composition of the complex of mercuric sulphate and acetone might offset the analytical advantage gained by its high molecular weight. The reply was that Van Slyke (*J. Biol. Chem.*, 1917, **32**, 456) had found the composition of the complex to be reasonably constant for a given ratio of H_2SO_4 to $HgSO_4$.

Replying to Mr. Williams, who had found very considerable quantities of carbohydrates in Manchurian soya lecithin, Mr. Hutt said that he had found up to 18% of sugars (mainly non-reducing) present in combination with the phosphatides. With regard to the difference between the figures obtained for lecithin content by calculation from the amount of phosphorus and by determination of acetone-insoluble matter he said that other methods of ashing had been tried, but that wet destruction had been found the most satisfactory.

The elutriation method was not applicable to crude oils. Phosphorus was usually determined gravimetrically on 50 to 100 g portions of the samples.

Photometric Determination of Silicon in Aluminium Alloys: Observations on Hadley's Method

By W. STROSS, M.D.

METHODS for the photometric determination of silicon in aluminium alloys have been based on its forming with molybdate the yellow[1,2,3] or the blue[4] complex. The former (*e.g.*, the method of Hadley[2,3]) were established by means of the Pulfrich photometer, and the latter with the Spekker absorptiometer. For large scale routine work the Pulfrich instrument, now practically unobtainable in this country, seems definitely less suitable as regards speed, ease of operation by various operators and eye strain than the Spekker photometer. The methods based on the yellow complex, however, seemed preferable because of their greater simplicity and speed. Hence, in adapting the first type of method for the Spekker instrument and for large scale routine work numerous details were modified in order to give a sufficient increase in speed, simplicity and accuracy. In well over 20,000 determinations on alloys of various types (*e.g.*, Duralumins, RR.50, 53, 56, 77, DTD.324, 423, 424, L.33, Si-hardeners, Mn- and Ni-hardeners, Y-alloys) the following technique was developed.

METHOD—Attack 100-mg samples of any range between 0·2 and 15% of silicon with 10 ml of 40% sodium hydroxide soln. in stainless steel beakers covered with steel or nickel lids; when the violent reaction has ceased, boil gently for 10 min. Then rinse down with 25 ml of water and add nitric acid slowly with vigorous shaking, continuing the shaking for several sec. after the addition. Take the following amounts of acid—(*a*) for less than 1·5% of Si: 25 ml of 4·9 *N* acid (23 ml for blanks); (*b*) for 1·5 to 3% Si: 21 ml of 7·2 *N* acid (19 ml for blanks); (*c*) for more than 3% of Si: 50 ml of 7·2 *N* acid (same amount for blanks). Under these conditions the pptn. of aluminium hydroxide is quite transitory, and no heating in order to re-dissolve it[2] is necessary. Filter, and then dilute (*a*) to 100,* (*b*) and (*c*) to 200 ml*; use Whatman No. 40 filters for (*a*) and (*b*), particularly for Y-alloys (with which partial reduction to molybdenum blue frequently occurs if coarser filters are employed; any fine particles of nickel passing through the filter appear to have a reducing effect), and for alloys rich in Mn. With No. 40 filters the use of sulphurous acid, followed by permanganate and then oxalic acid,[2] was never found necessary by us. Cool to between 20 and 22° C. To develop the colour, add to the solns. one-tenth of their vol. of 10% ammonium molybdate soln. Thus, add to the 100 ml of samples of type (*a*) (*i.e.*, with less than 1·5% of silicon) 10 ml of molybdate soln.; to those of range (*b*) (1·5 to 3% of silicon), the vol of which is made up to 200 ml, add 20 ml. The highest range (*c*) requires preliminary dilution; pipette 10 ml from the well-mixed contents of the 200-ml flask into a 50-ml measuring flask,* make up to 50 ml and add 5 ml of molybdate soln. Measure the extinction not less than 5 or more than 10 min. after addition of the molybdate, using a 4-cm cell, a mercury vapour lamp, and Ilford 601 Spectral filters. Insert two "Calorex" filters on the right-hand side of the instrument to protect the Ilford filters from heat over long periods of use. Set the instrument at 1·30 "water-to-water." Establish calibration graphs for alloys, the silicon content of which is known from careful gravimetric analysis. A tungsten lamp may be used with Ilford Spectrum Violet filter 601, but the curve flattens out, and the range of silicon contents covered is therefore much narrower than with the mercury lamp.

Messrs. A. Hilger advocate for the yellow molybdenum complex the combination of Chance's No. 8 (now called Chance's OV1) and Wratten No. 2 filters. This combination is about 3 times as sensitive as the 601 Ilford filters; the latter, however, gave considerably more concordant results, probably owing to the extreme sensitiveness of the former to the most minute turbidity.

Contrary to Hadley's recommendation,[2] it was found unnecessary to make up fresh solns. of sodium hydroxide and molybdate. The former can be stored for at least 2 weeks in stainless steel containers with tight-fitting lids,† the latter for longer in glass. It is, however, advisable to use a brand of molybdate having little tendency to form a white deposit,‡

* The so-called "sugar-analysis" flasks with wide necks and two marks are very convenient.
† Obtainable from Joseph Long, Ltd., Harrow.
‡ In our expts. "AnalaR" molybdate proved superior to other brands that were tried.

and to filter the amount required daily through double Whatman No. 42 filters, using a little paper pulp.

The use of 10% sodium hydroxide soln.[2] for the two lower ranges does not ensure complete solution of the silicon. Appreciable amounts (increasing with increasing Si content of the alloys) may be found on the filter, even with less than 1·5% of Si, although no appreciable absorption of dissolved silicon on filter-paper occurs. The use of 40% sodium hydroxide soln., recommended by Hadley for the highest Si range only, is therefore advocated throughout.

The use of stainless steel beakers* cuts out entirely the transference from nickel crucibles[2] to glass beakers; it also avoids a source of error arising from the strong affinity of the silicon for the nickel surface, on which (in spite of thorough washing with hot water and "bobbying") silicon tends to accumulate slowly with frequent use, as can be proved by running "blanks" from time to time. No such accumulation was found with the stainless steel, even when no "bobby" was used.

For large scale routine work a speedy, clean and sufficiently accurate measurement of caustic alkali and acid solns. is ensured by using a small nickel measure with a handle† for the alkali and special short pipettes† for the acid (see Fig. 1); both are filled by dipping them into the solns.

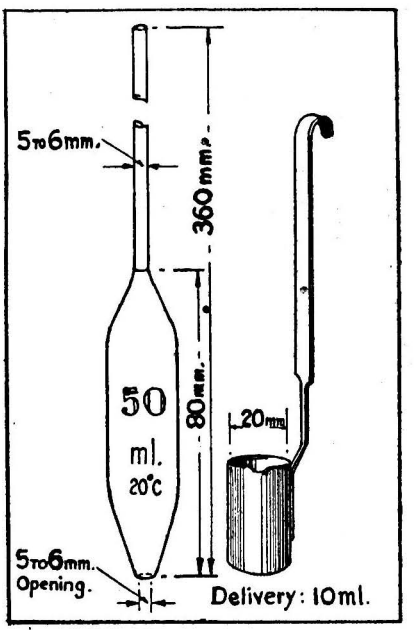

Fig. 1

Hadley states that the useful *p*H range in the final solns. is between 1·1 and 2·0; his optimum range is therefore likely to be about 1·5. He used a quinhydrone electrode. Although the acid-alkali balance that he recommends agrees fairly well with our optimum, we have found, using a glass electrode, the *p*H of the optimum range (see above) to be about 0·7 to 0·8. Dr. J. N. Agar, of the University Chemical Laboratory, Cambridge, kindly checked the *p*H of some of our solns. with both quinhydrone and glass electrodes. His results with both electrodes were consistent with each other and confirmed our findings.

The presence of the common alloying elements has very little influence upon the development of the colour; antimony soln., however, lowers considerably the extinction values if it is added before the molybdate. This, however, is of no practical consequence for the analysis of alloys containing up to about 0·5% of antimony (*e.g.*, B.A.35), apparently because most of the antimony remains undissolved.

I am indebted to the Directors of International Alloys, Ltd., for permission to publish these notes.

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Chemical Laboratory, International Alloys, Ltd. *October*, 1943

Notes

Application of Reindollar's Method to the Estimation of Saccharin

The increasing importance of sugar substitutes, particularly saccharin, has led me to study Reindollar's colorimetric test from the quantitative aspect. In that method the saccharin is treated under prescribed conditions with phenol sulphonic acid, whereby phenol sulphone phthalein, or phenol red, is formed, which in alkaline soln. produces a fine magenta colour.

* Obtainable from Townson & Mercer; the size 125 ml is convenient for ranges (*a*) and (*b*); 350 ml for (*c*).
† Obtainable from Joseph Long, Ltd., Harrow.

The influence of varying the temperature and time of reaction was studied, and the conditions prescribed by Reindollar, *viz.*, 2 hr. heating at 135–140° C., were found the best for maximum development of colour. No reaction took place at 100° C. in 2 hr., and after 2 hr. at 150° C. and above there was charring and swelling of the mass. If a metal air-oven is used it is important that the interior be lined with asbestos sheeting to prevent local overheating, which will give variable results and "smoky" colours that are difficult to match.

METHOD—Dissolve *ca.* 10 mg of the residue of the ethereal extract containing the saccharin in 1 ml of 10% sodium hydroxide soln. and make up to 100 ml with water. Take aliquot portions equiv. to 0·05 to 0·10 mg of saccharin. Evaporate the liquid in a small dish on a water-bath and to the dried residue add 2·5 ml of phenol sulphonic acid reagent, prepared by mixing equal weights of phenol and sulphuric acid. Heat the dish in an asbestos-lined air-oven at 135–140° C. for 2 hr. Treat known amounts of saccharin similarly. Remove the dishes and leave them to cool, and rinse their contents with a little hot water, into 100-ml Nessler cylinders. Dilute to 50 ml, make alkaline with 10 ml of 10% sodium hydroxide soln. make up to 100 ml and match the colours.

OBSERVATIONS.—(1) Blanks on extracts of various foodstuffs are good and equivalent to not more than 0·005 mg of saccharin. (2) Satisfactory gradation of colour is obtained over the range 0·01 to 0·10 mg. (3) It is possible to detect as little as 0·01 mg of saccharin. (4) Within the range 0·01 to 0·10 mg differences of 0·02 mg can be judged. (5) The colour is quite stable and lasts indefinitely. (6) Matching may be made by dilution, but standards prepared under the same conditions as the unknown are preferable. Experience has shown that 0·05 and 0·10 mg of saccharin are the most useful standards. (7) Standards may be prepared from a soln. of phenol red, but the recommended method is better.

INTERFERING SUBSTANCES.—A number of compounds that may occur in foodstuffs and might be extracted with the saccharin were examined for possible interference. It was found that amounts up to 2 mg of the following gave colours approx. of the order of the blank: salicylic acid, sodium *p*-chlorobenzoate, ethyl *p*-hydroxybenzoate, *p*-hydroxybenzoic acid. Amounts of benzoic acid[6] up to 10 mg gave colours equiv. to less than 0·015 mg of saccharin, and the colour due to 2 mg of cinnamic acid was equiv. to 0·07 mg of saccharin. None of these colours was of the true phenol red tint. Monier-Williams[3] has stated that cinnamic acid is not likely to be present in foodstuffs except where oils of cinnamon or clove have been used. Vanillin and caffeine interfere and will be discussed separately.

Vanillin—Reindollar's statement as to the influence of vanillin was confirmed. Vanillin melts at 80° C. and sublimes at 280° C. It was found that 8·4 mg of vanillin lost 2 mg in 15 min. at 100° C., 4·2 mg in 45 min., and the whole in 2 hr. Since the aliquot portions of the extracted saccharin are fixed with sodium hydroxide and dried prior to adding the phenol sulphonic acid reagent, vanillin, if present, will be partly or wholly lost in the drying. With a solution of vanillin in ether, 1 ml = 0·1 mg, it was shown that vanillin has a marked but variable influence upon the colorimetric method. It is probably unsafe to rely upon the volatility of vanillin to ensure its complete removal, and it is preferable to adopt Reindollar's method of extracting it from the residue with light petroleum. Saccharin is practically insol. in this solvent.[6]

Caffeine—When working with coffee extracts it was found that the caffeine extracted with the saccharin had a marked effect upon the colorimetric estimation. With such samples the caffeine must be separated by extraction from alkaline soln. with chloroform. The saccharin is then re-extracted from acid soln. with ether.

EXTRACTION—The foregoing work presupposes satisfactory extraction of the saccharin from the foodstuffs. Aqueous solns. of saccharin were first considered, and it was found that 92–95% of the saccharin could be recovered under the following conditions. To each 25 ml of the aqueous soln. add 3 ml of 10% hydrochloric acid and extract with 3 × 30 ml of ether. Wash the mixed ethereal solns. with 5 ml of water, separate the ether, dry and weigh the residue.

Many foods require no preliminary treatment before extracting, but with others proteins must be removed. Treatment with 25% lead acetate soln., as in the A.O.A.C. methods,[4] was adopted. In certain instances de-leading with sodium phosphate was used. Table I indicates the type of results obtained

TABLE I

	Saccharin, mg			Remarks
	Sulphate	L. & W.	R.	
25 ml sugar syrup 10 mg saccharin ..	9·3	—	—	Lead acetate and ether extraction of acid soln.
„ „ ..	—	9·2	8·7	De-leaded with sodium phosphate
„ „ ..	—	11·0	9·0	Direct extraction
25 g arrowroot 10 mg saccharin ..	—	8·2	10·6	Aqueous extraction of arrowroot; suction filtration
25 g cornflour 10 mg saccharin ..	17·7	7·3	—	Alcoholic extraction. Alcohol removed, extracted with ether
„ „ ..	—	2·5	0·6	Lead acetate and glacial acetic as in A.O.A.C. method

with syrup, cornflour and arrowroot. Comparison of the colorimetric method is made with the sulphate method[4] and the nesslerisation method of Lerrigo and Williams.[5] The syrup presented no difficulties. The results were satisfactory with and without treatment with lead acetate and with and without de-leading.

Several methods were employed in attempts to obtain quantitative extraction of the saccharin from starch foods. For arrowroot and cornflour the following method was adopted.

Treat 5–10 g of the food, or an amount to give 5–10 mg of saccharin, in a 100-ml flask with sufficient cold water to make a cream with starch products. Add hot water nearly to the mark, leave for 2 hr., make up to 100 ml and centrifuge. Pour off the supernatant liquid into a beaker. Grind up the residue from starch foods with sand and repeat the extraction and centrifuging twice more. Collect the supernatant

liquids and make up to a definite volume. Transfer to a separator, add 3 ml of 10% hydrochloric acid for every 20 ml of liquid, and extract with 3 × 30 ml of ether. No correction factor for the vol. of insol. matter is needed, since the extraction is exhaustive. Proceed as described under EXTRACTION (*supra*). Dissolve the residue in 1 ml of 10% sodium hydroxide soln., add 9 ml of hot water to assist solution. Take aliquot portions, 0·5 ml for the nesslerisation method[5] and 0·1 ml for the Reindollar modification. Determine the amount of saccharin by the two methods and calculate the saccharin %. Table II gives some typical results of expts. in which the amount of saccharin was unknown to the operators. (Caffeine was removed from the coffee extracts.) Table III indicates results with various foods, using 25 ml or g as appropriate and 10 mg of added saccharin.

TABLE II

Sample	Saccharin, %		
	Added	Lerrigo	Reindollar
A. Syrup	0·020	0·021	0·020
B. „	0·012	0·016	0·013
C. Jam	0·010	0·013	0·011
D. Coffee extract ..	0·018	0·020	0·017
E. „ „ ..	0·032	0·026	0·024
F. Plain flour	0·030	0·028	0·028
G. „ „	0·120	0·108	0·116

TABLE III

Sample	Saccharin, mg		
	Added	Lerrigo	Reindollar
Beer	10	7·0	10·0
Cider	10	12·4	10·0
Jam	10	9·0	8·0
Arrowroot	10	8·2	10·6
*Coffee extract	10	25·0	20·0
„ „	10	14·0	12·0

* Caffeine was removed from the second extract, but not from the first.

It is advisable to estimate the saccharin in unknown residues by the Lerrigo and Reindollar methods to provide against variations which sometimes occur in both methods. The estimation should be repeated if such variation does occur.

SUMMARY—(1) Reindollar's qualitative method for saccharin has been adapted for quantitative estimation.

(2) Simple aqueous solns. of saccharin upon extraction give satisfactory results by the sulphate, Lerrigo and Williams and modified methods.

(3) In absence of vanillin and caffeine the Reindollar method is more distinctive for saccharin than the Lerrigo and Williams method.

(4) Satisfactory recovery of saccharin is obtained in most instances. Wheat flour preparations require special treatment.

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COUNTY LABORATORY, COUNTY HALL
TAUNTON

E. G. WHITTLE

May, 1943

TWO NEW TESTS FOR BORON

SMALL amounts of boron can be readily detected by means of a reagent made by dissolving 0·5 g of the unsulphonated base of Solway Purple (Colour Index No. 1073) in 100 ml of conc. sulphuric acid. In testing for a small amount of boron, evaporate to dryness an aliquot portion of the test soln. previously made slightly alkaline with *N* sodium hydroxide, take up the residue in 0·1 ml of water and again evaporate on a watchglass. Add 2 drops of the reagent; the development of an intense deep blue colour indicates boron. The colour of the reagent is a dull weak green and the development of the blue colour with boron compounds is very marked.

Oxidising agents, especially nitrates, interfere. To remove such compounds, add 1 drop of conc. sulphuric acid and a crystal of hydrazine sulphate to the spot and evaporate to dryness. Fluorides present do not stop the colour development, but the blue colour takes 2 or 3 min. to develop and a drop of the reagent should be put at the side for comparison. One drop of a soln. containing 1 g of hydrated borax and 10 g of sodium fluoride in 10 litres of water gives the reaction distinctly after 2 min. Larger quantities of fluoride are removed by heating the spot with 2 drops of conc. sulphuric acid and a little silicic acid prior to adding the reagent. In absence of fluorides 0·1 ml of test soln. containing 1 g of hydrated borax in 100 litres of water gives the reaction. With the exception of oxidising agents and large amounts of fluoride no other compounds appear to interfere or give the reaction unless present in such quantities that their own colour masks the reaction and makes it indeterminate. The reagent should be freshly prepared for each series of tests and kept in a well-stoppered bottle when not in use.

Another test for boron depends on the production of an intense orange brown fluorescence in daylight upon addition of a 0·01% soln. of 1-amino-4-hydroxy-anthraquinone in conc. sulphuric acid. To 1 drop of the test solution in a small test-tube add 1 ml of the reagent and examine against a black velvet background. One drop of a soln. containing 1 g of hydrated borax in 10 litres is the limit of detection by this method.

I wish to thank I.C.I. (Dyestuffs), Ltd., for permission to publish this note.

11, Wilton Road
Crumpsall, Manchester, 8

J. A. Radley

October, 1943

A BOTTLE FOR SAMPLING WATER AT A GIVEN DEPTH

The bottle that I have designed for sampling at any given depth is much simpler than the multi-cord bottle with the usual brass spring and clip. It comprises a weighted bottle, to the stopper of which is attached at one end the lifting cord and at the other (inside the bottle) a rubber disc of special construction (see Fig. 1). For use, the stopper is inserted and the bottle is allowed to sink to the required depth; the

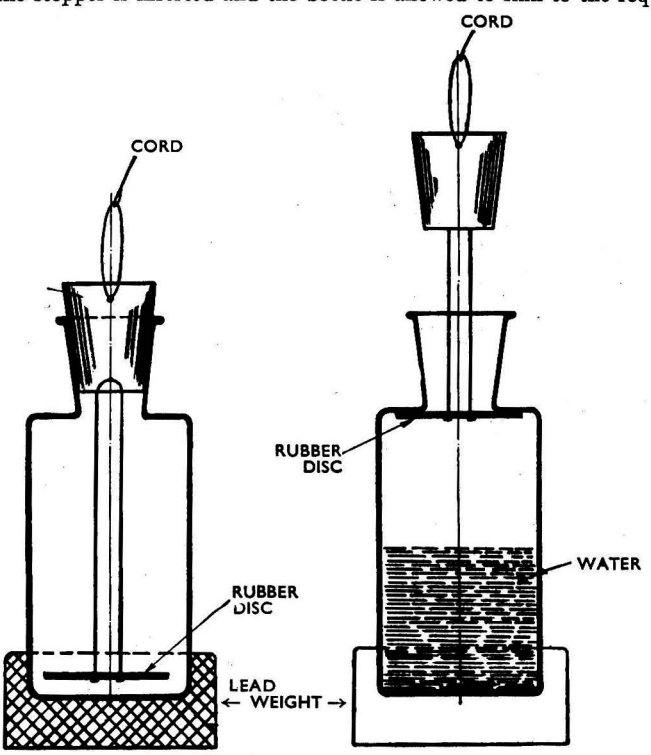

Fig. 1

cord is then given a sharp jerk, which removes the stopper, so that the bottle is filled with water and becomes heavy on the cord; when it is hauled to surface the internal disc seals the mouth.

The bottle is obtainable from Griffin & Tatlock, Ltd.

29, Wimmerfield Avenue
Killay, Swansea

D. A. Bishop

August, 1943

Ministry of Food

STATUTORY RULES AND ORDERS*

1943—No. 1653. The Bread (Control and Maximum Prices) (No. 2) Order, 1943. Dated November 23, 1943. Price 5d.

A number of alterations and additions are made in this consolidating Order replacing the Bread (Control and Maximum Prices) Order, 1943 (Analyst, 1943, **68**, 114).

"Rye bread" means bread in the production of which at least 25% of the flour used is rye flour and/or rye meal.

Schedule I—Substances other than National Flour which may be included in National Bread—White flour, oils and fats, water, salts, yeast, improvers of the nature of yeast food, any acid or acidic substance suitable for regulating the acidity of the dough, potato and potato flour, barm.

* A summary of some Orders. Italics signify changed wording. Obtainable from H.M. Stationery Office.

1943 No. 1691. Order, dated December 9, 1943, amending the Feeding Stuffs (Rationing) Order, 1943, and the Directions, dated October 23, 1943, supplementary thereto. Price 1d.

The effect of this Order is to bring within the definition of "rationed feeding stuffs" those by-products produced in the course of manufacturing pearl barley which contain not more than 20% of fibre.

— **No. 1738. Order dated December 18, 1943, amending the Feeding Stuffs (Regulation of Manufacture) Order, 1943.** Price 2d.

This Order, which came into force on January 1, 1944, removes the restriction to 25% of datum imposed by the Principal Order (S.R. & O., 1943, No. 1237) upon the manufacture of pig and poultry compounds (other than National Poultry Balancer Meal), alters the composition of permitted compounds by increasing the quantity of home-grown grains to be included, and requires the inclusion of cod-liver oil in National Pig Food No. 1.

1944—No. 42. The Food Standards (General Provisions) Order, 1944. Dated January 14, 1944. Price 1d.

— **No. 43. The Food Standards (Mustard) Order, 1944. Dated January 14, 1944.**
— **No. 44. The Food Standards (Self-raising Flour) Order, 1944. Dated January 14, 1944.**
— **No. 45. The Food Standards (Shredded Suet) Order, 1944. Dated January 14, 1944.**
— **No. 46. The Food Standards (Baking and Golden Raising Powder) Order, January 14, 1944.** Price for the four, 2d.

Ministry of Food Press Notice: Food Standards

These Orders are the first to be issued creating legal standards for foods in accordance with the policy announced in the White Paper on "The Labelling and Advertising of Foods." (Cmd. 6482.)

THE GENERAL PROVISIONS ORDER, which applies to all standards prescribed under Regulation 2 of the Defence (Sale of Food) Regulations, makes it an offence to sell a food for which a standard is prescribed unless the food complies with that standard. It is also an offence to sell a food which is so described as to lead an intending purchaser to believe that he is purchasing food of a description for which a standard is in force, unless it complies with the standard.

A warranty defence and the defence that some other person is responsible for the commission of the alleged offence, similar to the corresponding defences in the Food and Drugs Act, 1938, are provided for the protection of retailers. The provisions relating to taking samples and powers of inspection contained in the Defence (Sale of Food) Regulations, 1943, apply to proceedings in respect of offences against the Food Standards Orders. Under the Regulations, also, enforcement is entrusted to Food and Drugs Authorities, but whereas proceedings in respect of offences against the Regulations may not, in general, be instituted by Authorities unless the Minister's prior consent is obtained, it is provided in each of the four commodity Standards Orders now issued that consent is not necessary for prosecutions for infringements of the standards.

The following are summaries of the standards prescribed:

A. BAKING POWDER (S.R. & O., 1944, No. 46)—Not less than 8% of available carbon dioxide and not more than 1·5% of residual carbon dioxide.

GOLDEN RAISING POWDER—Not less than 6% of available carbon dioxide and not more than 1·5% of residual carbon dioxide.

In these two instances the definitions of "available" and "residual" carbon dioxide are based on a detailed indication of the method of determination.

B. MUSTARD (S.R. & O., 1944, No. 43)—Mustard of any description in powder form—

To consist of a blend of brown and white mustard flours without the addition of any other substances, and to yield not less than 0·35% of allyl *iso*thiocyanate after maceration with water for 2 hours at 37° C.

Compound Mustard or Mustard Condiment—To consist of a blend of brown and white mustard flours with not more than 20% of amylaceous flours and spices, and to yield not less than 0·35% of allyl *iso*thiocyanate after maceration with water for 2 hours at 37° C.

The standards do not apply to white mustard flour sold under the description "pickling mustard" nor to brown mustard flour sold under the description "brown mustard."

C. SELF-RAISING FLOUR (S.R. & O., 1944, No. 44)—Not less than 0·45% of available carbon dioxide and not more than 0·65% of total carbon dioxide. Definitions of "available" and "total" carbon dioxide, based on a brief indication of the methods by which they are to be determined, are included.

D. SHREDDED SUET (S.R. & O., 1944, No. 45)—Not less than 83% of fat.

These Orders come into operation as from March 16 on sales by manufacturers, on May 16 on sales by wholesalers and on July 16 on sales by retailers.

Department of Scientific and Industrial Research

INVESTIGATION OF ATMOSPHERIC POLLUTION

A SUMMARY Report for the year ended March 31, 1943, has been prepared for the information of the co-operating bodies (including the S.P.A.). The numbers of instruments maintained by the Co-operating Bodies were about the same as in 1941–42, *viz.*, deposit gauges, 99; automatic filters, 8; volumetric sulphur apparatus, 7; lead peroxide apparatus, 40; daylight apparatus, 6.

EFFECT OF FUEL ECONOMY ON POLLUTION—To test the effects of the national effort in the autumn of 1942 to economise fuel in the factory and in the home, observations for the winter of 1942–3 have been compared with the corresponding figures for the previous winter.

Deposit Gauge—Observations from 92 stations gave the following results for Great Britain as a whole:

	Rainfall	Tar	Non-tar carbonaceous matter	Ash	Sol. matter	Sulphates
Summer (Apr.–Sept.) ..	+9%	+10%	—	—	—	—
Winter (Oct.–March) ..	−6%	−9%	—	—	−6%	−6%

Where no figures are given the change was not statistically significant, being so small that it might be spurious. A change is regarded as significant if the probability of its being spurious is less than 0·05 (odds longer than 19 to 1 against). Allowance was made for the additional deposit brought by the increase in rainfall in 1942–3. The correction was estimated from a curve, obtained during the Leicester survey, showing the dependence of deposit on rainfall, and varied from 1 to 2%. There was no significant change in combustible matter or ash, but, taken as a whole, the results suggest that there was *ca.* 5% less pollution in the winter of 1941–2 than in the previous winter. The only significant change in summer was a 10% increase in the tar deposit.

Observations during 1934–8 at Kew Observatory have shown that closely concordant results may not be obtained from 2 gauges relatively close together. From these results it has been calculated that changes of less than 20% in the mean of 6 monthly observations are not statistically significant. On the other hand, a change greater than 20% in the summer or winter mean at any individual station is likely to be a real change. Among the individual stations the following changes of more than 20% were noted—Bradford North: (winter) decrease in all insol. deposits. Grimsby (summer and winter) 80–180% increase in all insol. deposits. Halifax, 5 stations: (winter) decrease in tar deposit. Leeds, Hunslet: (summer and winter) decrease in tar and ash. London, Victoria Park: (summer and winter) decrease in tar and sulphates. Manchester, Withington: (winter) increase in all insol. deposits. St. Helens (winter) decrease in all insol. deposits. Skipton: (summer and winter) increase in tar and ash. Southport, Bedford Road Park: (summer and winter) decrease in sulphates. Southport, Hesketh Park: (winter) decrease in all insol. deposits.

Automatic Filter—The automatic filters at Glasgow showed reductions of 34 and 52% for the monthly concns. of smoke for the 7 months September, 1942, to March, 1943, respectively. No significant changes were recorded by the other automatic filters, *viz.*, those at Stoke on Trent and at 3 London stations.

Lead Peroxide Apparatus—Records of the determination of sulphur dioxide by the lead peroxide method at 40 stations from September, 1942, to March, 1943, were compared with the corresponding monthly means of the previous year. There was a significant reduction of 11% in Great Britain as a whole. It may be assumed that the rate of sulphation of lead peroxide is proportional to the weight of coal burned, particularly if a long period of measurements and a large number of stations are being considered. The result would be consistent with a decrease in the coal consumption in Great Britain. Certain individual stations also showed a decrease in the yield of lead sulphate. Standard errors of the mean of 7 comparisons ranged from 3 to 17%, and the min. changes that could be regarded as significant from 6 to 34%. There were no significant increases, and the following significant decreases were noted during the 7 months as compared with the corresponding months in 1941–2—Barnsley, 18; Cardiff, 18; Edinburgh, 15; Glasgow, 15; Halifax, 13; Leicester, 18; Loggerheads, 23; London (County Hall roof), 18; London (Ravenscourt Park), 17; Sheffield, 17; Southport, 20; Stoke on Trent, 23%.

Volumetric Sulphur Dioxide Apparatus—The recorded results were examined in the same way as those from the lead peroxide method. The one significant change was a decrease of 17% at Newcastle upon Tyne.

Ministry of Fuel and Power

ANNUAL REPORT OF THE SAFETY IN MINES RESEARCH BOARD*

THIS, the 21st Annual Report, deals in the main with the progress made in various branches of safety research.

COAL DUST EXPLOSIONS—In a report of the Mines Department (Cmd. 6450) a survey was made of explosions attributed to coal dust since the general adoption of stone-dusting about 1921. Nothing has occurred since then to shake confidence in the efficacy of stone dust, applied in suitably large proportions, and properly mixed with the coal dust. Laboratory expts. have proved that a violent explosion of fire damp can initiate a self-propagating explosion in a cloud of very fine coal dust (*ca.* 85% passing through a 240 B.S. test sieve) intimately mixed with 75% or less of incombustible shale dust; for the coarser coal dust usually present in coal pits 75% would usually be an adequate safety provision. In laboratory expts. with actual road dusts, 68–69% of shale dust was necessary with one sample and 73–74% with 2 other samples. Had it not been for the war, regulations prescribing the use of 75% would doubtless have been enforced for the 4 or 5 coal mines that had major explosions during the last 20 years.

Effect of Incomplete Mixing of Stone and Coal Dusts—In expts. in which the 2 dusts were spread in alternate strips in a gallery, 4 ft. in diam., by means of a mortar charged with gunpowder and coal dust so as to eject a large flame with little violence, it was found that the amount of stone dust required to prevent propagation of the explosion was 4 times that required when the two dusts were mixed.

Composition of Dust raised by an Explosion—The mixed dust in an intake haulage road contained 70–71% of incombustible dust in the floor dust and 70% in the disturbed dust. So far, the tests have shown that the proportions of ingredients in well-mixed dusts are not materially altered when the dust is raised by an air blast or a blast from a cannon, but there is some separation as the dust settles; in one test the subsided dust contained *ca.* 4% less incombustible matter than the original mixture. The expts. support the conclusion that there is not much separation in the interval of time between the raising of the dust and the arrival of the explosion flame.

* Pp. 23. H.M. Stationery Office, 1943. Price 1s. net.

Highly Dispersible Stone Dusts—An investigation has been made of dusts which are fine enough to suppress an explosion and yet contain less than the usual proportion of ultra-fine particles. Such dusts separate in the air into a cloud of particles instead of remaining more or less aggregated; they are also disturbed more readily than ordinary fine dusts by a tangential current of air passing over the surface. In expts. with the Rotor Dust Extractor the dust was found to be increasingly dispersible with each successive treatment. Thus a shale dust containing 18% by weight of particles less than 10μ in diam. required a min. speed of air current of 3200 ft. per min. to raise it from a smoothed surface, and *ca.* 16% of the dust blown into the air in a standardised apparatus remained in suspension after 2 sec. After 3 successive passages through the separator the dust contained only 4% of particles less than 10μ in diam., and an air current of only 1800 ft. per sec. was sufficient to move it, and 33% of the dust blown into the air remained in suspension after 2 sec. Similar improvements were obtained with magnesian limestone and gypsum dusts.

Examination of Road Dusts Underground—A colorimetric method of estimating whether a road dust contains over 80% of incombustible dust has been used in several collieries. Two similar devices for assisting visual evaluation have given promising results. In one of these a little of a standard mixture of coal dust and the incombustible dust used in the pit is dropped on to the dust to be examined and pressed flat down on to it, and the colours of the standard and the surrounding dust are compared. The other device consists of a watchglass on the convex side of which 2 or 3 sectors of different shades of grey have been pasted, a clear space being left in the centre. The glass is pressed down on to the dust to be tested and the colour, as viewed through the clear space, is compared with those of the grey standards. A white incombustible dust, *e.g.*, limestone, gives the best results in these tests, but fairly good results are also obtained with gypsum and shale.

Reduction and Collection of Coal Dust in Mines—This is largely a problem of practical management in the pits. The Board has taken no active part in the work, but has given grants for the purpose to the British Colliery Owners Association.

FIREDAMP EXPLOSIONS—The possibility of firedamp being ignited by sparks of heat produced by friction between steel surfaces has been investigated by making tests in atmospheres enriched by different amounts of oxygen (29·2–34·9%). The results indicate that the relationship between probability of ignition and oxygen % is linear and that mixtures of methane and air cannot be ignited in this way under the experimental conditions devised. Other work included investigations on standards of safety for the use of electricity underground and an experimental research on the ignition of gas by mining explosives. It has long been known that the presence of nitrous fumes reduces the ignition temp. of firedamp by *ca.* 150° C. Expts. have shown that, whilst mixtures of firedamp and air blown into a tube ignited at 700° C. and upwards, when a little ammonium nitrate dust accompanied the firedamp a temp. of 430° C. was sufficient for ignition. The imperfect detonation of an explosive cartridge may therefore be an important factor in the ignition of firedamp.

FALLS OF GROUND—Investigations in the laboratory and the mine have been mainly concerned with questions of materials and the development of substitutes.

Bending Tests of Roof Bars—The following average results were obtained in about 20 tests on bars *ca.* 5 ft. by 6 × 3 in. section.

	Ash	Beech	Oak	Larch	Scots Pine
Aver. max. strength in tons	8·5	8·8	6·6	5·9	4·5
Deflection in the middle, inches	3·4	2·2	1·75	2·5	2·5

The ash, beech and larch bars were fairly uniform in their respective max. strengths, but the oak and Scots pine bars differed widely among themselves, nearly a quarter having less than 70% of the average strength.

Seasoning of Timber—Softwood increased in strength by 20 to 50% when seasoned underground in air at 77° F. and 47% saturation and at 85·5° F. and 64% saturation.

Other subjects discussed in the Report include concrete supports, roof control, haulage and wire ropes.

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

VII. CADMIUM

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J. H. SINGER

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Evaluation of the Modified Renard and Kerr Tests for the Determination of Peanut Oil. S. T. Voorhies and S. T. Bauer (*Oil and Soap*, 1943, **20**, 175–178)—The method of Renard (*Compt. rend.*, 1871, **73**, 1330), as modified by the A.O.A.C. (*Methods of Analysis*, 1940, 5th Ed., p. 439) and that of Kerr, *J. Ind. Eng. Chem.*, 1916, **8**, 904) as modified by Thomas and Yu (*J. Amer. Chem. Soc.*, 1923, **45**, 113) for the determination of arachis oil in admixture with other vegetable oils by evaluation of the arachidic acid content are discussed, and the results obtained by the application of both methods to a number of artificial mixtures and to a sample of olive oil are presented in 5 tables. The authors consider that neither test gives results sufficiently accurate to justify its use for the determination of arachis oil in vegetable oil mixtures and point out that the greatest discrepancies occur when the content of unsaturated fatty acids in the mixture is high (in mixtures of arachis and soya bean and of arachis and olive oil), a phenomenon attributed by Lewkowitsch (*Analysis of Oils, Fats and Waxes*, 1938, 6th Ed., Vol. I, p. 554) to the greater solubility in ether of the lead soaps of saturated fatty acids in presence of a large proportion of the lead salts of unsaturated fatty acids. It is noted that a similar conclusion has been reached by Pritzker and Jungkunz (*Mitt. Lebensm. Hyg.*, 1942, **33**, 75), who suggest that the isolated fatty acids be crystallised from 90% alcohol repeatedly until the m.p. is above 72° C. J. A.

Some Constituents of Malt Extract. J. G. Organ, J. Duncan, M. E. Westbrook, and F. Wokes (*Quart. J. Pharm.*, 1943, **16**, 275–281)—A series of 17 commercial and 2 experimental samples of malt extract were examined for diastatic activity, vitamin B_1, protein and reducing sugar contents. Apart from 3 fortified samples, the vitamin B_1 content ranged from 62 to 158 I.U. per 100 g, and the protein content from 3·19 to 9·70 g per 100 g. It was observed that, on the whole, extracts with low diastatic activity contain less vitamin and protein, the same correlation between diastatic activity and protein content being observed in 35 samples of commercial malt and oil. Of 45 samples of malt extract examined for protein content, only 8 were below the Pharmacopoeial min. of 4·5%, 23 contained between 5·6 and 7·0%, and the average was 5·6%. Malt extracts containing 9% of protein have been made and are satisfactory. It is suggested that, taking into consideration the human requirements of vitamin B_1 and the normal dosage of malt extract, a satisfactory content of the vitamin would be 125–250 I.U. per 100 g; of 12 normal samples examined, averaging 123 I.U. per 100 g, 6 reached the min. of 125 I.U. per 100 g, one of them containing 135 I.U. per 100 g; one fortified sample contained 300 I.U. per 100 g. It is pointed out that the vitamin B_1 content of malt extract may diminish appreciably during normal storage.

The suggestion that malt extract for malt and oil preparations should have had all the enzymes destroyed (Garratt and Woodhead, ANALYST,

1942, 67, 194) is criticised on the ground that this would normally mean the loss of most of the vitamin and protein value; it would seem preferable to replace the oil by a stable concentrate of vitamins A and D. J. A.

Liver Extracts for Parenteral Use. W. A. N. Markwell (*Pharm. J.*, 1943, **151**, 223)—Commercial samples of liver extracts gave the following results for sp.gr., total solids and *p*H.

Sample	Stated concn.	Sp.gr. at 15/15° C.	Total solids dried at 100° C.	Approx. *p*H
1	None given	1·009	1·50	6·0
2	25 : 1	1·090	30·80	5·4
3	2 U.S.P. units per ml	1·108	29·00	5·0
4	25 : 1	1·104	25·50	4·8
5	"Pure principle"	1·019	4·27	6·0
6	5 : 1	1·007	1·51	7·2
7	25 : 1	1·015	3·90	4·0
8	25 : 1	1·012	3·01	6·0
9	25 : 1	1·105	39·30	6·0
10	12·5 : 1	1·018	4·18	4·2
11	None given	1·134	29·05	6·0

It is pointed out that no satisfactory method of determining the haematopoietic activity of liver injection is available, the U.S.P. unit being ill-defined and open to several objections. No monograph for liver injection is included in the B.P. or its Addenda, while the U.S. Pharmacopoeia XII gives no guidance as to the method of manufacture and no test for toxicity. The variations recorded above in the content of total solids are noteworthy in connection with the work of Barfred (*Acta. Med. Scand. Supp.*, 1942, 131), who has shown that preparations with a high solid content tend to produce unpleasant reactions; nevertheless too great a degree of purification should be avoided, since often the factors that stimulate the production of anti-anaemic substances in the stomach are removed; moreover, highly purified extracts have little effect on tropical macrocytic anaemia, whereas less purified injections at once induce a strong increase of reticulocytes. It seems desirable that there should be some official regulation to ensure satisfactory clinical response, absence of undue toxicity and fair uniformity in the products. J. A.

Cardamon: A Study of its Sclerenchymatous Layer. J. W. Fairbairn (*Quart. J. Pharm.*, 1943, **16**, 243–249)—The varieties of cardamom fruits occurring in commerce can be distinguished with reasonable accuracy by the characters of their pericarps, but if the seeds are separate or in powder, the problem of identification is difficult. It has been found, however, that the number of cells per sq. mm of the sclerenchymatous layer is diagnostically significant. The following procedure is recommended. Reduce the seeds to No. 85 powder, add 0·2 g to 8 ml of 5% potassium hydroxide soln. and heat in a boiling water-bath for 15 min. Add 2 ml of 10 vol hydrogen peroxide and heat for a further 5 min. Filter, wash with water and then with 5 ml of 10% hydrochloric acid and mount in glycerin. Examine not less than 36 pieces of sclerenchymatous layer (diam. *ca.* 135μ) under the microscope, draw their outlines on paper at a magnification of *ca.* ×350, using a camera lucida, and represent the cells in the drawing of each piece by small circles. Cut out the drawings, weigh them, determine the weight of a known area of the same paper and thence calculate the area of the drawings; count the number of cells represented and calculate the number per sq. mm. The following results were obtained.

Variety	Cells per sq.mm.
Malabar (*Elettaria Cardamomum* var *minuscula*)	3926
Bangalore (do do.)	4526
Bengal (*Amomum aromaticum* Roxb.)	1851
Korarima (*Afromomum Korarima*) ..	5070
Cluster (*Amomum Kepulaga* Sprague and Burkill)	3640

The limit of error of the above method is estimated at ±8%. The area of sclerenchymatous layer per g of air-dried seed was determined for 2 samples of powdered Mysore cardamom, and the values 29·74 and 27·40 sq.cm. per g were obtained. J. A.

Stomatal Index: Its use for Distinguishing between Powdered Alexandrian and Tinnevelly Senna Leaf. J. M. Rowson (*Quart. J. Pharm.*, 1943, **16**, 24–31)—The stomatal index is given by the expression $I = \frac{S}{E + S} \times 100$, where S = no. of stomata per unit area and E = no. of ordinary epidermal cells in the same area. The sample is prepared by diffusing with a little alcohol, mixing with chloral hydrate soln., heating on a water-bath for 30 min. or until cleared and suspending in sufficient 50% glycerin to give convenient mounts. For the microscopic examination, a 4-mm objective and a ×5 eyepiece containing a 5-mm square micrometer disc are used and counts are made upon pieces of epidermal tissue of convenient size, all cells being included whose half area or more comes within the square field. Stomatal indices are calculated from observations on about 500 cells. Two guard cells are considered as one unit; a hair, or the cell giving rise to it, is considered as a unit. Elongated tabular cells of the extreme leaf margins and the epidermal cells from above the midrib are not included, the count of epidermal cells being restricted to those which are straight-walled and almost equally five-sided. Although there is a wide variation in the stomatal number per sq. mm within samples and between samples of the same species, the stomatal index is characteristic; values for Tinnevelly senna (*Cassia angustifolia*) range from 17·1 to 20·0, and those for Alexandrian (*C. acutifolia*) from 11·4 to 13·0.

The method is applicable to confection of senna and to compound liquorice powder. Samples are prepared for examination by digestion for 1 hr. with water on a boiling water-bath, allowing them to stand and decanting; for confection of senna the digestion is repeated; the insol. matter is collected in a Buchner funnel and cleared with chloral hydrate soln., and convenient mounts are made by suspension in dil. glycerin. J. A.

Stomatal Index: Its Use in Distinguishing between English and Indian Belladonna Leaf and for the Characterisation of Coca. J. M. Rowson (*Quart. J. Pharm.*, 1943, **16**, 255–264)—It has been shown (see preceding abstract) that the stomatal index may be used to distinguish between the powdered leaflets of the two species of senna, *Cassia acutifolia* and *Cassia angustifolia*, and it is now shown that similar data serve to differentiate English and Indian Belladonna leaf, derived from *Atropa belladonna* and *Atropa lutescens* respectively, and also the two species of coca, *Erythroxylum coca* and *Erythroxylum truxillense*. Leaves of belladonna are first cleared with chloral hydrate soln. by gently warming for a short time (or by macerating at room temp. overnight; strips of epidermis are then removed from both upper and lower surfaces, and mounted in dilute glycerin. Counts are made of the number of stomata and of epidermal cells in contiguous fields, using a 4-mm objective and a × 10 eyepiece containing a 5-mm square micrometer disc, the conventions mentioned in the preceding abstract. As the epidermal cells of belladonna are large and of wavy outline, each field containing about 20 cells, it is advisable to count 20 adjacent fields in 4 strips.

Suitable preparations of coca may be prepared by boiling portions of leaf in lactic acid for a few moments and mounting the entire portion in the same fluid. The epidermal cells of coca are small, and counts of 5 fields in 2 strips give totals of 500 to 600 cells. There is considerable variation in stomatal number per sq. mm of leaf surface within samples and between different samples of the same species, the observed range of values being—*A. Belladonna*, 77·5 to 176·5, aver. 113·1; *A. lutescens*, 61·5 to 173·8, aver. 93·2; *E. coca*, 230 to 565, aver. 354; *E. truxillense*, 210 to 385, aver. 264. This is paralleled by a similar variation in the number of epidermal cells, whence the stomatal index is relatively constant for each species, the observed values of the stomatal index being—*A. Belladonna*, 21·6 ± 1·30; *A. lutescens*, 17·6 ± 0·71; *E. coca*, 13·2 ± 0·98; *E. truxillense*, 10·1 ± 0·79. No appreciable difference was observed between samples of *E. coca* grown in Bolivia and in Ceylon, or between samples of *E. truxillense* derived from Peru, Java, or Ceylon. J. A.

Senna Leaflets: Their Palisade Ratio Values and Ranges. E. George (*Quart. J. Pharm.*, 1943, **16**, 226–232)—The palisade ratio (*cf.* Wallis and Dewar, *Quart. J. Pharm.*, 1933, **6**, 347) was determined separately for the upper and lower epidermis of Indian, Arabian and Egyptian senna leaflets. The first two (called Tinnevelly and Mecca or Bombay respectively) being derived from *Cassia angustifolia* and the Egyptian (Alexandrian) from *Cassia acutifolia*. The ratio was found to be uniform over the leaflet surface for both species, the upper surface having a value about 1·4 times greater than the lower in each instance. The ranges found are given below with the most probable (modal) values in heavier type:

Species	Groups of 4 epidermal cells	Individual cells
C. acutifolia, Upper surface	**4·5–9·5**–18·0	**2·0–9·8**–21·0
Lower ,, ...	**3·5–7·5**–14·5	**2·0–7·0**–19·0
Whole leaflet	**3·5–8·0**–18·0	**2·0–8·0**–21·0
C. angustifolia, Upper surface	**4·0–7·5**–12·0	**2·0–7·0**–17·5
Lower ,, ...	**2·5–5·1**–10·5	**1·5–5·4**–12·5
Whole leaflet	**2·5–5·6**–12·0	**1·5–6·5**–17·5

The lower epidermis of *C. acutifolia* and the upper epidermis of *C. angustifolia* both have palisade ratios very near to 7·5 and are indistinguishable from each other. The upper epidermis of *C. acutifolia* has a ratio of 9·5 as compared with 5·0 for the lower epidermis of *C. angustifolia*, whence these may be identified. The identity of a powder, even when so fine as No. 90, may be established from the mean of 20–30 determinations on epidermal fragments. A value above 7·5 indicates *C. acutifolia*, that for *C. angustifolia* being below this figure. If the powder is a mixture of both species it will be possible to find many cells giving a palisade ratio above 10 and many giving a value below 5. J. A.

Assay of Methylene Blue. G. J. W. Ferrey (*Quart. J. Pharm.*, 1943, **16**, 208–212)—Methylene blue can be pptd. with potassium dichromate as tetramethylthionine dichromate, $(C_{16}H_{18}N_3S)_2.Cr_2O_7$, which is very nearly insol. in water or dil. acetic acid containing a moderate excess of dichromate, but is slightly sol. in presence of sodium acetate. Unlike the ppt. in the A.O.A.C. iodine method (*Methods of Analysis*, 4th Ed., 1935, p. 559), the ppt. has a composition independent, within wide limits, of the excess of reagent, and, since a stable reagent is used, the procedure is more convenient than the titanous chloride method.

Volumetric—(*a*) Dissolve 0·1 to 0·4 g of methylene blue in 100 ml of water in a 200-ml graduated flask, add 50 ml of 0·1 *N* potassium dichromate and dilute to volume. Leave for 5 min. with occasional shaking and filter through a Whatman No. 41 paper. Add 2 g of potassium iodide and 50 ml of 10% sulphuric acid to 100 ml of the filtrate and titrate the liberated iodine with 0·1 *N* sodium thiosulphate (0·01066 g of anhydrous methylene blue ≡ 1 ml of 0·1 *N* potassium dichromate).

(*b*) It is preferable to titrate the whole of the filtrate; if this is done, the above procedure should be followed and the mixture filtered through a No. 3 sintered glass filter or a Gooch crucible, and the ppt. washed with 10 ml of water. A tendency to clog the filter can be prevented by heating the reaction mixture to 75° C. for 5 min. and cooling to room temp. before filtering; no decomposition occurs during this treatment.

Gravimetric—The ppt. obtained as in (*b*) above can be dried at 100° C. for 1 hr. and weighed; no further loss in weight occurs on heating at 110° C. for 2 hr. (1 g of ppt. ≡ 0·8150 g of anhyd. methylene blue).

The volumetric method is applicable to the assay of methylene blue or to its determination in tablets and pills. Acacia, dextrin, starch, lactose, dextrose or sucrose do not interfere, provided that the pptn. is carried out in the cold. In presence of starch the substance is dissolved in boiling water and the soln. is cooled to room temp. prior to the pptn. The dye can be extracted from insol. vegetable excipients, *e.g.*, gentian, liquorice or marshmallow, by boiling with successive small quantities of 10% acetic acid and decanting through

a plug of cotton wool or glass wool; the error introduced will not be greater than 2%. Where greater accuracy is required or in presence of hexamine, which vitiates the volumetric procedure, the gravimetric method should be applied. J. A.

Determination of Cantharidin. G. A. Guthrie and H. Brindle (*Quart. J. Pharm.*, 1943, **16**, 249–255)—Cantharidin can be quantitatively pptd. as barium cantharidate from aqueous solns. adjusted to *p*H 8, and the principle has been applied to the determination of small quantities present in galenicals and hair lotions. Adjust the reaction of the soln. under examination, expected to contain between 0·1 and 0·5 mg, to *p*H 8 by adding, if necessary, sodium hydroxide soln. until a pink colour forms with phenolphthalein followed by dil. hydrochloric acid until a faint pink just persists. Add a measured vol. of standard barium chloride soln. (0·02% to 0·05% $BaCl_2.2H_2O$) in excess, leave for 6 hr. and decant the liquid through a small plug of cotton wool. To as large an aliquot part as possible add an excess of 0·004 *N* or 0·01 *N* potassium chromate, leave for 3 hr. or longer, centrifuge or decant. To an aliquot part of the clear liquid add a few crystals of potassium iodide and 2 ml of hydrochloric acid (10%) and titrate with 0·002 *N* sodium thiosulphate. Make a blank determination on the potassium chromate soln. Calculate the result on the basis that 1·00 mg of barium chloride ≡ 0·941 mg of cantharidin.

Liquor Cantharidini B.P.C. (*Tinct. Cantharidini B.P.* 1914)—Remove the solvent from a 10-ml sample by evaporation in a current of air at 40° C. and apply the above procedure to the residue. *Hair Lotions*—Acidify with hydrochloric acid a vol. of the sample expected to contain from 0·5 to 1 mg of cantharidin, extract the latter with 15-, 10- and 5-ml portions of chloroform, remove the solvent by evaporation in a current of air at 40° C., transfer the residue of impure cantharidin by means of acetone to a metal lid such as is used for closing wide-necked bottles (the varnish having previously been burnt off) and evaporate the solvent at 40° C. Cover the lid with a watch glass containing cold water, float the assembly on a mercury bath at 105°–110° C. and from time to time substitute fresh watch glasses until examination under the microscope shows that no more cantharidin is being sublimed (45 min. to 2 hr.). Dissolve the cantharidin from the watch glasses by means of acetone, add a little dilute aqueous sodium hydroxide soln., remove the acetone by boiling, adjust the reaction to *p*H 8 with dil. hydrochloric acid and continue the determination as already described.

Solubility of Cantharidin in Water—When a sufficiently large quantity of cantharidin is available it can be determined gravimetrically, the ppt. of barium cantharidate being collected on a sintered-glass filter, washed with water and dried at 110° C. This procedure was used to determine the solubility of cantharidin in water, which was found to be 1 in 10,500 at 20° C. J. A.

Biochemical

Total Nitrogen Content of Egg Albumin and other Proteins. A. C. Chibnall, M. W. Rees and E. F. Williams (*Biochem. J.*, 1943, **37**, 354–359)—In the original Kjeldahl method the period of digestion occupied several hours, but with the introduction of such catalysts as selenium, the time of heating has been considerably reduced in the belief that the time lag inherent in the older methods has been thereby overcome. The application of these modified methods, especially on the microscale, to the estimation of nitrogen in biological preparations, has led to anomalous results, and values lower than those in the earlier literature have recently been reported This is attributed to the use of too short a time of digestion, and it is believed that with proteins and protein hydrolysates the heating should be continued for at least 8 hr. after the digest has cleared. It has also been observed that some proteins, *e.g.*, egg albumin, pepsin and insulin, are very hygroscopic in the anhydrous state and cannot be handled without special precautions. It is therefore recommended that moisture and nitrogen should be determined on separate samples of air-dried material. The following values were obtained for the nitrogen content of moisture- and ash-free proteins: egg albumin (native and coagulated), 15·76; edestin, 18·7; β-lactoglobulin, 15·58; casein, 15·73; excelsin, 17·94; amandin, 18·75; insulin, 15·54; pepsin, 14·8; horse carboxy-haemoglobin (not ash-free), 16·8%. F. A. R.

Anomalous Amino Nitrogen Values. H. E. Carter and S. R. Dickman (*J. Biol. Chem.*, 1943, **149**, 571–572)—In estimating amino and total nitrogen in crude penicillin preparations it was found that the manometric amino nitrogen value was almost double that for total nitrogen (micro-Kjeldahl). Chrysogenin, which contains no nitrogen, gave an apparent amino nitrogen value of 2·73%. Several other non-nitrogenous compounds also gave appreciable amino nitrogen values. It was found, for example, that by the standard Van Slyke manometric technique at 24–28° C. resorcinol apparently contained 9·4% of amino nitrogen, whilst resorcinol monomethyl ether, catechol, hydroquinone and phenol all gave values exceeding 1%. These results show that amino nitrogen values on compounds of unknown structure must be interpreted with caution. It appears probable that the anomalous results are due to the formation of nitrogen by reduction of the nitrous acid. F. A. R.

Observations on the Acid Haematin Method for the Estimation of Haemoglobin in Blood. C. A. Ashford (*Brit. Med. J.*, 1943, ii, 575–577)—The disadvantages of the acid haematin (Sahli) method are discussed (see also Clegg and King, *Brit. Med. J.*, 1942, ii, 329) and a standard procedure is recommended whereby the tubes containing the blood sample and 0·1 *N* hydrochloric acid are fitted with condensers, immersed in a water-bath at 40–45° C. for 10–15 min., rapidly cooled in melting ice and compared with a standard. This is shown to give substantially the same colour as standing at room temp. (14–18° C.) for 2 hr. (100% acid haematin production), while heating for 5 min. at 100° C. gives a 25% increase in colour and should be avoided, as turbid solns. often result. The expts. are summarised in 2 tables and a graph. The choice of a standard is considered and for an occasional determination a properly calibrated glass standard of the Newcomer type (*J. Biol. Chem.*, 1919, **37**, 465) is recommended; the calibration should be in g of haemoglobin per 100 ml of blood or in terms of the Haldane scale, owing to the varying meanings attaching to "100% Sahli" (Ashford, *Brit. Med. J.*, 1942, i, 163). A standard prepared by dilution of a 3% stock soln. of acid haematin was found to be unsatisfactory, being more turbid than the fluid prepared from fresh blood, and an alternative procedure, whereby blood of

known oxygen capacity is diluted with 0·1 *N* hydrochloric acid as required, is suggested; it is stated that the rate of formation of acid haematin is unaffected by the storage of blood in a refrigerator up to 2 weeks. The use of ox blood as standard in the assay of human blood is shown to be permissible, and expts. to this end are summarised in a table, which also shows that the method can be accurately applied to a wide range of anaemic bloods. It is not considered likely that the amounts of carboxy-, cyan- or met-haemoglobin in human blood would appreciably affect the colour. J. A.

The Dicarboxylic and Basic Amino-acids of Edestin, Egg Albumin and β-Lactoglobulin. A. C. Chibnall, M. W. Rees and E. F. Williams (*Biochem. J.*, 1943, **37**, 372–388)—Dicarboxylic and basic amino acids in protein hydrolysates are estimated by a method in which these amino acids are quantitatively isolated as pure derivatives. It is essential not to use any reagent that cannot be quantitatively removed at a later stage without appreciable loss of nitrogen. For example, cuprous and cupric salts may be used, as these can be removed as the corresponding sulphides, but neither sulphates nor chlorides must be added, at all events, in the early stages of the separation, as they cannot be removed as barium sulphate or silver chloride until the hydrolysate has been cleared of cystine and most of the dicarboxylic acids. The hydrolysate is first freed from cystine as the cuprous mercaptide of cysteine and the major part of the dicarboxylic acid is then isolated by Foreman's lime-ethanol process. Next the bases are removed by pptn. with phosphotungstic acid and estimated gravimetrically by the method of Tristram (*Biochem. J.*, 1939, **33**, 1271) as modified by Block (*J. Biol. Chem.*, 1940, **133**, 67). The mono-amino acids are then removed by treatment with copper carbonate by the method of Town (*Biochem. J.*, 1941, **35**, 417). After removal of copper the filtrate is subjected to a second lime-ethanol treatment and pptn. and the bases are again pptd. The method was applied to hydrolysates of edestin, egg albumin and β-lactoglobulin, with the following results:

	Edestin		Egg albumin		β-Lactoglobulin	
	N as % total protein-N	Wt. as % of protein	N as % total protein-N	Wt. as % of protein	N as % total protein-N	Wt. as % of protein
Glutamic acid ..	10·54	20·70	9·70	16·10	13·14	21·51
Aspartic acid ..	6·77	12·0	5·43	8·13	6·68	9·88
Arginine	28·85	16·71	11·44	5·63	5·95	2·89
Histidine	3·50	2·41	2·50	1·45	2·69	1·54
Lysine	2·44	2·37	6·15	5·06	12·07	9·75

The overall losses of nitrogen were 2·95, 2·86 and 1·25% of the total protein nitrogen respectively. F. A. R.

Use of the o-Phenanthroline Ferrous Complex as an Indicator in the Ceric Sulphate Titration of Blood Sugar. D. A. MacFadyen and D. D. Van Slyke (*J. Biol. Chem.*, 1943, **149**, 527)—In the method of Miller and Van Slyke (*J. Biol. Chem.*, 1936, **114**, 583) for the estimation of blood sugar, the ferrocyanide formed by reduction of ferricyanide is titrated with ceric sulphate. The oxidation-reduction indicator originally recommended is now unobtainable, but it has been found that *o*-phenanthroline is satisfactory for this purpose. The indicator is used in the form of a ferrous complex prepared in 0·025 *M* soln. as follows. Dissolve 14·85 g of *o*-phenanthroline monohydrate, $C_{12}H_8N_2.H_2O$, in 1 litre of a soln. containing 6·85 g of $FeSO_4.7H_2O$. The colour change from golden-brown to light-yellow at the end-point in the sugar titration is sharp, and the end-point is stable for at least 1 min. F. A. R.

Colorimetric Estimation of Total, Free and Ester Cholesterol in Tissue Extracts. G. Popjak (*Biochem. J.*, 1943, **37**, 468–470)—Kelsey's method (*J. Biol. Chem.*, 1939, **127**, 15; ANALYST, 1939, **64**, 365) for the estimation of total, free and ester cholesterol sometimes gives inaccurate results, apparently owing to the presence of other substances which give a colour with the Liebermann-Burchard reagent. Furthermore, recoveries of added cholesterol are not always quantitative. The method has therefore been modified as follows: *Total cholesterol*—Evaporate an alcohol-ether extract of the tissue, containing about 0·5 mg of cholesterol, and add 10 ml of a 1 : 1 mixture of alcohol and ether and 0·2 ml of 40% potassium hydroxide soln. Heat to the b.p. and maintain at 75° C. for about 2 hr. Add a drop of phenol red and acidify with hydrochloric acid of such conc. that about 1 ml is required. Evaporate the sol. almost to dryness on a hot-plate and dissolve the salts of the residue in 0·5-1 ml of water. Remove the soln., add 10 ml of light petroleum (40–60° C.) and shake the flask vigorously. Decant the extract into a centrifuge tube and concentrate by immersing the tube in hot water. Extract the residue in the flask with three further quantities of boiling light petroleum and add the extracts to the centrifuge tube, concentrating each in turn, the final volume being about 0·5 ml. Precipitate the cholesterol by adding 4 drops of 5% hydrochloric acid and 4 ml of a 0·25% alcoholic soln. of digitonin, leave overnight, and separate the cholesterol digitonide by centrifuging for 5 min. Decant the supernatant liquid, which can be used for the estimation of total fatty acids, wash the digitonide with 3 ml of a 1 : 2 mixture of acetone and ether and again centrifuge. Wash the digitonide with two 3-ml portions of ether and then add 7 ml of benzene. Immerse the tube in water at 80–82° C. for 45 min. with addition of benzene from time to time, keeping the vol. above 5 ml. Finally, concentrate to 2 ml and while the liquid is still hot add 9 ml of light petroleum with thorough mixing. After 10 min. remove the pptd. digitonin by centrifuging, and wash three times with light petroleum. Evaporate the combined extracts to dryness on a hot-plate. Dissolve the cholesterol in 5 ml of chloroform, immerse the flasks in water at 18° C. and, after a few min., add 2 ml of a freshly prepared 20 : 1 mixture of acetic anhydride and conc. sulphuric acid, also cooled to 18° C. At the same time prepare suitable standards, and after keeping them in a dark place for 60 min., compare the colours in a colorimeter. If total fatty acids are to be determined, decant the supernatant liquid from the cholesterol digitonide into another centrifuge tube and concentrate to 1 ml. Add the acetone-ether

washings, again concentrate to 1 ml, and then add the ether washings; this results in the pptn. of the excess of digitonin. Centrifuge, decant the supernatant liquid and evaporate to dryness. Determine the fatty acids by the dichromate oxidation method of Bloor (*J. Biol. Chem.*, 1938, **77**, 53). *Free cholesterol*—First separate the phospholipids from the alcohol-ether extract of the tissue, since phospholipids are pptd. by digitonin. Evaporate the extract to dryness on a hot-plate and thoroughly extract with light petroleum. Concentrate the extract to 2 ml in a centrifuge tube and add 7 ml of acetone and 3 drops of saturated alcoholic magnesium chloride soln. Remove the pptd. phospholipids by centrifuging, and decant the supernatant liquid into another centrifuge tube. Wash the phospholipids with two 2-ml portions of acetone and concentrate the combined extract and washings to 0·5 ml. Add 4 ml of digitonin soln. and 4 drops of 5% hydrochloric acid and leave overnight in a sealed jar. Remove the cholesterol digitonide by centrifuging and save the supernatant liquid for the estimation of ester cholesterol and of fatty acids derived from neutral fats and cholesteryl esters. Continue the process as described for total cholesterol. *Ester cholesterol*—This is estimated in the supernatant liquid from the cholesterol digitonide pptn. Precipitate the excess of digitonin as described above, decant the supernatant liquid, evaporate and saponify, and estimate the cholesterol by pptn. with digitonin as described for total cholesterol. Excellent agreement was obtained for the recovery of cholesterol added to alcohol-ether extracts of known cholesterol content. F. A. R.

Xanthine Oxidase Activity of Cow's Milk. A. N. Worden (*Nature*, 1943, **152**, 505–506)—Xanthine oxidase activity was determined (*a*) by the decolorisation of methylene blue and (*b*) manometrically. (*a*)—Samples (1 ml) suspended in a phosphate buffer of *p*H 8·0 were allowed to react anaerobically with xanthine or hypoxanthine in presence of 0·001 *N* methylene blue in a total vol. of 5 ml. With a final substrate concn. of 0·0004 *M* the activity was low when tested within a few hr. of milking, but rose spontaneously during the next 34 hr. This contradicts the work of Booth (*Biochem. J.*, 1938, **32**, 494), whose results may be due to the fact that the samples he tested may have become more active as a result of routine cooling on the farm. The author cites expts. demonstrating the rise in activity which occurs on cooling or shaking. (*b*)—The oxygen uptakes per hr. per mg of dry matter of 1-ml samples suspended in a phosphate buffer of *p*H 8·0 were determined in presence of various substrate concns. With a typical uncooled milk the activities rose from 2·72, 3·28, 3·50 and 6·24 (2 hr. after milking) to 5·76, 6·88, 9·20 and 9·52 μ-litre (3 days after milking) in presence of 0·0008, 0·0016, 0·0032 and 0·0064*M* xanthine, respectively. The samples were from Guernsey, Red Poll and Shorthorn cows (Dec. 1938—July, 1939, March-June, 1941) and were preserved with thymol and stored at 0–2° C. Tests made at different stages of single milkings showed that both the activity and the spontaneous rise increase with the fat content. These phenomena may be due to the release of activity accompanying the aggregation of fat globules and the consequent disruption of the surface membrane, which is composed of a complex phospholipid and membrane protein. J. G.

Micro-method for Estimating Vitamin A by the Carr-Price Reaction. H. Hoch (*Biochem. J.*, 1943, **37**, 425–429)—In an attempt to reduce the errors due to fading in the Carr–Price reaction for vitamin A, a method has been developed in which the reaction mixture is photographed in light of a selected wavelength together with a series of standard tubes, and the photographic film is then analysed by means of a photoelectric photometer. As standards, solns. of cuprammonium sulphate in water, ranging from 2·145 to 0·167 g of $CuSO_45H_2O$ per 100 ml are used, the series decreasing regularly by 20%. Put 0·4-ml quantities, containing 6 ml of conc. ammonia and 15 g of ammonium sulphate per g of hydrated copper sulphate, into small glass tubes (2 mm internal diam., 2·9 mm external diam., and 30 mm in length), and seal each tube. Calibrate them against solns. of vitamin A naphthoate (2 to 22 I.U./ml) of known concn. For measurement of β-carotene, prepare a similar series of standards with potassium dichromate soln. ranging from 0·595–0·01865 g/100 ml and containing 1 ml of conc. hydrochloric acid per 150 mg of dichromate. Calibrate these tubes against solns. of pure β-carotene in light petroleum, b.p. 40–50°. Pipette the extract to be tested into a special reaction tube made by fusing together a piece of glass tubing equal in diam. to that of the standards, and a glass tube of 7 mm internal diam., and evaporate the solvent in a stream of nitrogen at 50–55° C. Rinse the tube with a small amount of light petroleum and again evaporate. Add 0·005–0·03 ml of light petroleum and centrifuge. Calculate the vol. of the soln. from the height of the column and estimate the carotene content by comparison with the dichromate standard. Next evaporate the light petroleum in a current of nitrogen, take up the residue 3 times with pure chloroform, evaporate to dryness to remove all light petroleum and finally dissolve the residue in 0·015–0·105 ml of chloroform. Add acetic anhydride from a capillary pipette to give a final concn. of 1·0–1·5%, and then place the tube in position between a camera and a source of light. Between the light and the tube insert a diffusing glass (2 mm thick), a "Calorex" filter (2 mm thick), a "Signal Green" filter (1 mm thick), and then another diffusing glass. Between the tube and the camera (fitted with an enlarging lens to give an image of about natural size) insert a Wratten 26 red gelatin filter. Add the antimony trichloride reagent (saturated soln. in chloroform containing 1·5% of ethyl alcohol) from a capillary pipette with a long and very fine point, at such a rate that the addition occupies about ½ sec. Two sec. later make an exposure of 1½ sec. with subsequent 1-sec. exposures at 1-sec. intervals. Determine the vol. of the liquid. Analyse the photographic films electrophotometrically under a microscope with a photoelectric cell mounted at the other end. Record the galvanometer readings as the film is moved across the stage, and plot the difference between the reading of the maxima and the background against concn. of copper sulphate in the standards. Read the value of the unknown soln. from the line connecting the values for the standards which, under the conditions used, approximates to a straight line. It is well-known that the colour in the Carr–Price reaction fades at a variable rate with different samples; when fading begins as soon as the coloured compound is present the true value can only be obtained by applying to the observed maximum a correction derived from the fading rate. With blood, however, it was found that the corrections required to be applied in this way are within ±5% for the majority of extracts, and so can be ignored. The blue colour

given by carotene with the Carr–Price reagent was generally too small to affect the vitamin A results, but when the carotene content was appreciable the calibration line was extrapolated graphically to reach the point where the calibration line for vitamin A crosses the abscissa, and this value was subtracted from all figures of the calibration curve for carotene. F. A. R.

Chemical Estimation of Tocopherols in Liver and Muscle: Tocopherol in Urine and Faeces. L. R. Hines and H. A. Mattill (*J. Biol. Chem.*, 1943, **149**, 549–554)—The method of Devlin and Mattill (*J. Biol. Chem.*, 1942, **146**, 123) for the estimation of tocopherol in muscle proved unsatisfactory for its estimation in liver tissue. A modified method for liver, urine and faeces is now described. The original method failed to work with liver tissue because the presence of vitamin A prevented the cholesterol in the liver from being completely adsorbed on Florisil, with the result that the tocopherol extract contained cholesterol, and this led to high values on development of the colour. It was shown that vitamin A present in liver extracts could be destroyed by treatment with 85% sulphuric acid, and that the resulting extract, when passed through a second column of Florisil, gave satisfactory readings in the colorimeter. In the modified method, therefore, the extract is first treated with acid and alkali and then adsorbed on two columns of Florisil. After this treatment the extracts gave max. colour intensity after 10 min.; with muscle extracts a single adsorption proved sufficient. Shred 8–10 g of liver tissue in 60 ml of Skellysolve B and 70 ml of redistilled abs. alcohol in an ice-cold Waring Blendor. After centrifuging, re-extract the tissue 3 times. Transfer the combined extracts to a 2-litre separating funnel and add 600 ml of water. Shake the funnel gently, add sufficient alcohol to break the emulsion and then extract the aqueous alcohol phase with three 150-ml portions of Skellysolve B. Evaporate the combined extracts under nitrogen at reduced pressure and dissolve the residual oil in 25 ml of Skellysolve E. To 10 ml of this soln. add 2 ml of 85% sulphuric acid and centrifuge for 5 min. Transfer as much of the supernatant liquid as possible to another centrifuge tube, add 5 ml of 1% potassium hydroxide soln. and centrifuge for 10 min. Evaporate 7 ml of the supernatant liquid to dryness under nitrogen, dissolve the residue in 7 ml of benzene and run 6 ml of the soln. through a 70 × 12 mm column of Florisil, saturated by washing with 10 ml of benzene. Wash the column with 3–5 ml of benzene and evaporate the filtrate to dryness under nitrogen. Dissolve the residue in 6 ml of benzene and pour 5 ml on to a second column of Florisil, wash the column as before and evaporate the filtrate to dryness. Dissolve the residue in 5 ml of Skellysolve E, add 20 ml of αα′-dipyridyl reagent (250 mg of $FeCl_3.6H_2O$ and 500 mg of αα′-dipyridyl in 1 litre of glacial acetic acid) and measure the colour after 10 min. in a Klett-Summerson photoelectric colorimeter with a No. 52 filter; calculate the results from a calibration curve. Recoveries of known amounts of α-tocopherol added to liver tissues ranged from 96 to 103%. F. A. R.

Bacteriological

***Azobacter* Species capable of Infecting Beer. J. Tošić and T. K. Walker** (*J. Inst. Brewing*, 1943, **49**, 236–279)—*Azobacter* is the generic term approved, in modern nomenclature for the vinegar bacteria. Hitherto the only species isolated in pure culture from English beers were *A. viscosum* (Baker, Day and Hulton, *J. Inst. Brew.*, 1912, 651), *A. capsulatum* (Shimwell, *id.*, 1936, 585) and *A. turbidans* (*id.*, 194[illegible], 882; 1942, 82). In the authors' study of the capacity of various species to infect sterile beer the most active were *A. acetigenum* and *A. acetosum* (Henneberg, *Handbuch der Gärungsbakteriologie*, 1926) and *A. capsulatum* (*loc. cit.*). Next, in order of development, came *A. ascendens* (Henneberg, *loc. cit.*), whilst *A. aceti* and *A. pasteurianum* (Hansen, *Compt. rend, Lab. Carlsberg*, 1879, 96; 1894, 191), found in Danish beers, grew relatively slowly. *A. gluconicum*, *A. kützingianum* (Hansen), *A. suboxydans* (Kluyver and de Leeuw, Bergen's *Manual of Determinative Bacteriology*, 1939, p. 231), and *A. xylinum* failed to grow. The species *A. viscosum* causes "ropiness" in beer (Day and Baker, *Centr. Bakt. Par.*, 1913, **36**, 433), and *A. peroxydans* (Visser't Hooft, *Thesis*, Delft, 1925) is remarkable for being devoid of catalase. Since *A. kützingianum* and *A. suboxydans* were isolated originally from Continental beers, their failure to develop in the present expts. may be due to previous long cultivation in an artificial environment. Temperature had considerable influence on the rate of development. In every instance growth occurred at 14° C., but was more rapid at 25° C.

Isolation of Lactic Acid producing Bacteria from Beer. T. K. Walker and A. Parker (*J. Inst. Brewing*, 1943, **49**, 280–287)—In studying the control of "beer disease" bacteria by means of hops, a representative selection of them was necessary, and in the present paper the method adopted for their isolation, mainly anaerobically, from ales and beers, is described. The method recommended by Snieszko, which was chosen for this work, consists essentially in growing cultures in pairs of Petri dishes of equal size, luted together with plasticine, one of the cultures being strongly aerobic and providing nearly anaerobic conditions for the other by its respiration. The aerobic organism used was *Serratia marcescens* and it was grown on nutrient agar, whilst the bacteria to be isolated were grown on unhopped beer-gelatin inverted over the agar. When these sealed pairs of plates were incubated at 18–20° C., in some expts. colonies appeared in 1 week, whilst in others 3 weeks were required. From 9 different beers and one sample of yeast 34 cultures were obtained, the majority of which were found to belong to the family *Lactobacteriaceae*. A detailed examination of the characteristics of these organisms is being made. D. R. W.

Water

Colorimetric Determination of Fluoride in Waters with Thorium and Alizarin. N. A. Talvitie (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 620–621)—A thorium-alizarin lake has been used in the determination of fluoride by volumetric (Willard and Winter, *Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 7; ANALYST, 1933, **58**, 242) and colorimetric (van der Merwe, *Onderstepoort J. Vet. Sci. Animal Ind.*, 1940, **14**, 359) procedures, the fluorine being first isolated by distillation. This has been adapted to the estimation of fluoride in potable waters without preliminary distillation, and the method resembles those now in use in which a zirconium lake is used. The buffered thorium reagent is 0·001 *M* with respect to thorium nitrate

and 1 *M* with respect to sodium sulphate, formic acid and sodium formate. The alizarin soln. (0·00025 *M*) contains 0·0855 g of alizarin monosodium sulphonate per litre. The standard fluoride soln. contains 0·0221 g of sodium fluoride per litre (1 ml ≡ 0·01 mg of fluorine). To prepare standards, add 5 ml of alizarin soln. to 0, 2, 4, 6, 8, 10 and 12 ml of standard fluoride soln. in 100-ml Nessler glasses marked at 110 ml. Dilute to 110 ml with water, add 5 ml of thorium reagent, mix well and leave for 30 min. To prepare the sample, titrate 100 ml of the water and 5 ml of alizarin soln. with 0·3 *N* nitric acid to a pure yellow colour, dilute the liquid to 110 ml in a Nessler glass, add 5 ml of thorium reagent and mix well. After 30 min. compare the colour of the sample with those of the standards. Although the effect of some individual interfering ions is negligible, their combined effect is significant and may be considered additive if the sample contains not more than 100 mg of any one ion. Approx. effects of the common ions are—100 mg of sulphate ion ≡ +0·02 mg of fluorine; 100 mg of calcium or magnesium ion ≡ −0·01 mg of fluorine; 100 mg of nitrate ion ≡ −0·005 mg of fluorine. Since the ionic wt. of nitrate is approx. that of bicarbonate, the nitrate introduced by titration may be considered equal to the bicarbonate content of the water. Sodium and potassium have no effect. When phosphate or aluminium is present the fluoride must be isolated by distillation (Willard and Winter, *loc. cit.*). Phosphate may be recognised by the turbidity due to thorium phosphate, aluminium by failure to obtain the pure yellow colour when titrating with nitric acid. Iron in sufficient amount will interfere and may be removed by aerating and filtering the water (made alkaline if necessary). Silica in amounts up to 20 mg does not interfere. Waters containing organic colouring matter may be treated by the Walpole method (*Biochem. J.*, 1911, **5**, 207), using 100 ml short-form Nessler glasses in a simply constructed comparator in which the sample glass rests above a glass of distilled water and the standard glass rests above a glass of duplicate sample containing 5 ml of thorium reagent but no alizarin. Difference of temp. between sample and standard causes an apparent difference in fluorine content of 0·002 mg per °C. The standards are stable in darkness for *ca.* 1 week. A. O. J.

Fixing and Determining Oil in Feed Water and Boiler Water. C. A. Noll and W. J. Tomlinson (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 629–632)—In the determination of oil in water samples two sources of error are encountered, *viz.*, the inclusion in the isolated oil of other substances such as sodium sulphate and sodium chloride, and the separation of the oil from the water on standing. Oil deposited on the sides of the sample container is difficult to remove even with the help of an organic solvent. The following method, based upon that of Scott ("*Standard Methods of Analysis*," Vol. II, 5th Ed., p. 2078), avoids these sources of error. Collect water at the sampling point in a 500-ml volumetric flask, transfer the sample to a stoppered 500-ml bottle, rinse the flask with oil-free water and add the rinsings to the sample. Treat the sample with 10 ml of ferric chloride soln. (*infra*) and, after shaking, with 10 ml of ammonium hydroxide and shake well again. The sample is now "fixed" and may stand for a few days before the oil is determined. Boil the mixture for 1 min., cool below 40° C., filter with gentle suction through an ignited alundum thimble and transfer all the ferric hydroxide floc to the thimble. Wash the floc with water until free from chloride and dry the thimble by gentle suction and finally in an oven at 105° ± 1° C. for 1·5 hr. Extract the cooled thimble in a Soxhlet extractor with ether, evaporate the solvent, and weigh the dried residue of oil. The oil recovered by this method is free from sulphate and chloride but does not include any saponifiable fraction of the original oil. As a rule this saponifiable fraction is very small. To prepare the ferric chloride soln., dissolve 10 g of pure ether-washed iron in 30 ml of hydrochloric acid diluted with 400 ml of water. When solution is complete add conc. nitric acid with stirring until oxidation of the ferrous salt is complete. Boil the soln. for *ca.* 30 min. and dilute to 1 litre. To prepare the ammonium hydroxide soln., dilute 360 ml of conc. ammonium hydroxide (sp.gr. 0·9) to 1 litre. All vessels used in sampling and testing must be rinsed with alcohol and ether to remove oil. A. O. J.

Agricultural

Determination of Potash in Fertilisers or Base Goods in Absence of Ammonium Salts and Organic Matter. P. M. Shuey (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 633–634)—The proposed method removes calcium, iron and aluminium as phosphates and yields accurate and concordant results in absence of ammonium salts and organic matter. Boil 5 g of the prepared sample in a 500-ml flask (or 2·5 g in a 250-ml flask) with 350 ml (or 200 ml) of water for 30 min. Add 30 ml of 2% sodium phosphate soln. (or 6 ml of a 10% soln.) and then dil. sodium hydroxide soln. until the mixture is permanently alkaline to litmus. If the soln. becomes acid on standing, add more sodium hydroxide but avoid a large excess. Dilute the cooled mixture to volume, filter, treat the aliquot portion (≡ 0·25 g) with platinic chloride and hydrochloric acid and proceed as directed in the official A.O.A.C. method for determination of potash in potash salts. Immediately before filtering it is best to grind the ppt. lightly with a small pestle to remove excess of platinum chloride and facilitate the subsequent purification with ammonium chloride soln. When the sodium hydroxide soln. is not freshly made, it is advisable to know the amount of dissolved glass therein, and to make the necessary correction if the ppt. of chloroplatinate is not finally dissolved in hot water. The formation of lumps during the preliminary boiling may be prevented by adding water to a portion of the sample and then adding small portions of the sample and water alternately, swirling the mixture after each addition of water. A. O. J.

Determination of Mustard Oils in Crucifers. M. A. Stahmann, K. P. Link and J. C. Walker (*J. Agric. Res.*, 1943, **67**, 49–63)—Rochlin (*Phytopath. Z.*, 1933, **5**, 381) attributed the resistance of certain species and varieties of *Cruciferae* to the clubroot organism (*Plasmodiophora brassicae* Wor.) to the high toxicity of the *iso*thiocyanates in their mustard oils. To investigate this conclusion the authors have studied certain strains of black mustard (*Brassica nigra*), turnip (*B. rapa*), white mustard (*B. alba*[*h*] Boiss.), cabbage (*B. oleracea* var. *Capitata*) and horseradish (*Amoracea rusticana* Gaertner). *Isolation of Mustard Oils*—The fresh cortical tissue (3 kg) was ground to a pulp, divided into four 750-g portions and immediately steam-distilled. The distillates were collected in 5-litre receivers containing 50 ml of conc. ammonia soln.

in 200 ml of water, each condenser being fitted with an adapter which dipped below the level of the liquid in the receiver. The combined distillates (*ca.* 6 litres), containing excess of ammonia, were stirred for 2 hr. and left for 24 hr. at room temp. to complete thiourea formation and then extracted for 12 hr. with peroxide-free ether in a continuous liquid-liquid extractor. The ethereal soln. was concentrated to *ca.* 50 ml, 100 ml of 95% ethanol were added, and the residual ether was distilled off. The alcoholic soln., containing the thiourea fraction corresponding with the mustard oil in the distillates was decolorised with carbon (excess being avoided), concentrated under reduced pressure to about 25 ml, and transferred to a crystallising dish, which was heated on a steam-bath, and water was added until the first appearance of turbidity; after cooling and concentration of the liquid *in vacuo* crystalline diamond-shaped plates separated and were filtered off. Yields of 0·2 to 0·8 g depending on the variety were obtained from 3 kg of turnips. The products, recrystallised from a mixture of ethanol and water, had m.p. 136°–137° C. The m.p. will not distinguish between the thiourea obtained from *d*-secondary-butyl *iso*thiocyanate (m.p. 134° C.) and that from β-phenethyl *iso*thiocyanate (m.p. 137° C.); they may be distinguished by converting the isolated thiourea into the corresponding urea by a modification of the method of Bertram and Walbaum (*J. prakt. Chem.*, 1894, **50**, 555).

The ethereal soln., obtained as described above and concentrated to *ca.* 50 ml, was transferred to a 500-ml flask by means of 25 ml of ethanol and *ca.* 300 ml of water, and the flask was heated to *ca.* 60° C. to remove the last traces of ether. *N*/10 silver nitrate (100 ml) was then added, and the flask was swirled and left for 24 hrs. to complete the sulphide pptn. The contents were then diluted to 500 ml and mixed and the silver sulphide was filtered off. One hundred ml of the filtrate were acidified with 10 ml of 3 *N* nitric acid and titrated with *N*/10 ammonium thiocyanate, ferric ammonium sulphate being used as indicator. The mustard oil content was calculated as β-phenethyl *iso*thiocyanate from the amount of silver removed as silver sulphide. The following results were obtained.

Species	Variety	Susceptibility to clubroot	Mustard oil %
Turnip	Snowball	Highly resistant	0·019
,,	White Milan	,,	0·015
,,	Purple Top White Globe	,,	0·021
,,	Purple Top Milan	,,	0·024
,,	Cowhorn	Moderately resistant	0·020
,,	Shogoin	Very susceptible	0·017
Black mustard	No. 3	Resistant	0·010
,,	No. 14	Susceptible	0·016

No relationship could be found between the resistance to clubroot and the mustard oil content.

Determination of Allyl Isothiocyanate *from Hydrolysis of Sinigrin*—Viebeck and Brecker's modification of the iodine absorption procedure (*Monatsh. Pharm.*, 1936, **11**, 149, 203) gave accurate and reproducible results. The allyl *iso*thiocyanate is converted into allyl thiourea by means of excess of ammonium hydroxde and the iodine absorption of the allyl group is determined. Prior to the iodine absorption it is necessary to separate the allyl thiourea from the hydrolysis mixture by extraction with ether after the treatment with ammonia. It was found that allyl *iso*thiocyanate, which is present as the glycoside sinigrin in the seeds of black mustard and in horse radish, does not occur in the roots of turnip, cabbage, or white mustard seed. On the other hand, β-phenethyl *iso*thiocyanate was found in the root tissue of each of the above-mentioned crucifers. It has the same general degree of toxicity towards fungi as allyl *iso*thiocyanate. Although both *iso*thiocyanates are highly toxic to clubroot organisms, they do not seem to inhibit infection or development of the disease in the tissues that contain them.

New Properties of Inorganic Dusts [Testing Dust Insecticides]. **H. V. A. Briscoe** (*J. Soc. Arts*, 1943, **91**, 593–607)—*The weevil as "reagent"*—The grain weevil (*Calandra granaria*) is used in a standard toxicity test of the potency of dusts. Mix 50 g of English wheat, in equilibrium with air of *ca.* 70% R.H. and containing *ca.* 14% of moisture, with a weighed amount (usually 1% of the wheat) of the dust to be tested. Select 50 adult weevils at random from a batch of several thousand, put them on the prepared wheat in a boiling-tube, close the tube with a muslin cover and keep it in a cupboard thermostatically held at 23° C. At suitable intervals, usually 2–3 days, separate the weevils from the wheat by means of 10– and 40-mesh sieves and count the dead. Then return the weevils to the tube with the original wheat and continue the expt.; with every group of tests use at least one control containing no added dust. Plot the number % of deaths against time. The "effectiveness" of a given dust is measured by the time taken to kill, *e.g.*, 50% of the weevils.

Chemical and physical properties of dusts—A series of tests on a commercial precipitated silica, treated with various reagents, showed that the chemical properties are not significant, and other expts. proved that the lethal action of mineral dusts is essentially physical or mechanical. Their main effect is the result of an action on the outer casing of the insect, causing loss of water. The weevil living on grain contains 50% of water and if the amount falls to *ca.* 30% the insect dies. The rate of loss in weight of a batch of starving weevils is greatly increased by dusting them, and it has been proved (*e.g.*, by means of minute hair hygrometers) that this loss consists almost entirely of water. Effective dusts do not absorb water from the insects; many, *e.g.*, diamond and carborundum, are incapable of doing so. They function by promoting the normal loss of water to the surrounding air; the loss is always 2 to 3 times the normal amount when the insects are coated with an effective dust.

Examination of Experimental dusts—Crystalline powders, *e.g.*, carborundum, are best dispersed on the slide in a drop of saponin soln., and then allowed to dry and mounted in Canada balsam. Quartz powders, which are almost invisible in most media, should be dispersed as well as possible and mounted dry. A method generally applicable is to disperse a known weight of the dust in a suitable liquid, to take small aliquot parts of the dispersion and dilute it with the same liquid, and then, in a tube of special construction, to centrifuge out the solid on to a cover glass laid on the flat removable base of the tube. This secures uniform and random distribution of the particles, so that photographs of the deposit have a definite quantitative significance.

Characteristics of a good insecticidal dust—Expts.

described in detail, have shown that a good dust insecticide should be: (1) fairly hard, preferably above 6·5 on Moh's scale, *i.e.*, harder than Pyrex glass; (2) very fine, with particles mostly under 10μ in diam. and preferably with a high proportion within the range 1–5μ; (3) free from SiO_2, to avoid silicosis hazard to men handling the grain; (4) cheap, readily available and free from poisonous elements, *e.g.*, arsenic or lead. It was found that coal ash from powdered fuel was not fine enough and that London clay, cements, china clay and the wastes from its extraction were ineffective, whilst iron oxide, though moderately effective, was unsuitable because of its colour. Reasonably effective dusts were yielded by limestone, basalt, Whin Sill granite, low grade iron ores, serpentine, etc., but the most promising material was coal ash clinker, which when wet ground proved very effective. Fine grinding with any liquid—ether, benzene, carbon tetrachloride or water, produces dusts which are much more effective than dry-ground dusts, possibly owing to their particles being more uniform. Other expts. showed that the insecticidal value of a dust is enhanced if the surface is crystalline or angular and is reduced as the surface becomes less angular or more amorphous. The lethal mechanism of dusts is attributed not to blockage of the spiracles but to their increasing the transmission of water through the hard cuticle. An analogous effect could be observed with artificial membranes so devised as to simulate in some degree the shell of the insect.

Organic

Use of Silver Vanadate in Micro-combustion of Organic Compounds. G. Ingram (*J. Soc. Chem. Ind.*, 1943, **62**, 175–176)—Errors in the combustion of nitrogenous organic compounds, particularly those containing such elements as chlorine and sulphur, are frequently due to the inefficiency of lead peroxide in decomposing oxides of nitrogen; they can be avoided by using as the oxidation filling for the combustion tube silver orthovanadate suspended on pumice granules. This filling is prepared as follows. Filter a soln. of 18 g of vanadium pentoxide in 2 *N* sodium hydroxide, add an excess of silver nitrate soln., boil, and filter off the yellow ppt. Distribute 5 g of the dried ppt. on 8 g of 10- to 14-mesh pumice stone by heating, with stirring, until melted. Heat strongly for 4 hr., with occasional stirring, to remove all volatile products, place in the combustion tube and heat for 1 hr. before use. As this filling becomes exhausted it changes from yellow to reddish-brown. With organometallic compounds the life of the filling is considerably increased by using an initial plug of copper oxide gauze. Fillings made by suspending oxides of cerium or manganese on silver chromate are also capable of destroying oxides of nitrogen but have no particular advantage over the standard lead peroxide filling, since they become exhausted after 8 combustions. Mixtures containing iron, copper, and uranium were tried without success. E. M. P.

Lead Tetraacetate Oxidations in the Sugar Group. R. C. Hockett, M. T. Dienes and H. E. Ramsden (*J. Amer. Chem. Soc.*, 1943, **65**, 1474–1477)—Criegee's observation that *cis*-1.2-glycols are more rapidly oxidised by lead tetraacetate than *trans*-glycols (*Ann.*, 1933, **507** 159) led to a study of the correlation between oxidation rates and configurations in a series of pyranosides which contained $\alpha.\beta.\gamma$-triol structures with fixed spatial relationships among the hydroxyl groups (Hockett and McClenahan, *J. Amer. Chem. Soc.*, 1939, **61**, 1667). The findings were: (1) ultimately at least 2 mols. of lead tetraacetate are consumed by a vicinal triol. By-reactions, such as the oxidation of formic acid, often prevent the consumption of oxidant from ending sharply at 2 mols. (2) If 2 of the 3 hydroxyl groups are adjacent and in *cis* relationship oxidation is more rapid than if each hydroxyl is *trans* to its neighbours. (3) An α-hydroxyaldehyde is attacked by lead tetraacetate, but often slowly. (4) An α-hydroxyaldehyde is relatively rapidly oxidised if another hydroxyl, γ or δ to the carbonyl, permits of cyclic hemiacetalization to a pseudoglycol structure. These facts should allow the elucidation of the configuration of certain molecules, including the distinction between pyranosides containing a *trans-trans* configuration within the ring and those having one or more pairs of *cis* hydroxyl groups. Isolation of the final oxidation products does not elucidate the original configuration of carbon atoms which are eliminated or lose their asymmetry in the oxidation.

The present study records the oxidation rates of 8 sugar derivatives: β-methyl-*d*-xylopyranoside, β-methyl-*d*-glucopyranoside, laevoglucosan, trehalose, α-methyl-*l*-sorbopyranoside, α-methyl-*d*-glucopyranoside, α-methyl-*d*-mannopyranoside, and styracitol. The structures of styracitol and polygalitol are discussed.

Experimental details are as follows. *Prepn. of acetic acid*—The water content of the medium influences the rate of oxidation by lead tetraacetate and must therefore be controlled. Boil U.S.P. acetic acid with chromic acid to remove aldehydes, fractionate through an efficient column, and titrate by the Almy, Griffin and Wilcox modification (*Ind. Eng. Chem., Anal. Edn.*, 1940, **12**, 392) of Karl Fischer's method (*Z. angew. Chem.*, 1935, **48**, 394); the water-content is usually about 0·60%, which has an observable effect on the oxidation rate. Add the calculated quantity of acetic anhydride and heat under reflux for 3 hr. in an apparatus protected from the atmosphere. Titration then usually shows 0–0·05% of water. This dried acetic acid is used both as a reaction medium and for preparing standard lead tetraacetate solns., which are dispensed from an all-glass automatic burette with a 2-litre reservoir and efficient protection from atmospheric moisture. *Samples*—Finely powder the samples and dry at 60° C. *in vacuo* before use. *Procedure*—The conditions have been standardised as follows. (1) The lead tetraacetate soln. is about *N*/10. (2) The sample is 0·00025 mole. (3) The vol. of standard soln. containing 0·0038 mole of lead tetraacetate is calculated (ratio of oxidant to sample is 15·2/1). (4) Dissolve the sample in a 100-ml flask in a vol. of acetic acid equal to 99 ml minus the vol. of standard soln. to be added. (5) Add the calc. vol. of standard lead tetraacetate soln.; note the time from the first contact of the oxidising agent with the substrate. (6) Make the soln. up to 100 ml with acetic acid and place in a thermostat at 25° C. (7) Remove samples at noted time intervals with a 10-ml pipette and drop into 25-ml vols. of a soln. containing about 0·5 g of sodium iodide and 5 g of sodium acetate. (8) Titrate the liberated iodine with 0·0200 *N* sodium thiosulphate. (9) Plot the results as the ratio of moles of oxidant consumed per mole of substance against time in hours. E. M. P.

Characteristic Reactions of Citric and Tartaric Acids. A. Steigmann (*J. Soc. Chem. Ind.*, 1943, **62**, 176)—Tests for citric and tartaric acids are based on their reaction with urea to form hydroxypyridines and hydroxypyrroles respectively, followed by condensation of these products with formaldehyde to yield characteristic dyes. Procedures are as follows. *Citric acid*—(*a*) Melt 1–2 g of urea in a small crucible and mix-in 0·1–0·5 g of citric acid. Raise the temp. rapidly to 175–180° C.; with slower heating, the max. temp. must be lower, but not below 170° C. Dissolve the yellow product (solns. of which show yellowish-green fluorescence) in 5 ml of 50% acetic acid. Draw a line with this soln. on lignocellulose paper (news-print); a red-brown colour appears after a few min.; if the paper is first treated with dil. (1 : 1) hydrochloric acid the reaction is almost immediate and the colour is redder. (*b*) Heat the citric acid and urea mixture to 190–195° C., dissolve in 5–10 ml of 50% acetic acid, and add 0·5–1 ml of 40% formaldehyde. The product dyes wool or silk in yellow shades remarkably fast to acids, alkalis, peroxides, soap and light, but not very fast to chlorine. No organic acid tested so far interferes with either (*a*) or (*b*), and the reactions are specific for citric acid. *Tartaric acid*—Melt together 1–2 g of urea and 0·2–0·5 g of tartaric acid and heat rapidly to 190°–200° C. Cool to 120° C., add 0·05–0·1 g of *p*-dimethylaminobenzaldehyde and 2 ml of 50% acetic acid, and heat to 100° C. An orange-red dye results. Under the same conditions the urea and citric acid melt (produced at 175°–180° C.) gives a violet dye. Malic and glycollic acids give yellower shades than tartaric acid and require temps. of 210°–215° C. for the melts. The test for tartaric acid is negative in presence of citric acid, but in mixtures of the two the former can be detected by the resorcinol and sulphuric acid reaction (violet-red), which is negative with citric acid. For small quantities of the acids, add 0·02–0·04 g of *p*-dimethylaminobenzaldehyde to 1–2 g of molten urea, followed by 0·1–1 ml of test soln.; heat to 170°–185° C. for citric acid and 200–220° C. for tartaric acid (more dilute solns. require higher temps.); on adding 0·5 ml of 50% acetic acid and 2–3 ml of water the characteristic colours are produced.

The dye from tartaric acid is an optical sensitiser for silver chloride (green sensitiser), whilst the citric acid dye is a desensitiser, although they appear to have no photographic value. Bathe a silver chloride (gaslight) paper for 2 min. in a soln. of the dye at *p*H 5·0, rinse for 1 min. in distilled water, dry, and expose under a colour sensitometer with a sample of the same paper which has been washed for 3 mins. with distilled water and dried. E. M. P.

Determination of Glycerol by the Pyridine-Acetylation Method. J. C. Moore and E. W. Blank (*Oil and Soap*, 1943, **20**, 178)—In the method of Shaefer (*Ind. Eng. Chem., Anal. Ed.*, 1937, **9**, 449) 50 ml of an aqueous soln. containing not more than 2·5 g of glycerol or glycol are carefully concentrated to about 10 ml by distillation through a 3-bulb Snyder column, any salts which form hydrates or compounds with pyridine are removed, 100 ml of anhydrous pyridine are added, the mixture is distilled slowly until the temp. reaches 110° C., and the alcohol is determined in the residue, or in an aliquot part of its soln. in pyridine, by the pyridine-acetic anhydride method. Schaefer's method is recommended for the determination of glycerol in soaps, sweet-waters, soap lyes, etc. It is noted that, with a pure sample of glycerol, the dichromate method and the acetylation method give practically identical results, the ratio

$$\frac{\text{glycerol by acetylation}}{\text{glycerol by dichromate oxidation}}$$

being constant; the average in a large number of determinations was 0·993. By using this factor, the method becomes invaluable for checking oxidation values on dil. glycerol solns. J. A.

Determination of *p*H of Textile Materials. H. R. R. Wakeham and E. L. Skau (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 616–618)—The usual methods for the determination of *p*H of textile materials depend upon measurement of the *p*H of an aqueous extract of the fabric, and it is assumed that the effect of moisture in the sample is relatively insignificant. Expts. showed that the *p*H of the extract is a function of the amount of water used, and that the smaller the amount of water used the nearer the *p*H of the extract to the true *p*H of the fabric. Under the standard A.S.T.M. conditions (21° C. and 65% relative humidity), textiles may contain up to 20% of moisture. By the method to be described, the same piece of fabric is treated with varying vols. of water, and by extrapolation the *p*H of the water present in the fabric under the standard conditions is found. Macerate *ca.* 2 g of the air-dried sample, cut into small pieces, with 3 ml of water at room temp. until the mass is uniformly wetted. Leave for 30 min. and then, after stirring, press the cloth against the side of the vessel until *ca.* 1 ml of liquid can be poured into the 5-ml cup of a Beckman *p*H meter, and measure the *p*H on the standard 2·5-in. electrodes of the meter. Return the liquid to the vessel and add to it another 1 or 2 ml of water. Stir the mass and after 15 min. measure the *p*H in the same manner. Repeat the process until a number of values have been obtained (*e.g.*, with 3, 5, 10, 20 and 30 ml of water). Plot the *p*H values at these dilutions against the vol. of water added and extrapolate the curve back to the zero ordinate, *i.e.*, to the value corresponding with the moisture in the sample in its air-dried condition. In this way the *p*H of the moisture present in the sample is ascertained. The curves show that only in some instances does the *p*H of the extract equal the extrapolated value. Some materials show nearly identical extrapolation values and widely different extract values at the higher dilutions. As the extracts become more dilute, the *p*H approaches that of pure water, but with certain sulphur-dyed cotton fabrics the *p*H was found to rise farther above 7 with greater dilution. The explanation of this is not yet known. Statistical examination of the results showed that the value obtained at the 5-ml point for a single observation is subject to an error of 0·05 and that this error would increase somewhat for smaller amounts of water. For a given set of observations the average error involved in the extrapolation would be of the order 0·1 *p*H unit. Greater accuracy is obtainable by averaging several measurements on similar samples. Some inaccuracy in extrapolation may occur when the curve has a high curvature at the points of higher concn. The extreme *p*H obtained by extrapolation would be that of the sat. soln. It is suggested that the *p*H of fabric or textile material might be defined as the *p*H of the water present under the A.S.T.M. standard conditions. This value is the one obtained by the method described with samples brought to the standard conditions in a conditioning room. A. O. J.

Inorganic

Dithizone Method for the Rapid [Colorimetric] Determination of Copper. G. H. Bendix and D. Grabenstetter (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 649–652)—The method is suitable for the examination of canned foods, tinplate and solders. Interference by all metals except platinum, palladium, gold, silver, mercury, bismuth and stannous tin is eliminated by extracting the copper as dithizonate complex from an aqueous soln. of *p*H 2·3. The reaction products of gold, silver, mercury, bismuth and stannous tin are destroyed by shaking the extract with an acid soln. of potassium iodide which has been decolorised with sodium thiosulphate. The transmission at 520*mμ* of the final soln. is compared with that (100%) of a "blank" in a (Coleman) spectrophotometer, and the copper content is determined from a calibration curve. *Reagents*—Water and pure nitric acid redistilled in Pyrex apparatus. Cresol red, 0·02 g in 100 ml of water. Buffer soln.: Dissolve 38 g of citric acid and 21 g of disodium hydrogen phosphate dodecahydrate in water, shake with a conc. soln. of dithizone in carbon tetrachloride, remove the excess of dithizone by washing with carbon tetrachloride and dilute the aqueous layer to 250 ml. Dithizone soln.: 15 mg of pure compound in 1 litre of carbon tetrachloride. Potassium iodide: Dissolve 10 g in 450 ml of water, add 5 ml of *N* hydrochloric acid and then 0·1 *N* sodium thiosulphate drop by drop until the colour of iodine is discharged. Shake with successive 10-ml portions of dithizone soln. until the dithizone is not discoloured, wash the aqueous layer with carbon tetrachloride and dilute to 500 ml. *Procedure*—Pipette a suitable volume of the sample soln. into a 150-ml separating funnel and add sulphuric acid (10%) to bring the vol. up to 25 ml if necessary. Add 2 drops of cresol red soln. and then conc. ammonia soln. until the yellow end-point is reached. Add 2 ml of buffer soln. and 10 ml of dithizone reagent and shake (preferably mechanically) for 10 min. Transfer the carbon tetrachloride layer to another separating funnel and shake for 2 min. with 10 ml of potassium iodide soln. Again transfer the carbon tetrachloride layer to another separating funnel and shake for 2 min. with 25 ml of ammonia soln. (1 : 200) to remove excess dithizone. Determine the transmission at 520*mμ*. *Application of method*—(*a*) *Food*—Heat 20–50 g of the mixed sample in a 500-ml conical flask until charring just begins, cool, add 10 ml of conc. sulphuric acid and heat to fuming. Add conc. nitric acid drop by drop until the contents of the flask are colourless or only straw yellow. Cool, dilute to 50 ml, "fume," repeat to remove nitrogen oxides and finally dilute in a standard flask. (*b*) *Steel*—Dissolve 1 g of drillings in 100 ml of sulphuric acid (1 : 9), oxidise with nitric acid, cool and dilute to 250 ml. (*c*) *Solder*—Dissolve 1 g in conc. hydrochloric acid and bromine, filter off the lead chloride and adjust the *p*H to 2–3. Earlier dithizone methods are discussed and the experimental background of the present procedure is given. L. A. D.

Iodimetric Estimation of Zinc in Magnesium Alloys. C. C. Casto and A. J. Boyle (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 623–624)—Lang's iodimetric procedure (*Z. anal. Chem.*, 1929, **79**, 161–170), based on the reaction

$$3ZnSO_4 + 2K_3Fe(CN)_6 + 2KI \rightarrow K_2Zn_3[Fe(CN)_6]_2 + 3K_2SO + I$$

has been modified. The zinc is determined by titration with standard sodium thiosulphate soln. after removal of copper by means of test lead and manganese by pptn. as dioxide; iron is converted into a complex citrate. If the alloy contains less than 2% of zinc, concentration is effected by means of hydrogen sulphide separation (see [*b*] below), or standard zinc sulphate soln. may be added. *Reagents*—Potassium ferricyanide soln., 0·2 *M*. Sodium or ammonium citrate soln., 10%. Sodium thiosulphate soln., 0·1 *N*. Manganous sulphate soln., 1 g of Mn per litre. *Procedure*—(*a*) Zinc content not less than 2%—To 2 g of alloy in a 400-ml beaker add 50 ml of water and 35 ml of sulphuric acid (1 : 2) in small portions. If copper is present boil for 15 min. with 5–10 g of granulated test lead, filter, and wash the residue. To the filtrate add manganous sulphate soln. to bring the manganese content up to 10 mg. Add not more than 1 g of ammonium persulphate and boil for 30 min. Filter (a porcelain filter crucible is suggested) and wash thoroughly, keeping the volume of the filtrate below 150 ml. Add 10 ml of citrate soln. for each 1 mg of iron present and neutralise (methyl orange) with ammonia soln. Make distinctly acid with sulphuric acid (1 : 2); the total volume should not exceed 200 ml. Cool to room temp., add 1 to 2 g of potassium iodide and leave for 10 to 15 min. Add 2 to 3 ml of 1% starch soln. and just discharge any blue colour by adding sodium thiosulphate soln. Add potassium ferricyanide reagent in 1 to 2 ml portions and titrate slowly with standard sodium thiosulphate soln. after each addition until the starch-iodine colour is faint. Continue until the colour is not intensified by addition of ferricyanide soln. and complete the titration to a sharp (1 drop) sulphur-yellow end-point. A light green end-point indicates that copper or iron has not been completely removed or converted into a complex. The end-point should be stable for at least 30 sec., but on standing the ferricyanide slowly liberates iodine. (*b*) Zinc content less than 2%—Dissolve 10 g of the sample in 100 ml of sulphuric acid (1 : 1) and dilute the soln. to 300 ml. Neutralise with ammonia soln. and make just acid (methyl orange) with 6 *N* sulphuric acid. Pass a rapid stream of hydrogen sulphide for 45 min. and filter. Without washing, return the paper and its contents to the beaker, add 5 ml of conc. nitric acid and 10 ml of perchloric acid (70%) and boil until dense fumes of perchloric acid are evolved. Cool, dilute to 100 ml and add 5 to 7 ml of conc. sulphuric acid. Alternatively, destroy the filter-paper with 5 ml of conc. nitric acid and 10 ml of conc. sulphuric acid. Complete the determination as under (*a*) above, omitting the procedure for removal of manganese. Use the empirical factor 1 ml of 0·1 *N* sodium thiosulphate ≡ 9·97 mg of Zn. Any cadmium present must be removed, *e.g.*, by adding 40-mesh aluminium to the slightly acid soln. This procedure will remove copper if cadmium is also present. Lang prevents the effect of cobalt by adding potassium cyanide and that of nickel by adding sodium potassium tartrate and aluminium sulphate, but these elements usually occur in such small amounts in magnesium alloys that they do not interfere. The method is as accurate as other standard procedures for zinc determination and is applicable to a wide variety of magnesium alloys. L. A. D.

Determination of Tin with Mercuric Chloride. J. G. Fairchild (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 625–626)—Tin can be determined by weighing

the mercurous chloride formed on adding stannous chloride to mercuric chloride. With pure solns. the procedure is rapid, accurate and applicable over a range of 0·1 to 70% of tin. The application of the method, which is especially suitable for tin in low-grade ores, to concentrates of cassiterite and ores containing complex tin sulphides, is described. Lead and antimony are the only elements likely to interfere, but their relative insolubility in dil. acid after reduction with zinc makes any interference negligible in most instances. *Method*: (1) *Concentrates of Cassiterite*—Fuse 1 g of 200-mesh material with sodium pyrosulphate, extract with 50 ml of 5% nitric acid and simmer for at least 1 hr. Filter off the insol. residue, wash with hot dil. nitric acid and ignite in a 30-ml porcelain crucible. Half fill the crucible with sodium cyanide, fuse cautiously until the tin is reduced to metal and finally heat for 2 min. in a full Bunsen flame to make the metal form visible beads. Extract the melt with water and wash the beads by decantation. Dissolve in 50 ml of hot (1 : 1) hydrochloric acid and 10 ml of sulphuric acid in a covered vessel (digestion for several hours at 60° C. is necessary). Filter off the small residue, re-fuse it with sodium cyanide, re-extract, and combine the two solns. and make up to 250 ml. Reduce 25-ml aliquot portions with 2 g of 20-mesh zinc in a conical flask fitted with a steam trap. When the tin is reduced to a spongy floating mass add a further 0·3 g of zinc and 12 ml of hydrochloric acid and heat gently until both tin and zinc are completely dissolved. Close the flask, cool quickly, and pour the solution into 30 ml of water containing 1 g of mercuric chloride. Allow the ppt. to settle for 30 min., filter on a Gooch crucible, wash first with 1 : 4 hydrochloric acid, and twice with hot water, and dry the ppt. for 30 min. at 105° C. (2) *Cassiterite ores*—Digest a 4-g sample with 20 ml of 1 : 1 sulphuric acid and 15 ml of hydrofluoric acid for several hours in a covered platinum dish. Evaporate to "fuming" twice, then add 40 ml of 1 : 1 nitric acid and heat until solution is complete. Transfer the soln. to a beaker, dilute to 400 ml and leave to simmer for 1 hr. Filter off the residue, fuse with cyanide once only, as described above, and make up to 100 ml. Take a 25- or 50-ml aliquot portion according to the richness of the ore and complete as in (1). (3) *Complex sulphides* (including concentrates of the tetrahedrite group containing some stannite or teallite)—Decompose a 2-g sample with nitric acid and evaporate nearly to dryness. Add 200 ml of water, digest and allow the residue to settle. Filter, wash the residue with 1 : 1 nitric acid followed by hot water and return the filter and residue to the original beaker. Destroy the paper with sulphuric and nitric acids, then transfer the soln. to a platinum dish, add hydrofluoric acid and evaporate to "fuming." Dissolve the residue in 20 ml of 1 : 4 hydrochloric acid and make up to 100 ml. Reduce a 50-ml aliquot portion with 4-5 g of zinc for 30 min., add 25 ml of hydrochloric acid and boil for 5 min. Close the flask, cool quickly and filter (to remove antimony and lead) into 15 ml of mercuric chloride soln. Wt. of mercurous chloride ppt. × 0·2514 ≡ wt. of tin (Sn). *Abstractor's Note*—The factor 0·02514, also given in the original paper, is evidently a mistake.

C. F. P.

Modified Basic Succinate Estimation of Aluminium in Magnesium Alloys. A. J. Boyle and D. F. Masser (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 621–622)—A modification of the Willard and Tang basic succinate procedure is described. The method is as accurate as the benzoate-oxine method of Stenger, Kramer and Beshgetoor (*id.*, 1942, **14**, 797) and less easily affected by impurities. One pptn. is sufficient to separate aluminium from most bivalent metals. The resulting ppt. is dense and readily filterable. *Succinate reagent*: urea, 10 g; ammonium chloride, 5 g; succinic acid, 5 g; in 300 ml. water. *Method*—Dissolve 1 g of the sample in 50 ml of 1 : 5 hydrochloric acid and filter to remove metallic copper. Add a few drops of 10% ammonium bisulphite soln. and 2 ml of phenylhydrazine to the heated filtrate (to prevent interference from ferric iron) then 300 ml of succinate reagent, and boil gently. Make the soln. just alkaline to methyl orange with freshly filtered (1 : 1) ammonium hydroxide or 5% ammonium carbonate soln. and continue boiling gently for 90 min. (no "bumping" occurs). Filter on a coarse paper and remove any ppt. which adheres to the beaker by dissolving it in 20 ml of 1 : 1 hydrochloric acid, re-pptng. with ammonia, boiling for 1 min. and pouring through the filter. Wash the ppt. 6 times, with 1% ammonium chloride soln. and ignite at 1300° C. for 1 hr. in an unglazed porcelain crucible. If more than 0·2% of silicon is present, dissolve the sample in perchloric acid, evaporate to "fuming," cool, dilute and filter before proceeding wit the basic succinate method.

C. F. P.

Determination of Rare Earths in Scheelite. J. K. Marsh (*J. Chem. Soc.*, 1943, 577–578)—Add the powdered mineral (50 g) to fused sodium hydroxide (80 g). Leach the cold melt, collect and wash the residue, dissolve it in a very slight excess of dil. nitric acid, filter off any small residue, and add sodium hydroxide soln., drop by drop, to the filtrate until a small ppt. is obtained. Collect and dissolve it in nitric acid, remove a slight hydrogen sulphide ppt. and again ppt. with sodium hydroxide. Dissolve the ppt. in nitric acid, ppt. the soln. with oxalic acid (double treatment), ignite and weigh the rare earths. The individual earths are determined in the ppt. by arc spectrography. A sample rich in rare earths (Forbes Reef, Swaziland) gave 0·05%.

W. R. S.

Separation of Europium and Samarium. J. K. Marsh (*J. Chem. Soc.*, 1943, 531–535)—Separation of samarium-rich mixtures can proceed from the mixed amalgam (ANALYST, 1942, **67**, 404) or from the mixed acetates. *Amalgam*—This was shaken in a separating funnel (closed with a Bunsen valve to maintain a hydrogen atmosphere) with hot dilute acetic acid and 0·2 ml of sulphuric acid until it was nearly exhausted; two further small additions of sulphuric acid were made during the operation, the products of which were: an orange ppt. of europous sulphate, a samarium acetate filtrate, and mercury still containing a little samarium. The ppt. contained 5% of the metals present in the amalgam and 97% of the europium; 6 mg of europium were found in the acetate filtrate, and 0·93 g of samaria containing 0·2% of europium were recovered from the mercury. The metal content of the amalgam treated was equivalent to 18 g of oxides. For the europium determination, the arc spectra of the oxides obtained from the mixed oxalates were compared with those of standard mixtures. *Acetate solutions* were treated directly with reducing agents (amalgams of zinc, barium, samarium, or sodium). Samarium acetate equivalent to 60 g of samaria in 450 ml of solution

was gently shaken with 50 ml of mercury, 0·5 ml of sulphuric acid, and 8 successive 0·4-g portions of sodium in the form of amalgam, with an intervening filtration to recover the sulphate ppt. after each treatment with amalgam. The first 4 crops contained the bulk of the europium, the last was spectroscopically free from it. Finally the mercury contained only a little samarium free from europium. The bulk of the samarium was recovered from the acetate soln. by crystallisation, yielding 54·3 g of samaria free from europium. Europous chloride is sparingly soluble in fairly strong hydrochloric acid, which ppts. it as $EuCl_2.2H_2O$; this may be obtained by the action of hydrochloric acid upon the amalgam. W. R. S

Qualitative Analysis of Mixed Halides. A. J. Jones (*Quart. J. Pharm.*, 1943, **16**, 171–178)—The scheme proposed by Benedict and Snell (*J. Amer. Chem. Soc.*, 1903, **25**, 809) is modified in such a way that the limits of accuracy are known and reliance can be placed on the indications obtained. Dissolve 50 mg of the salt in 9 ml of water, add 1 ml of glacial acetic acid and 2 drops of 1% sodium nitrite soln. and shake with carbon disulphide or carbon tetrachloride. The equiv. of 1 ml of 0·001 *N* iodine will produce a good pink colour. Iodine having been detected, dissolve 50 mg of the salt in 8 ml of water, add 1 ml of 5% potassium iodate soln. and 1 ml of glacial acetic acid and boil off the liberated iodine, reducing the vol. of the soln. to 4 ml. Add 6 ml of 10 *N* sulphuric acid and leave for 15 min. If no bromine is obvious, extract with 5 ml of chloroform, separate and shake the chloroform thoroughly with 3 ml of modified Denigès reagent. The equiv. of 1 ml of 0·001 *N* bromine gives a definite pink colour. Boil the aqueous soln. from the above separation for *ca.* 1 min. to remove all bromine, dilute to 30 ml, add 10 ml of nitric acid B.P. and 5 drops of 2% silver nitrate soln.; the equiv. of 2 ml of 0·001 *N* sodium chloride gives a distinct turbidity, while in absence of chlorine the soln. is clear. Under the above conditions silver iodate is not pptd. until the mixture has stood for some time; if necessary, the iodate can be removed by diluting the aqueous soln. from the bromine test to a convenient vol., treating 5/6 of it with 10% sodium sulphite soln. until the iodine liberated is nearly removed, and the colour of the soln. remains a full golden-yellow, adding the remaining sixth and boiling off the free iodine. The iodate is reduced thereby to a negligible quantity and the soln. can be diluted and the test for chloride applied.

Modified Denigès Reagent—Mix 40 ml of sulphuric acid B.P. with 60 ml of water and allow to cool. Dissolve 0·1 g of fuchsin in 100 ml of water. Mix the cold solns., dilute to 200 ml and leave until the colour of the mixture is golden-orange. For use, mix with an equal vol. of glacial acetic acid. J. A.

Photometric Estimation of Silicon in Magnesium and Magnesium Alloys. A. J. Boyle and V. V. Hughey (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 618–619)—The molybdenum blue colour developed by sodium sulphite reduction at *p*H 7·0–7·3 of the silicomolybdic acid complex is used, the transmission of the soln. being determined at a wavelength of about 610$m\mu$ in a Lumetron 402 E absorptiometer. Aluminium, zinc and iron are prevented from interfering by adding ammonium tartrate. *Reagents*—Ammonium molybdate, 100 g (81·0% MoO_3) per litre. Sodium sulphite, 170 g of anhyd. salt per litre. Ammonium tartrate, 400 g per litre. Reagents are prepared in paraffin-lined flasks. *Procedure*—Dissolve 2 g of metal in a quartz or Vycor beaker by dropwise addition of 50 ml of nitric acid (1 : 1), and boil for 15 min. Cool, dilute to 100 ml and take an aliquot part containing not more than 0·05 mg of silicon. Adjust the *p*H to *ca.* 4 with ammonium carbonate soln., add 5 ml of ammonium molybdate reagent and adjust the *p*H to 2·4–2·7 with *N* hydrochloric acid. (A commercial *p*H meter is used for all the *p*H adjustments.) After 2 min. add 25 ml of sodium sulphite reagent, leave for 10 min., add 10 ml of ammonium tartrate reagent and dilute to 100 ml. Leave for 50 min. and determine the transmission. If more than 0·2% of copper is present dissolve the sample in cold 6 *N* sulphuric acid, filter out the copper, boil the filtrate with 10 ml of conc. nitric acid and continue as above. Slight loss of silicon hydride results from introducing this modification. Less than 0·2% of copper and the amounts of lead, manganese, tin, titanium and nickel normally found in magnesium alloys do not interfere. Phosphate interferes, but is rarely present in sufficient concn. to cause trouble. Reasonable accuracy is claimed over the range 0·002–0·05% of Si, but it is expected that the method can be used to determine larger proportions of silicon as well. L. A. D.

Physical Methods, Apparatus, etc.

Systematic Polarographic Metal Analysis. J. J. Lingane (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 583–590)—The half-wave potentials and diffusion current constants of arsenic, antimony, bismuth, tin, lead, cadmium, zinc and copper in 9 different supporting electrolytes have been determined. These metals were studied as a group because they are so commonly associated in nonferrous alloys and other commercial products and also because, with the exception of zinc, they are usually separated as a group by hydrogen sulphide pptn. in common analytical schemes. This study forms part of a larger programme for developing systematic schemes of polarographic analysis that can be applied to the qualitative and quantitative analysis of any mixture of the commoner metallic elements. A graphical method of presenting the data on half-wave potentials is illustrated; this is of more convenience than numerical data when planning analytical procedures. A number of new facts in connection with the characteristics of waves of analytical importance have been discovered during the course of the investigation. A description is included of a method of applying diffusion current constants so as to avoid the need of calibrating each particular dropping electrode with known concns. of the substances to be determined. B. S. C.

Determination of Phenol and *m*-Cresol in Coal Distillation Products with the Spekker Absorptiometer. T. S. Harrison (*J. Soc. Chem. Ind.*, 1943, **62**, 119–123)—The analytical method prescribed by the Standardisation of Tar Products Tests Committee (No. C.C. 15–38, p. 226) is based on Chapin's colorimetric test for phenol; this depends on a red colour being formed when Millon's reagent is added to a soln. containing phenol. A similar test on a mixture of the 3 cresol homologues produces a strong yellow colour; this is shown to be due almost entirely to the *m*-isomeride. The principal objects of the investigation were to speed up the process for the extraction of phenol from creosote oils and to eliminate the personal error in

colour comparison. A satisfactory extraction treatment has been developed, using a single wash with an excess of warm 10% caustic soda soln. The photometric measurements with the absorptiometer have been investigated in detail, and a satisfactory method has been developed for the determination of phenol which is more convenient, rapid and accurate than the S.T.P.T.C. processes. A similar method is described for the determination of *m*-cresol, and a technique has been developed which will enable both phenol and *m*-cresol to be determined in the one extract. B. S. C.

Determination of the Rugosity of Granular Solids. R. H. S. Robertson and B. S. Emödi *Nature*, 1943, **152**, 539–540)—Rugosity (roughness or angularity) is defined as the ratio of the measured specific surface to the hypothetical surface area which the grains in question would have if they were all spherical. The specific surface may now be measured rapidly by the air-permeability method of Lea and Nurse (*J. Soc. Chem. Ind.*, 1939, **58**, 277); the particle size distribution by means of an accurately-calibrated set of sieves (or preferably for higher accuracy, from a frequency curve). It is shown how the coefficient of rugosity may be calculated from $ab/6\Sigma(x/y)$, where a is the measured surface area of 1 g of material (in C.G.S. units); b, the density of the material; x, the proportion of the total mass in any particular sieve fraction (*vide infra*); y, the diam. of the particles in this fraction. Further, $x = \pi.z.y^3.b./6$, where z is the number of particles in the fractions of diam. y. The method has proved of value in work on the properties of synthetic steel moulding sands (the rugosity coeffs. of which are usually 1·05–1·40) and base-exchange materials, on the flow of dry powders, and on the rheological properties of insol. powder-liquid systems. Other likely applications are indicated. J. G.

Reviews

THE METABOLISM OF FAT. By IDA SMEDLEY-MACLEAN, M.A., D.Sc., F.I.C. Pp. vi+104. London: Methuen & Co., Ltd. 1943. Price 5s.

To give a critical and balanced account of current knowledge and views on fat metabolism in the animal within the compass of a hundred small octavo pages, and withal to produce an interesting, well written and readable volume is an extremely difficult task. Dr. Smedley-MacLean has nevertheless done all of these things, and the result is a little monograph which can be read with profit either by a physiologist or by those whose knowledge is less specialised and whose interests are more general. The book contains references to communications by more than two hundred investigators, yet it is no catalogue of scientific memoirs, but a connected story built around those studies which the author has deemed pertinent to the discussion of her subject.

It would doubtless be impossible to satisfy everybody in a book of this kind. Dr. Smedley-MacLean has clearly tried to utilise all the more modern work in this field, and has been able to show where some of the earlier theories must now be discarded. Whether she has given sufficient weight to the evidence now available from what may be called the organic chemistry side of fat studies will be a matter of opinion. For instance, in the very full discussion of the possible methods of fat synthesis *in vivo*, much attention is given to the "two by two" possibilities of synthesis of long carbon chains from a C_2 unit, but the chance that oleic acid may be the most favoured primary product, although noted (p. 12), is said "not to hold," because the double bond ($\Delta 9:10$) in hexadecenoic acid is not also centrally placed. This seems to overlook the possible need to distinguish between what may be termed primary, and derived or secondary, processes in the synthesis; for the hexadecenoic acid which is almost as widespread, but by no means so abundant, as oleic acid in nature might be derived from the latter by further chemical change.

Admittedly many of the theories and speculations in this field are not directly susceptible to proof. This seems to make it more rather than less desirable to look for additional help from other than purely biochemical studies. Thus the relative amounts of such acids as oleic, palmitic, stearic, linoleic and many others which occur in different natural lipids is worthy of more consideration than has yet been accorded to it by the biochemist. What, for example, is the significance of the circumstance that in nearly all animal fats, one molecule of palmitic acid is present for, very approximately, two molecules of C_{18} (oleic and stearic) acids? Many other instances could be cited where contemplation of the specific composition of different natural fats might well give a basis for biochemical speculation at least as trustworthy as some of the supposed facts of a less purely chemical nature on which so many theories have blossomed and, too frequently, faded away as time went on. Of course, as the author stresses (p. 74), conclusions based on mere chemical constitution "must be treated with caution"—a view to which none can object provided that it is extended equally to the frequently less rigid data obtained by more strictly biochemical studies, wherein the actual facts are usually far more difficult to settle.

Probably space prevented more attention being given to the milk fats, one of the most interesting groups of animal lipids from the constitutive standpoint. They contain, for example, a whole range of Δ9:10 ethenoid acids below oleic, and it should not be forgotten that the presence of the lowest of these, decenoic acid, in butter was first indicated as long ago as 1912 by Dr. Smedley-MacLean herself.

In addition to its literary merits, the book is well produced and its price is very moderate. Anyone interested in scientific knowledge at large would profit by perusing it; those concerned in any way with fats, or with nutrition, ought not to be without it.

T. P. HILDITCH

REPORTS ON THE PROGRESS OF APPLIED CHEMISTRY. Vol. XXVII. Pp. 545. Issued by the Society of Chemical Industry, London. 1942. Price to members 7s. 6d., to non-members 20s.

A large proportion of the research in Applied Chemistry carried out during war-time must necessarily be withheld from publication; nevertheless, the interest of these familiar Reports is fully maintained with this issue. As in previous volumes, a team of specialists record the advances made in 1942 in that branch of the subject with which each is particularly associated and, even though in some instances the reports consist mainly of commentaries on published work, much of the matter being the subject of patents, they should prove to be of great interest to all engaged in the profession of chemistry.

With few exceptions, the reports include discussions of improved methods of analysis. The report headed "Foods" is particularly well supplied in this respect, containing a section on "General Food Analysis" in addition to discussions of more specialised methods in the surveys devoted to individual products. No place is accorded to the analysis of iron and steel or of non-ferrous metals in the reports dealing with these subjects; the work on the determination of essential constituents in alloys published in the year under review, to cite but one example, should surely deserve mention.

The volume is provided with a complete name-index and an adequate subject-index, and the paper and print are excellent in view of the difficulties which beset publishers at the present time. The binding is uniform with that of previous issues.

JOHN ALLEN

ANALYTICAL METHODS COMMITTEE

The following Reports may be obtained direct from the Editor of THE ANALYST, The Close, Weedon, Aylesbury, Bucks. **(not through Trade Agents)**, at the price of 1s. 6d. each to Members of the Society, 2s. each to non-Members. Remittance must accompany the order, and be made payable to "The Society of Public Analysts."

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Report No. 6. Determination of Citral in Lemon Oil.
Report No. 7. Determination of Solubilities.
Report No. 8. Determination of Cineole in Essential Oils. (2) Camphor Oil. (3) Other Oils.
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Sub-Committee on the Determination of Unsaponifiable Matter in Oils and Fats and of Unsaponified Fat in Soaps:

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Report No. 2. Determination of Unsaponified Fat in Soaps.
Report No. 3. Determination of Free Alkali in Soaps.
Report No. 4. Determination of Free Alkali and Silica in Silicated Soaps.
Report No. 5. Determination of Rosin in Soaps.

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Report No. 1. Assay of Lobelia (*Lobelia Inflata*).
Report No. 2. Assay of Gelsemium.
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THE Council has approved the following notice by the Publication Committee, which is here given in condensed form.

The Society publishes papers concerned with all aspects of analytical chemistry, inorganic and organic, as, for example, food and drugs analysis, analysis of water (including its bacteriological examination), gas analysis, metallurgical assays, biological standardisation and micro-analysis. Papers on these and allied subjects may be submitted for presentation and publication; they may:

(1) Record the results of original investigations into known methods or improvements therein;
(2) Record proposals for new methods and the investigations on which the proposals are based;
(3) Record analytical results obtained by known methods where these results, by virtue of special circumstances, such as their range or the novelty of the materials examined, make a useful addition to analytical information;
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Conciseness of expression should be aimed at; clarity is facilitated by the adoption of a logical order of presentation, with the insertion of suitable paragraph or sectional headings.

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

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

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

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

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

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

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

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

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

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