FEBRUARY, 1934 Vol. 59, No. 695

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

Deaths

WE greatly regret to have to record the deaths of the following members of the Society:

John Joseph Bryant.
John Haworth, on January 19th.
Thomas James Hutchinson.
William Marshall (Public Analyst for Stockport), on January 16th.
George Tate, on November 24th.

Obituary

WILLIAM PARTRIDGE

WILLIAM PARTRIDGE was born in Westminster, was educated at the United Westminster (now Westminster City) School, and proceeded in 1897 to Finsbury Technical College with a Holl Scholarship. After completing the 3 years' course there he became assistant to the late William Chattaway, and after Chattaway's death joined Mr. C. G. Moor in private practice, but for a good many years past he had worked independently. Jointly with Mr. Moor he held the appointments of Public Analyst for the County of Dorset and the Boroughs of Poole and Penzance, was also Public Analyst and Official Agricultural Analyst for the County Borough of Burton-on-Trent, and for the past year had been Public Analyst for the Metropolitan Borough of Fulham. He also acted as temporary Public Analyst for the County of Cornwall for nine months after the death of the late Benedict Kitto. Another of his official appointments was that of lecturer on the Analysis of Food and Drugs at the South-Western Polytechnic, Chelsea, and also, at a later date, lecturer in Chemistry (Public Health) at King's College, London.

Apart from his official work, Partridge was engaged in analytical practice, and also devoted a good deal of time to private coaching and to literary work. His books include *Aids to Bacteriology*, *A Dictionary of Bacteriological Equivalents*, and *The Bacteriological Examination of Disinfectants*, but he will be chiefly remembered in this connection as the joint-author of *Aids to the Analysis of Food and Drugs*. The revision for the 4th edition, published in 1918, was almost entirely his work.

He joined the Society of Public Analysts in 1903, and was a very active member, serving on the Council in 1917–18 and 1928–29, and as Vice-President in 1930–31. He rarely missed the meetings at Burlington House, or the informal dinners which precede them, often took part in the discussions, and contributed several papers to the Society, and very many useful and practical "Notes" that were published in the ANALYST. Nothing gave him greater pleasure during the last few years of his life than serving on the Publication Committee of our Society.

With very decided opinions, odd prejudices, nervous hesitating speech and many quaint ways, Partridge possessed "personality" in a marked degree. No one could be a more loyal friend, and it was characteristic of him that he "went all out" in helping anyone to whom he was drawn by sympathy or some common interest.

A very severe attack of influenza in 1919 seriously affected the health of one who was never robust. It was noticed that he was not present at the meeting of the Society on December 6th of last year, and it came as a great shock to his many friends to learn that he had been taken ill on that day and had passed away on the 11th December, in his 53rd year. He leaves a widow, but had no children.

ERNEST M. HAWKINS

THE CHEMICAL (AS DISTINCT FROM PHYSIOLOGICAL) TESTS FOR VITAMINS

The following papers were contributed to the discussion on this subject at the meeting on November 1st, 1933:*

The Chemical Evaluation of the Vitamins

BY A. L. BACHARACH, M.A., F.I.C., AND
E. LESTER SMITH, D.Sc., F.I.C.

THE limitations set to the subject of this paper present an interesting paradox when the history of vitamin research is considered. In descending order of importance the methods that have been used for elucidating the properties of the vitamins, considered as a group, are the biological, the physical, and the chemical. Yet it is not intended here to consider biological methods at all, and physical methods will be discussed only in so far as they bear upon those that are more specifically chemical. We have thus to direct our attention to those properties that have been so far of least importance in advancing our knowledge.

That very paradox, however, carries with it considerable grounds for optimism. The fact that analysts are to-day able to discuss the chemistry of the vitamins suggests that we have advanced a very considerable distance along the road to their isolation and complete chemical identification.

* For discussion see p. 93 *et. seq.*

There is a sense in which all biological tests are chemical, and all chemical tests biological. Every biological test itself depends upon a reaction, or a series of reactions, sometimes bewilderingly complex, taking place in the body of the experimental animal; a chemical test, whether gravimetric, volumetric, or colorimetric, must depend upon the observations of a chemist, and the chemist himself becomes an experimental animal used in the test.

It will be necessary, therefore, to draw distinctions that have a practical use rather than a philosophic basis; this point may be illustrated with a particular example.

It is well known that the antimony trichloride reaction with substances containing vitamin *A* can be refined by measuring not the colour itself produced with antimony trichloride, but the two spectral bands that give rise to the colour. This refinement of the "chemical" blue test is generally regarded as a spectroscopic test, that is to say, a physical one. The direct spectroscopic test for vitamin *A*, whereby, without the use of any chemical reagents other than solvents, we measure the intensity of an absorption band at $328m\mu$ invariably shown by products containing vitamin *A*, is clearly a non-chemical test, differing from the antimony trichloride test in that no reagents are used. In these notes neither it nor the spectroscopic modification of the antimony trichloride test will be described further, and this limitation is made for the purely practical reason that spectrographic or spectrophotometric technique is not available at present to many analysts.

Before summarising briefly our present knowledge of the chemical composition of the various vitamins it may be of use to make one or two general observations on the analytical procedure that must be adopted for their determination.

We have to deal here with the estimation, in the most favourable cases with the determination, of substances that may legitimately be described as "trace" substances, however fundamental their importance to human and animal health. From the point of view of the analyst, the estimation of a vitamin in a food, natural or reinforced, is a problem of the same nature as the estimation of some of those metals that have also been proved essential to life, though they are present at very low concentrations in most natural products. The determination of any main constituent of food—fat, protein, carbohydrate, mineral matter, and water—is carried out either without actually separating it completely from the other constituents, or after methods of separation that actually break down completely the other constituents. Reduction methods for determining sugars in complex solutions and the ordinary Kjeldahl nitrogen determination illustrate these procedures.

Such methods are inapplicable to the determination of trace substances. With rare exceptions it is not merely necessary to separate the trace substance wholly or partly from the main constituents, but it is necessary to do so by processes sufficiently mild to prevent the destruction of the substance being determined. The analyst's style is, in a sense, severely cramped in these circumstances, although the problems they present have led to some of the most beautiful of all analytical methods.

The determination even of such substances as the alkaloids, many of which give characteristic, if not completely specific, colour reactions, involves at least

a partial separation from natural sources, though the alkaloidal content of plants is, in terms of weight, far higher than the content of vitamins, apart from a few exceptional cases. It is also a permissible generalisation to say that, the more we leave estimation and approach determination of trace substances, the more specific are the chemical reactions involved, and the more closely must we approach actual isolation of the substance to be evaluated.

It is, therefore, to be expected that our progress towards the successful chemical determination of vitamins in foods and other products, will go side by side with a general advance in methods for their isolation. In what follows, the vitamins will not be treated in alphabetical, that is, in chronological, order, but divided, according to the conventional classification, into water-soluble and fat-soluble. No reference will be made to the physiological properties that constitute the ultimate basis for their characterisation, but their composition, as far as it is known, is stated below, as well as their approximate concentration in certain sources. These facts are given partly for convenience of reference and partly to illustrate the general observations made above.

VITAMIN *B* (B_1, B_2, etc.).—The most probable formula for vitamin B_1 appears to be

$$C_{12}H_{20}O_2N_4S \quad or \quad C_{12}H_{18}O_2N_4S.$$

It is certainly a basic substance, though not a primary amine, and it seems now to be generally agreed that sulphur is present in the molecule, and is split off when the vitamin is heated with alkali. The occurrence of a ring system is highly probable, but there is little more that can be said definitely about its chemical nature save that it is relatively labile to oxidation at p_H values above 7, and relatively stable in acid solution.

APPROXIMATE CONCENTRATION OF VITAMIN B_1 IN TYPICAL SOURCES

	Parts per 10^6
Dried yeast	16
Wheat germ	5
Fresh yeast	4

Recent work by Kuhn and his associates (*J. Soc. Chem. Ind.*, 1933, **52**, 981) indicates that vitamin B_2 is probably identical with a crystalline orange-brown fluorescent pigment, lactoflavine, that can be isolated from milk and whey (see Booher, *J. Biol. Chem.*, 1933, **102**, 39). Ovoflavine, present in egg-white, appears to be the same substance. The most probable formula is $C_{17}H_{20}O_6N_4$.

Four of the oxygen atoms are in hydroxyl groups that can be acetylated. Two of the nitrogen atoms are in the grouping –NH–CO–NH–, probably belonging to a ring system. The other nitrogen atoms seem to be tertiary and correlated with the conjugated double bonds that determine the colour.

APPROXIMATE CONCENTRATION OF VITAMIN B_2 IN TYPICAL SOURCES

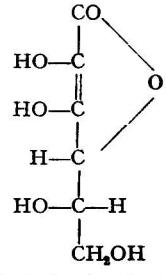
	Parts per 10^6
Liver or kidney	20–40
Yeast (fresh)	15
Muscle	2·5–5
Egg-white	1·5–3
Cow's milk	1·2–1·5

Of the remaining vitamins of the *B* group, B_3, B_4, B_5, and substance "*Y*" (Chick and Copping, *Biochem. J.*, 1930, **24**, 1764), practically nothing is known

save the rather close association of vitamin B_4 with adenine; it is, however, doubtful whether the association is in any way chemical. All that has been shown is that separation of adenine and vitamin B_4 from natural sources is accomplished by the same method.

VITAMIN *C* can now be identified with a sugar acid known as ascorbic acid, and having the formula

ASCORBIC ACID (VITAMIN *C*)



3-keto-*l*-gulonolactone
$C_6H_8O_6$

Contrary to expectation, it is the first vitamin not merely to have assigned to it a definite molecular formula, but actually to be synthesised by purely chemical means (Ault, Baird, Carrington, Haworth, Herbert, Hirst, Percival, Smith, and Stacey, *J. Chem. Soc.*, 1933, 1419).

APPROXIMATE CONCENTRATION OF VITAMIN *C* (ASCORBIC ACID) IN TYPICAL SOURCES

	Parts per 10^6
Paprika (fresh)	2000
Lemon-juice and orange-juice	500 to 750
Potato (fresh)	150
Milk (best)	25

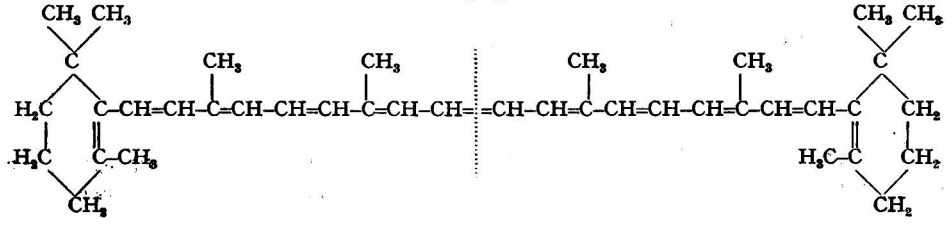
FAT-SOLUBLE VITAMINS.—Of the fat-soluble vitamins, under which heading for convenience we include carotene, vitamin *E* can be briefly dismissed, since little more can be said of its composition except that it appears to be closely related, as are so many substances of physiological activity, with the sterols.

APPROXIMATE CONCENTRATION OF VITAMIN *E* IN TYPICAL SOURCES

	Parts per 10^6
Evans's concentrate assumed ..	10^6
Wheat germ oil	< 2500
Wheat germ	< 250
Lettuce leaf	< 250

To β-carotene has been assigned the formula

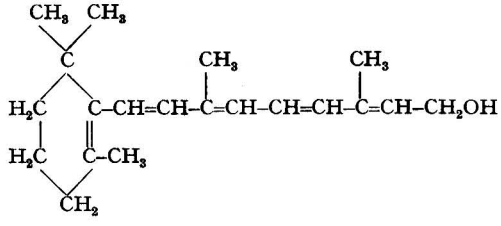
β-CAROTENE
$C_{40}H_{56}$



α-Carotene differs from this in that the positions of the double bonds are changed so that the molecule now has an asymmetric carbon atom. It is very likely that vitamin *A* is a partial oxidation product of half the carotene molecule, having the formula

VITAMIN *A*

$C_{20}H_{30}O$



The close association of carotene with vitamin *A* is inferred partly from the fact that the conversion of the latter into the former has been abundantly proved to take place in animals of several species, and is held to be so converted by the human organism, and partly from chemical work on degradation products of pure carotene and of highly purified concentrates of vitamin *A*. Both α- and β-carotenes have vitamin *A* activity, but the former only half that of the latter.

APPROXIMATE CONCENTRATION OF VITAMIN *A* IN TYPICAL SOURCES

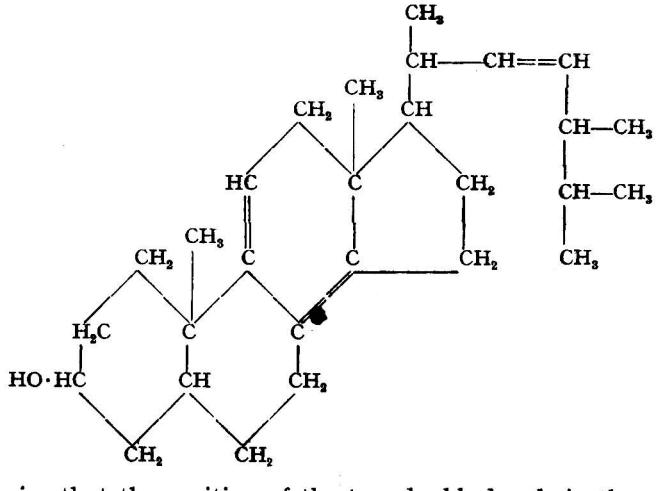
	Parts per 10^6
Various concentrates	2×10^5 to $9 \cdot 5 \times 10^5$
Halibut liver oil	10000 to 15000
Cod-liver oil	100 to 500
Butter-fat (best)	*5
Milk (best)	*0·2

* Assuming no activity due to carotene.

VITAMIN *D* is an isomer of ergosterol whose formula is

ERGOSTEROL (ANHYDROUS)

$C_{28}H_{44}O$



with the proviso that the position of the two double bonds in the ring system is not finally established. The nature of the isomeric change that takes place when

ergosterol is converted to calciferol, to intermediate products and to photochemical degradation products is largely unknown. In certain respects calciferol differs from other sterols, particularly in the fact that it does not form an insoluble digitonide.

APPROXIMATE CONCENTRATION OF VITAMIN *D* (CALCIFEROL) IN TYPICAL SOURCES

	Parts per 10^6
Irradiated ergosterol..	2×10^5
C.L.O. concentrate (Zucker) ..	2500 to 5000
Cod-liver oil	2 to 8
Butter (best) ..	0·08
Milk	0·003

CHEMICAL REACTIONS.—We may now consider briefly the chemical reactions that have been used, or suggested, as bases for the estimation or determination of the vitamins. It is possible to pass very briefly over most of these, as they appear to have little practical value at the moment.

A suggested chemical test for vitamin B_1 in rice polishings and concentrates from that source has been described by Spruyt (*Mededeel. Dienst Volksgezondheid Nederland.-Indië*, 1930, **19**, 46; *C.A.*, 1930, **24**, 5802; *Chem. Weekblad*, 1930, **27**, 298; ANALYST, 1930, **55**, 460). After removal of possible interfering substances with norit and salicylic acid, the vitamin solution is treated with phosphotungstic acid, and the resultant precipitate reduced with zinc and hydrochloric acid, the intensity of the brown colour so produced being, it is claimed, a function of the vitamin B_1 content.

Apparently no work on this test has been carried out in this country, but it has been used by certain investigators in India (Acton, Ghosh and Dutt, *Ind. J. Med. Res.*, 1933, 103), who appear to find it to be of some value and to give results not inconsistent with biological assays. Attempts by us to apply it to active yeast extracts failed, partly, no doubt, because of the inadequacy of the description given in Spruyt's original paper.

There appear to have been no proposals for chemical tests for any other vitamins of the *B* complex; in view of our scanty knowledge of their chemistry, this is hardly surprising.

Vitamin *C* is a strongly reducing substance, and is therefore capable of being titrated with a number of oxidising reagents, such as iodine and certain dye-stuffs, notably 2.6-dichlorophenolindophenol. The iodine test is frequently used for estimating the degree of purity of samples of pure ascorbic acid, and is made the basis of routine control for the concentration of orange juice in certain Californian factories. Its main disadvantage is, of course, its lack of specificity; but it would seem to be useful, not only for such control purposes as the one mentioned, but also as a confirmatory test to that with the indophenol indicator. The last-mentioned was due originally to Tillmans, and has been rendered more specific by a modification due to Birch, Harris and Ray (*Biochem. J.*, 1933, **27**, 590). Though probably the most valuable test for vitamin *C* yet devised, it still leaves something to be desired in the way of specificity, partly because certain other substances undoubtedly behave like ascorbic acid (Birch, Harris and Ray, *loc. cit.*), when the test is carried out under the best conditions so far devised, and

partly because some foodstuffs appear to contain substances that interfere with the reduction of the indicator (Svirbely, *Biochem. J.*, 1933, **27**, 960).*

The so-called Beszonoff test for vitamin *C* must also be mentioned, in passing, if only for historical reasons. This test was some years ago the subject of rather violent controversy, but there is little doubt that it is a not very specific test for phenolic substances; our present knowledge of the constitution of vitamin *C* makes it quite clear that its apparent validity in some circumstances can have been due only to coincidence (*cf.* ANALYST, 1921, **46**, 462).

For vitamin *E* there is, naturally, no chemical test whatever available.

For vitamin *D* no chemical test is available, except in the assay of one material, and one that is not, strictly speaking, a natural product. Since calciferol is produced by the method worked out at the National Institute for Medical Research, consisting in the treatment of ergosterol-free irradiated ergosterol with *m*-dinitrobenzoyl chloride, it should be possible to make use of this reaction for estimating the amount of vitamin *D* present in irradiated ergosterol. This can, in fact, be done, provided the irradiated material is sufficiently rich in calciferol, and provided a particle of the dinitrobenzoate is available, either in the hands of the analyst or in the air, for inoculation purposes. After complete removal of unchanged ergosterol from the irradiated material the methods recommended by the workers at the Institute can be followed almost exactly, and the separated dinitrobenzoate can be weighed, and its degree of purity quite accurately established by observation of its appearance and determinations of melting-point and optical rotation. We think that this procedure can legitimately be described as a genuine chemical assay, though it suffers from the considerable disadvantage that maximum separation of calciferyl dinitrobenzoate takes at best several days. Even so, there is almost always to be found by biological assay some residual antirachitic activity in the mother liquors. Nevertheless, it is satisfactory to be able to report that a number of biological assays, both by Dr. K. H. Coward at the Pharmaceutical Society's Laboratories and by ourselves, have shown very good agreement indeed with chemical assays carried out on the same material. For example, a recent assay on irradiated ergosterol gave the following results:

Biological assay:	
(Pharmaceutical Society):	$14{\cdot}5 \times 10^6$ units per grm.
(Glaxo Research Laboratory):	$13{\cdot}0 \times 10^6$ units per grm.
Chemical assay:	$12{\cdot}3 \times 10^6$ units per grm.*
(*Residual resin, probably contains calciferyl dinitrobenzoate equivalent to about	1×10^6 units per grm.)

Not only is this method difficult to apply to relatively low grade specimens of irradiated ergosterol, but so far it has been found entirely inapplicable to any natural source of vitamin *D*. Indeed, some recent experimental work has been interpreted to signify that there are in nature at least two other antirachitic substances differing from the synthetic calciferol. The work of Nussmeyer and

* See also an important paper by Bessey and King (*J. Biol. Chem.*, 1933, **103**, 687; ANALYST, 1934, 122).

Massengale (*J. Biol. Chem.*, 1930, **87**, 415, 423), of Steenbock, Kletzien and Halpin (*J. Biol. Chem.*, 1932, **97**, 249), of De Vaney, Munsell and Titus (*Poultry Science*, 1933, **12**, 215), of Bethke, Record and Kennard (*J. Nutrition*, 1933, **6**, 413), of Ender (*Zeits. f. Vitaminforsch.*, 1933, **2**, 241), and of Hess and Supplee (*Proc. Soc. Exp. Biol. Med.*, 1930, **27**, 609) on the one hand, and the work at Reading on the other (Kon and Booth, *Biochem. J.*, 1933, **27**, 1302), have been considered by some to indicate that the vitamin *D* in cod-liver oil and the vitamin *D* in butter-fat differ not only from each other but also from calciferol. At any rate, calciferol has never, so far, been identified in natural sources, though its antirachitic potency for human subjects has been definitely established by clinical observations (Spence, *Lancet*, 1933, Oct. 21st, 911).

There is no inherent reason why a substance with such definite properties as calciferol should not lend itself to chemical estimation. It is highly desirable that means for determining this substance should be worked out, for it is the least abundant vitamin in natural foodstuffs at the present day, and the one in which most people's diet is most likely to be deficient. It is also the only vitamin that has been added on any extensive scale to inactive foodstuffs, and means for establishing its presence and estimating its amount, other than biologically, are not unnaturally desired by Public Analysts and others.

BLUE TEST FOR VITAMIN *A*.—The so-called blue test for vitamin *A* will now be considered in rather more detail than the other tests so far surveyed. The reasons for this are two: first, of all the tests mentioned it is certainly the one that has been most extensively studied, and is most extensively used, both in biochemical research and in manufacturing routine; secondly, it is the one of which we, in our laboratories, have perhaps the most experience. We have indeed contributed something, we hope, to improving the technique of the test (Smith and Hazley, *Biochem. J.*, 1930, **24**, 1942). A very useful bibliography of the literature on the subject was given recently in this journal (ANALYST, 1932, **57**, 306).

It is hardly necessary to do more than mention, in passing, that the test is based upon the observation, made several years ago, that many cod-liver oils give a blue or purple colour with dehydrating agents like concentrated sulphuric acid. The most important step in advance of this was the discovery by Rosenheim and Drummond that, of all such reagents, arsenic trichloride gave the most intense and the least fleeting colour. A further modification, now well-known as the Carr-Price test, consisted in the replacement of arsenic trichloride by antimony trichloride, with a slight reduction in the sensitiveness of the test but a great increase in its usefulness.

Many papers have been published showing that the vitamin *A* content of cod-liver oil and other natural substances, biologically determined, is not simply correlated with the blue value of the oil determined by the Carr–Price technique. It is because of this that the Foreword to the 1932 British Pharmacopoeia is careful to disavow any intention that the limit colour test for cod-liver oil should be taken to indicate a limit for vitamin *A* content, or, indeed, to have any connection with it. Most workers in this field would now agree that the disclaimer in the Pharmacopoeia has to-day become over-cautious, consequent upon our increased knowledge.

We now know sufficient about this test to be able to make certain definite statements about it.

(i) In the vast majority of cases a high blue value indicates a high vitamin *A* content.

(ii) In the vast majority of cases a low blue value indicates a low vitamin *A* content.

(iii) The absence of blue value means the absence of more than a trace of vitamin *A*.

(iv) The intensity of blue given by a cod-liver oil is not in direct proportion to its concentration in the solutions used for the antimony trichloride test.

(v) The main reason why the colour intensity-concentration curve is not a straight line is the presence, in the saponifiable part of oils and fats, of substances inhibiting the production of the blue colour.

(vi) This fact explains why rich sources of vitamin *A* show a more nearly linear relationship of concentration and colour intensity than poor sources; a smaller weight of the substance is taken with rich than with poor sources, so that smaller amounts of the inhibiting substances are present in the test solution.

(vii) A preliminary concentration of the vitamin *A*-containing fraction of any natural product will increase the accuracy of the test, that is to say, will increase the closeness of its correlation with the content of vitamin *A*, biologically determined.

(viii) The most important method of concentration adopted for this purpose is the separation from the fatty constituents of the unsaponifiable matter, which should contain the whole of any vitamin *A* present if the process has been carried out with the necessary precautions.

(ix) There is some evidence from direct ultra-violet spectroscopy that a certain amount of vitamin *A* is lost even under the conditions laid down by Smith and Hazley (*loc. cit.*). The increased blue value obtained after saponification may well be the resultant of two separate effects, increased colour intensity due to removal of certain inhibitors and loss of vitamin during saponification. (See also Evers, and Crews and Cox, ANALYST, this issue, pp. 82, 85.)

(x) Vitamin *A* in fish liver oils is present mainly in an esterified form (Bacharach and Smith, *Quart. J. Pharm.*, 1928, **1**, 539), insoluble in ethyl alcohol; the vitamin itself is a primary alcohol, and is completely miscible with ethyl alcohol. It might be thought that the lack of linearity between concentration and colour intensity was a property of the ester as against the alcohol, but we have satisfied ourselves experimentally that this is not the case.

The importance of the different solubilities of the esterified and the unesterified forms of the vitamin lies in the fact that it is possible to extract an oil with alcohol without removing appreciable quantities of its vitamin *A*. Chevallier has shown that the ultra-violet absorption at 328$m\mu$ is affected by the presence of free fatty acids (Chevallier and

Chabre, *Biochem. J.*, 1933, **27**, 298), so that it is necessary to carry out an extraction with ethyl alcohol on oils of high acidity before their spectroscopic examination. It has not been shown that such a procedure effects any improvement in the colour test, but the point may be worth investigating. It would, of course, be necessary to remove all traces of ethyl alcohol from an oil so treated, since alcohol is known to affect the stability of the blue colour produced with antimony trichloride. On the whole, separation of the unsaponifiable matter from oils has the advantage of removing free acid and most other interfering substances, and takes no more time than the direct test, if this has to be carried out in such a way as to enable a dilution curve to be prepared.

(xi) The reaction (that is the intensity and stability of the colour produced) is influenced by a number of factors, and the conditions for the test laid down in the 1932 Pharmacopoeia have been devised to standardise these factors as far as possible. Provided the Pharmacopoeial conditions are observed, the same observer using the same apparatus can obtain results of a considerable degree of reproducibility.

(xii) Spectroscopic examination of the two bands, whose combined effect is measured as a blue value on the tintometer, broadly substantiates the conclusions just stated, with an important exception.

(xiii) The relative intensities of the two bands have been found to differ for materials that apparently possess the same blue value. It has also been observed that the relative intensities of the two bands alter with increasing concentration of the vitamin *A* from a given natural source; in other words, that the increasing intensity of each band does not run *pari passu* with the increasing vitamin concentration as determined biologically and by direct spectrophotometry on the material.

(xiv) Certain substances, such as indole and skatole and their derivatives, have the property of greatly diminishing the intensity of one band ($617m\mu$) with only slight effect on the other ($583m\mu$).

(xv) Several of the limitations of the test are, in fact, inherent in the use of the tintometer rather than in the application of the reaction as such. Thus it has been shown by many workers that other substances produce blue or similar colours with antimony trichloride (*cf.* Levine and Richman, *J. Biol. Chem.*, 1933, **101**, 373); the reaction appears to be associated with the presence of conjugated double bonds. Very few of the colours produced, however, are likely to be mistaken for that due to vitamin *A*, since most of them differ either in shade or in stability. In no case has spectroscopic examination of the colours, where it has been carried out, revealed bands at 617 and $583m\mu$, except with known sources of vitamin *A*. It is, therefore, the tintometric as against the spectroscopic modification of the test that limits its specificity in this respect.

(xvi) A factor limiting the usefulness of the colour test is that, for purely physiological reasons, the tintometer, used almost exclusively for

measuring blue values, has a rather limited range of accuracy. Below 4 and above 10 we have found it hardly possible, and below 2 and above 14 quite impossible, to obtain consistent readings, even on the same solution, owing to matching difficulties; this makes the colour-concentration curves liable to considerable error at low and high concentration values. It is, therefore, necessary, when applying the test to oils and concentrates appreciably richer in vitamin *A* than low-grade cod-liver oils, to modify the B.P. technique, if it is to give quantitative results, and not merely to be used in a limit test, for which it was devised.

(xvii) The blue glasses used in the tintometer transmit light that is spectrally different from that transmitted by the blue colour produced in the test. It is probably for this reason that different observers often do not match the same solution with absolutely identical glasses. There are indications that, where personal variations occur, they are constant and additive. Mr. R. T. M. Haines, of the Crookes Laboratories, tells us that he can reproduce the values of other observers that normally differ from his, provided he knows what they call a "match"; it becomes simply a question of adding or subtracting a fairly constant figure from his own match. This point would also repay further investigation.

If the difference in reading between observers is caused by their different sensitivity to the two spectral bands produced, it is justifiable to say that again the tintometer rather than the reaction limits the utility of the test.

(xviii) A further point of difficulty arises in the use of the neutral glasses supplied in the tintometer for dulling colours that are too bright to be matched. Every neutral glass in the tintometer sets consists of superimposed red, blue and yellow glasses, each standardised to contain the same number of units of colour. There has been some controversy on the advisability and legitimacy of deducting the amount of blue in the neutral glasses used from the total blue colour shown by a solution on matching. Two quite different levels of vitamin *A* may be attributed by different observers to a very bright solution, according to whether the blue share of the neutral glass is or is not deducted from the total blue units. The standard practice at present seems to be against deducting the blue value of the neutral glasses, but we are still unconvinced of the logic of this practice. A recent paper by Morgan (*Biochem. J.*, 1932, **26**, 377) describes a modified technique of computing vitamin *A* potency from the values of the blue and yellow glasses used in the match, and was itself criticised in a note by one of us (Smith, *J. Soc. Chem. Ind.*, 1932, **51**, 1042). The matter should be regarded as still *sub judice*. The difficulty, of course, does not arise if the blue colour is estimated spectroscopically.

We have attempted above to set out as completely as possible all the salient facts about the nature of the reaction of vitamin *A* with antimony trichloride and the technique used in measuring "blue values." There is, further, some indication that certain oils have rather anomalous physical and chromogenic properties,

and it would seem at present undesirable in important cases to rely solely upon physical and chemical criteria, if it is a question of comparing one oil or concentrate with another. Such anomalous behaviour as has so far been reported or experienced by ourselves is invariably in the direction of diminishing the apparent vitamin *A* content of an oil as determined by the colour test, whether on the oil itself or on its unsaponifiable matter. Exhibitors of the blue colour, as distinct from inhibitors, do not appear to be of importance, except in so far as other substances giving a blue colour may be present in a source of vitamin *A*. Even carotene, which, perhaps, of all substances most nearly simulates the behaviour of vitamin *A* when treated with antimony trichloride, can be distinguished from it spectroscopically; the absorption bands produced by carotene and antimony trichloride are different from those produced by vitamin *A* and antimony trichloride; further, the blue colour produced with carotene is relatively permanent.

The difference in physical and chemical properties between carotene and vitamin *A* has considerable analytical value, for it enables a distinction of importance from the point of view of dietetics to be drawn between the two sources of vitamin *A* activity in a substance like milk-fat, where both are present in amounts of the same order. Several different procedures have been recommended, and have in general led to similar results, as a means of discriminating between the two substances. The carotene can be estimated directly by means of the absorption coefficient of its characteristic bands in the visible spectrum; the total "apparent" vitamin *A* content can be estimated either biologically, or by means of the antimony trichloride test, and the amount of "apparent" vitamin *A* actually due to carotene deducted from the result of biological or physico-chemical test. Alternatively, the carotene can be removed by adsorption, and the amount of vitamin *A* determined by estimation of the band at 328$m\mu$, which cannot be done in the presence of carotene, as this possesses an overlapping band in this region of the ultra-violet. Methods of this kind have been adopted by Gillam, Heilbron, Morton, Bishop and Drummond (*Biochem. J.*, 1933, **27**, 878), by Baumann and Steenbock (*J. Biol. Chem.*, 1933, **101**, 547), and by Booth, Kon, Dann and Moore (*Biochem. J.*, 1933, **27**, 1189).

It will be clear that there are still many pitfalls awaiting the unwary user of the antimony trichloride colour test. Nevertheless, it can be truly said that it is difficult to exaggerate its importance and value, especially to all those who have applied the test to the preparation and standardisation of vitamin *A* concentrates; they will surely agree that without it difficulties of manufacture and control would have been enormously increased, and this could only finally express itself in an increased price to the public of standardised vitamin *A* preparations. It is possible now to use the blue test, suitably adapted, as a routine process in the control of factory operations and to have recourse to the spectrophotometer and the rat for final and more specific confirmation of results obtained with the blue test. A similar procedure is now possible, in certain circumstances, in the large-scale concentration of vitamin *C*, but we are not yet in so fortunate a position in the manufacture and control of any other vitamin.

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Notes on the Determination of Vitamin *A*

By NORMAN EVERS, B.Sc., F.I.C.

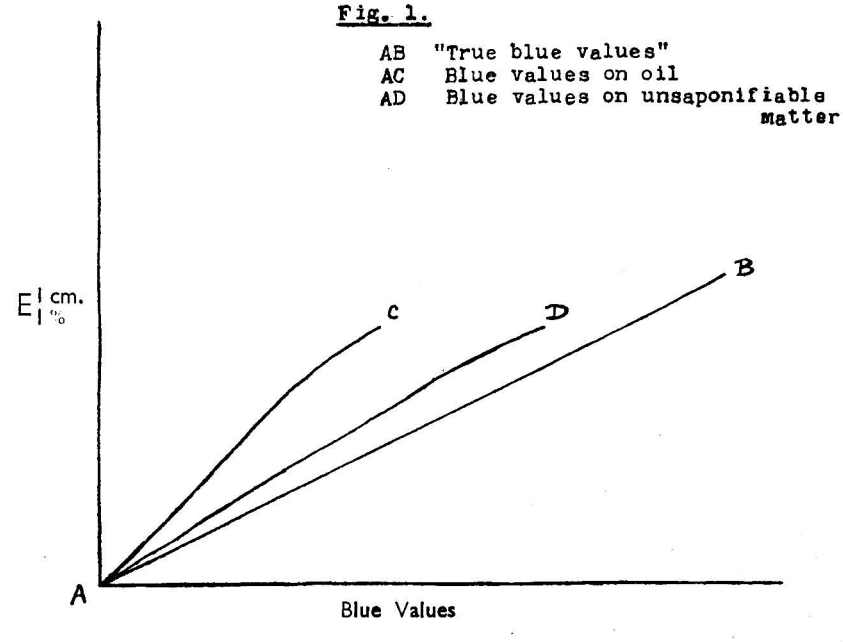
I. Cod-liver Oil. The Relationship between the "Blue Value" obtained on the Original Oil, the "Blue Value" obtained on the Unsaponifiable Matter and the "True Blue Value" of Cod-liver Oil

With reference to the technique of the antimony trichloride colour test, it seems desirable to emphasise the necessity of adjusting the concentration of the oil solution so that the final reading in the tintometer is taken at 5 blue units. Serious differences may be caused by taking readings even at 4 or 6 blue units.

Dyer (Analyst, 1933, **58**, 709) has reported on a series of 39 samples of cod-liver oil on which he determined the blue values of the original oils and of the unsaponifiable matter extracted by the method of Smith and Hazley (Analyst, 1931, **56**, 265). At the same time Wilfred Smith and the author (*Quart. J. Pharm.*, 1933, **6**, 329) published a graph for a series of cod-liver oils showing the relationship of the blue values determined on the original oils and the intensity of absorption at 328$m\mu$.

Fig. 1.

AB "True blue values"
AC Blue values on oil
AD Blue values on unsaponifiable matter



If we regard the blue value of pure vitamin *A* as 80,000 and the intensity of absorption at 328$m\mu$ as $E^{1\,cm.}_{1\%} = 1600$ (Carr and Jewell, *Nature*, 1933, **132**, 92), we can express this as a linear relationship graphically by the line AB in Fig. 1. Assuming that the value of $E^{1\,cm.}_{1\%}$ at 328$m\mu$ is a quantitative measure of vitamin *A*, it is possible to calculate by means of this graph the "true blue value" of an oil from the value of $E^{1\,cm.}_{1\%}$ 328$m\mu$.

These values have been calculated for a series of 48 samples of cod-liver oil. The results are expressed in the following table:

TABLE I

Oil	$E^{1\,cm.}_{1\%}$ 328$m\mu$	Blue value on original oil (x)	"True blue value" (y)	Ratio $y : x$
1	0·31	7·2	15·2	2·111
2	0·40	7·4	19·6	2·649
3	0·43	8·6	21·0	2·442
4	0·42	9·0	20·2	2·244
5	0·59	4·4	28·3	2·482
6	0·53	12·7	25·7	2·024
7	0·84	13·2	40·6	3·076
8	0·71	13·4	34·0	2·537
9	0·73	13·4	35·2	2·627
10	0·77	13·6	37·4	2·750
11	0·75	14·0	36·0	2·571
12	0·67	14·4	32·4	2·250
13	0·75	14·4	36·4	2·528
14	0·67	14·7	32·4	2·204
15	0·71	15·1	34·4	2·278
16	0·82	16·0	39·4	2·463
17	0·93	16·0	45·2	2·825
18	0·67	16·1	32·4	2·012
19	0·91	16·8	44·0	2·620
20	0·63	17·0	30·5	1·794
21	0·71	19·0	34·6	1·821
22	0·89	19·4	43·0	2·216
23	0·93	21·5	45·3	2·107
24	0·77	21·5	37·0	1·721
25	0·83	22·0	40·2	1·827
26	1·37	22·8	67·4	2·956
27	1·08	23·0	52·4	2·278
28	0·91	24·0	44·1	1·837
29	1·06	24·0	52·0	2·167
30	1·11	24·0	54·2	2·258
31	1·17	24·0	57·4	2·392
32	1·17	25·0	57·6	2·304
33	1·05	26·5	51·4	1·940
34	1·28	26·5	63·0	2·377
35	1·16	27·2	56·8	2·088
36	1·38	27·5	68·2	2·480
37	1·17	28·0	57·0	2·036
38	1·05	28·2	51·4	1·823
39	1·17	28·6	57·4	2·007
40	1·22	29·0	59·6	2·055
41	1·25	31·5	61·6	1·956
42	1·55	31·6	77·2	2·439
43	1·29	32·0	63·4	1·981
44	1·18	33·0	58·0	1·758
45	1·42	37·8	71·0	1·875
46	1·53	44·0	76·2	1·732
47	1·31	48·0	64·6	1·345
48	1·37	48·0	68·0	1·417

The mean value of log y—log x for this series was found to be 0·33645 or log 2·17. Hence, on the average, the "true blue value" of the oil is 217 per cent. of the blue value determined on the oil itself. Applying the same method, Dyer (*loc. cit.*) found that for his 39 oils the blue value of the oil when determined on the unsaponifiable matter was on the average 161·5 per cent. of the blue value determined on the oil itself. Hence it appears that the blue value determined on the unsaponifiable matter is intermediate between the blue value determined on the oil itself and the "true blue value." From this it may be concluded that Smith

and Hazley's method does not remove the whole of the material which is responsible for the reduction of the antimony trichloride colour.

Another point which emerges from these results is that there is a tendency for the ratio $y : x$ to become less for oils of high blue value, *i.e.* these values more nearly approach "true blue values." This is to be expected, since oils of very high blue value such as halibut liver oils lie on or near the line AB in the graph. Dyer (*loc. cit.*) found that the ratio of the blue value determined on the unsaponifiable matter to the value determined on the oil was on an average the same for oils of high and low blue value. Hence blue values determined on the unsaponifiable matter also approach nearer to "true blue values" for oils of high blue value.

II. A PLEA FOR GREATER UNIFORMITY IN EXPRESSING RESULTS FOR VITAMIN *A*.

Of the numerous methods of expressing results for vitamin *A*, the following list includes the more important, but cannot be said to be exhaustive:

(*a*) *Biological Units.*—(i) The *carotene unit*, which has been officially adopted as the international unit of vitamin *A*. Owing to difficulties in obtaining uniform results with carotene, this standard has not yet come into practical use.

(ii) The *U.S.P. Biological Unit.*—These units are usually meant when "biological units" are mentioned.

(*b*) *Units based on Chemical or Physical Tests.*—(i) The "blue value" determined on the oil itself (B.P. method).

(ii) The "blue value" determined by the method of Drummond and Hilditch (*Empire Marketing Board Report*, ANALYST, **1931**, **56**, **533**), which gives values about one-half of those given by (i).

(iii) The "blue value" determined on the unsaponifiable matter.

(iv) The number of "blue units" per mgrm. of oil.

(v) The intensity of absorption at $328m\mu$ expressed as $E\,{}^{1\,cm.}_{1\,\%}$.

(vi) The percentage of vitamin *A* calculated from $E\,{}^{1\,cm.}_{1\,\%}$ at $328m\mu$, regarding this value for pure vitamin *A* as 1600.

In addition to these, there is a method much to be deprecated, *viz.* the multiplication of the blue value by an arbitrary factor to give "biological units." I have even encountered an extension of this method in the use of a second factor to convert the "blue value" into units of vitamin *D*!

It is easy to imagine the confusion of the buyer of cod-liver oil when he is faced with this variety of expressions. Needless to say, there is a tendency for the seller to use the system of units which gives the most impressive figure.

Having regard to the simplicity and rapidity of the physical or chemical tests, and to the fact that they are at least equal to the biological test in accuracy, it seems desirable that, at any rate for commercial purposes, one of these methods should be generally adopted. The spectrographic method has the disadvantage of requiring a special apparatus, which, even at the greatly reduced cost of the simplified form exhibited by Professor Drummond, is still a serious item of expenditure.

The "blue value" when determined on the unsaponifiable matter has been shown by Coward, Dyer and Morton (*Biochem. J.*, 1932, **26**, 1593; ANALYST, 1932, **57**, 368) to give values which agree better with the biological values than the "blue value" determined on the original oil.

In spite of this, it appears doubtful whether the trouble of preparing the unsaponifiable matter is really worth while. The graph in the paper by the author and Wilfred Smith (*Quart. J. Pharm.*, 1933, **6**, 329) shows that the blue value determined on the oil does give an approximate idea of the vitamin *A* content of the oil, and one that is sufficiently accurate for most commercial purposes. Further, there is the fact that it is based on the method described in the British Pharmacopoeia.

Taking all things into consideration, there seems to be little reason with ordinary commercial oils to go beyond the blue value determined on the oil itself by the B.P. test suitably adapted, except in special cases where greater accuracy is desirable.

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The Relationship between the Carr-Price Value and the 328$m\mu$ Absorption Coefficient of Preparations containing Vitamin *A*

BY SYDNEY K. CREWS, A.I.C., AND STANLEY J. COX

SINCE 1926 it has been the practice in these laboratories to examine all products containing vitamin *A* for their chromogen-content by the Carr–Price antimony trichloride reaction,[1] and during this period many hundreds of determinations have been made annually. The information thus afforded has proved a valuable aid in making progress which would have been impossible in so short a space of time had the biological method of feeding tests been the sole means of evaluation.

Much work has been published recently in this country by Heilbron, Morton, Gillam and others[2,3,4] dealing with the selective absorption shown in the ultra-violet region of the spectrum by products containing vitamin *A*, and also with the differentiation of chromogenic substances by the spectroscopic examination of the blue colour produced by the antimony trichloride reagent.[5] Good evidence has been adduced to show that vitamin *A* activity is due to only one of these chromogens, namely that with an absorption of 328$m\mu$ in the ultra-violet. We have, therefore, examined our vitamin *A* products by spectrophotometry as well as by determination of chromogen content by the Carr–Price method.

The antimony trichloride test for cod-liver oil adopted in the B.P. 1932 is essentially the Carr–Price test. The quantity of oil taken for the B.P. test is

0·04 grm.; the original test was on 0·04 c.c. The antimony trichloride reagent is a saturated solution of antimony trichloride in dry alcohol-free chloroform; the original reagent was a saturated solution in B.P. chloroform containing a small amount of absolute alcohol. The colour produced on addition of the reagent to the chloroformic solution of the oil is matched in a glass cell having a square cross-section of 1 cm. side; in the original test a tube or cell of 8 mm. across was used.

For some considerable time before the publication of the B.P. 1932, we, in conjunction with other workers in this line, had been using the square-sectioned cell of 1 cm. side, and also had used dry alcohol-free chloroform for preparing the reagent. Throughout all our experiments, we have used the Lovibond Tintometer for measuring the intensity of the blue colour produced in the reaction. We have always matched the colour at the point of maximum intensity, and used red or yellow glasses, or both, where necessary, in addition to blue glasses, in order to obtain an exact colour match.

The details of the Carr–Price test are now fairly well known, but one or two points in the actual working may be of interest. It has been found that the best results are obtained when the actual tintometer readings are between 4·0 and 6·0 of blue, the observation being made at the moment of maximum colour intensity and not at the end of any time period. Either daylight or artificial daylight may be used, but if the latter, then a difference of 0·1 blue must be distinctly visible. The Tintometer Company make an attachment for their instrument which we find very suitable. The use of a specially designed automatic pipette for the antimony trichloride reagent is a great advantage, as this solution is rather corrosive, besides being hygroscopic. Another point is that one should not look down the eyepiece of the tube of the tintometer when the reagent is being poured into the cell, or the deeper blue produced by the first few drops of the reagent will make the final match more difficult.

The use of each eye alternately is an advantage, as ocular fatigue is thus minimised. In the final match about three seconds is the maximum time to look at the colours; after this the eye becomes less sensitive and good matching is impossible.

When these conditions are observed, there is no difficulty in obtaining concordant results by various workers, even in different laboratories.

The B.P. 1932 test is only a limit test to prevent the use of cod-liver oils containing less than a minimum of chromogenic substances, and the conditions laid down for the test are well defined. The test as described in the Pharmacopoeia, however, is not suitable for the evaluation of oils of higher chromogen-content or for vitamin *A* concentrates. For these, the concentration of the chloroform solution of the oil should be such that 0·2 ml. mixed with 2 ml. of the reagent produces a colour lying between 4·0 and 6·0 Lovibond blue. The result is then calculated to the proportionate colour for 0·04 grm. Owing to the curve of colour production, the colour calculated in this way will be quite different from the actual colour produced by 0·04 grm. in the quantities of chloroform and reagent used in the test.

While a comparison of the blue value with the results of spectro-photometric and biological tests shows considerable variation with cod-liver oils, this does not

apply to purified preparations of vitamin *A*. Remarkable changes occur in many oils on keeping. A very freshly prepared oil may have at first a negligible blue value which then rises, at first quickly, then more gradually, to a peak value, and, finally, with age, generally falls again. Both the rise and the fall may be accelerated by certain oxidising agents.

A determination made on the unsaponifiable fraction of a liver oil by the Smith and Hazley method (*Biochem. J.*, 1930, **24**, 1942; ANALYST, 1931, **56**, 265) usually gives a higher figure than one made directly, and affords a truer indication of the vitamin *A* content. We have occasionally examined oils which give even as much as three times the blue value by this method as by a direct determination on the original oil, and, judging by our spectrographic work on concentrates, we are inclined to think that even after hydrolysis the vitamin-content indicated by the antimony trichloride method may still be too low. For example, one of the oils recorded gave a Carr-Price value of 10·0, which increased to 30·0 on the unsaponifiable fraction, yet the E figure showed a value of 1·0, which in a purer concentrate would correspond with a blue value of about 50·0. There are, therefore, inhibiting substances present in the oil which cause the low blue value, and these substances are, in a large measure, removed by hydrolysis.

Although other chromogenic substances which give a blue colour by the Carr–Price test are often present in cod-liver oils, we have never found an oil of which the blue value is above what may be expected from the absorption at 328$m\mu$. The explanation appears to be that the inhibiting substances present cause a greater depression of the blue colour than any increase due to chromogenic substances other than vitamin *A*. It follows that the figures given by the Carr–Price method indicate the minimum content of vitamin *A*.

The absorption at 328$m\mu$ in the ultra-violet corresponds with that given at 572–584$m\mu$ by the blue compound resulting in the antimony trichloride reaction. The absorption at 328$m\mu$ can be determined with greater accuracy than that at 572 to 584$m\mu$, owing to the instability of the antimony trichloride blue substance, and has the additional advantage of more readily providing a permanent photographic record. In the present paper this value has been correlated with the blue value of many vitamin *A* products derived from various sources—fish-liver oils, mammalian-liver oils, concentrates, etc. It was pointed out by Morton that the spectrographic absorption in concentrates is less than would be expected from colorimetric determinations of the blue value, as compared with cod-liver oil, and our results obtained during the routine examination of a large number of samples confirm this, as will be seen from the tabulated results on p. 89.

APPARATUS.—The apparatus employed consists of the latest type of Bellingham and Stanley ultra-violet quartz spectrograph fitted with a B. and S. short-focus rotating sector photometer mounted directly on the instrument.

Illumination is effected by a condensed spark between tungsten steel electrodes 4 mm. apart. A voltage of 10,000 was obtained from 105 volt A.C. mains by the use of suitable transformers and a condenser. The current for driving the motor rotating the sector photometer was also taken directly from the mains by the use of suitable resistances.

The spectrograms were recorded on either plates or flat cut films (10×4 in.) with H. and D. speed of about 700. We have found either Imperial Eclipse plates or Barnet portrait films to be particularly suitable.

The determination is made by taking a series of pairs of photographs of the spectrum, each pair consisting of one spectrogram taken through a solution of the substance and another taken through the same thickness of the solvent. By means of a sector photometer consisting of two rotating shutters, one of which is adjustable for the size of opening, the time of exposure of the individual halves of each pair of spectrograms is varied so that the relative exposures through the solvent are decreased quantitatively. In this way points can be found on each pair of photographs where the density of the two halves is the same, showing that at these points the intensities of the two beams of light, multiplied by the relative exposures, are equal. As the ratio of the exposures decreases, the points of equal density of the spectrograms become closer together, until at the head of the band they become coincident. This point is used for the calculation of the "extinction coefficient," E, which is the logarithm of the ratio of the intensity of the transmitted light to the incident light.

After a series of exposures has been made through a number of pre-determined openings of the sector, the development, fixation and drying are carried out in the usual way. The negatives are inspected on a suitably illuminated viewing box, and the "cross over" points are marked, and from these the "extinction coefficients" are calculated.

In the case of vitamin *A* preparations, it is usual to calculate to a 1 per cent. concentration and a 1-cm. cell, and to specify the particular wave-length or band at which absorption has taken place. This is usually expressed as $E^{1\,cm.}_{1\%}\cdot 328m\mu$.

The solutions used were adjusted to concentrations such that readings of approximately between 0·8 and 1·2 were obtained for a 1-cm. quartz cell.

A small correction for the absorption due to substances other than vitamin *A* may be made if required, but in the Table given on p. 89 this has been omitted, as its value is very small.

The solvents used for these experiments were absolute alcohol and cyclohexane, which had been specially purified for spectrographic work, and showed no appreciable U.V. absorption above 220$m\mu$. The concentrations varied from 1·0 per cent. with oils to 0·0006 per cent. with vitamin *A* preparations of high potency.

CAROTENE AND VITAMIN *A*.—Spectrophotometry has confirmed the results recorded by others who have shown that the ultra-violet absorption of Vitamin *A* is not due to carotene or to similar colouring matters. A spectrogram of a sample of pure carotene B.D.H. shows that absorption occurs in a totally different part of the spectrum from that given by vitamin *A*, namely, at about 450$m\mu$, which is in the visible region, and that the absorption in the ultra-violet region is negligible.

A comparison of the curve with those obtained for products containing vitamin *A* shows that in all cases the peak of the curve for vitamin *A* apparently lies between 325 and 330$m\mu$, although it may be rather flat.

VITAMIN *D*.—The curves obtained for vitamin D ("calciferol," with a potency between 40,000 and 45,000 units per mgrm.) have their peak at 265 to 270$m\mu$.

TABLE I

Product	Blue value	Blue value on unsap. fraction	$E^{1\,cm.}_{1\%}$ 328 mμ.	B.V. / E	B.V. unsap. / $E^{1\,cm.}_{1\%}$ 328 mμ.
"Cattle" oil	0·6		0·3	2	
Cod-liver „	1·6		0·35	4·57	
„ „	7·6	12·0	0·4	19	30
Medicinal cod-liver oil ..	7·2	15·0	0·65	11	23·07
„ „ „ ..	11·2		0·7	16	
„ „ „ ..	12·0		0·75	16	
„ „ „ ..	10·8		0·8	13·5	
„ „ „ ..	8·8	18·8	0·8	11	23·5
„ „ „ ..	8·0		0·8	10	
„ „ „ ..	9·2	32·0	0·85	10·8	37·65
„ „ „ ..	12·0		0·9	13	
„ „ „	20·0		0·9	22	
„ „ „ ..	20·0		0·95	21	
„ „ „ ..	17·6	20·0	1·0	17·6	20
„ „ „ ..	10·0	30·0	1·0	10	30
„ „ „ ..	13·2		1·1	12	
„ „ „ ..	22·0	30·0	1·1	20	27·3
„ „ „ ..	28·0		1·2	23	
„ „ „ ..	28·0	40·0	1·2	23·3	33·3
„ „ „ ..	13·2	22·5	1·2	11	18·9
„ „ „ ..	20·0		1·4	14·2	
„ „ „ ..	28·0		1·4	20	
„ „ „ ..	32·0	46·0	1·6	20	28·75
Med. cod-liver oil	20·0		1·7	12	
„ „ „	32·8		1·7	19	
Dog-fish liver oil	60·0		1·9	32	
Coml. fish-liver oil	66·0		2·4	27·5	
„ „ „	110		3·3	33	
„ „ „	200		5·5	36	
„ „ „	197		6·0	33	
„ „ „	570		10·8	53	
Vitamin *A* concentrate ..	530		8·0	66·25	
„ „ ..	550		12·0	45·8	
„ „ ..	720		16·0	45	
„ „ ..	900		18·0	50	
„ „ ..	1000		20·0	50	
„ „ ..	1400		28·0	50	
„ „ ..	2000		44·0	45·45	
„ „ ..	2900		60·0	48·3	
„ „ ..	3000		45·0	66·7	
„ „ ..	4800		100	48	
„ „ ..	5760		94	61	
„ „ ..	7000		146	48	
„ „ ..	10000		180	55	
„ „ ..	1050		28·0	39	
„ „ ..	1400		28	50	
„ „ ..	2000		44	44	
„ „ ..	3000		37·5	80	
„ „ ..	3000		45	66·7	
„ „ ..	4800		100	48	
„ „ ..	5760		94	61	
„ „ ..	7000		146	50	
„ „ ..	7000		141	50	
„ „ ..	10000		180	55	
„ „ ..	11000		225	50	
„ „ ..	22000		480	46	
„ „ ..	27000		550	49	
„ „ ..	40000		800	50	
„ „ ..	50000		1100	49	
„ „ ..	57000		1160	49	
„ „ ..	63000		1128	55	
„ „ ..	76000		1360	56	
„ „ ..	76000		1440	52·8	
„ „ ..	80000		1600	50	

TABLE II

VITAMIN *A* CONCENTRATES

Mean ratio of blue components of colour to yellow and brightness

Strength of concentrate	Number of samples	Blue	Yellow	Brightness
100–500	16	1	0·305	0·10
500–1000	27	1	0·32	0·12
1000–2000	14	1	0·33	0·13
2000–10000	9	1	0·34	0·16
10000–50000	26	1	0·34	0·17
50000–80000	39	1	0·35	0·19

CONCENTRATES.—As will be seen from Table I, the B.V./E ratio becomes much more constant as the B.V. increases. An interesting point which has emerged from the determination of the chromogenic contents of concentrates is the difference in the colour analysis of the blue compound after the addition of the antimony trichloride. With oils, a pure blue or violet blue is possible, but with concentrates the colour is a bright greenish-blue.

Table II gives the colour analysis for the colour produced with various concentrates with Carr–Price values ranging from 100 to 80,000, from which it will be seen that for the highest concentrate examined the colour produced was of the order 1·0 blue + 0·35 yellow+0·19 brightness.

From a consideration of the ratio B.V./E it will be seen that:

(*a*) With cod-liver oils of blue value up to 20 the figure usually lies between 12 and 20, but when the value is determined on the unsaponifiable fraction the figure shows a marked increase, and is about 30.

(*b*) With cod-liver oils of blue value up to 60 the values approach 30.

(*c*) With high value commercial fish liver oils up to 600 the figure is in the neighbourhood of 50.

(*d*) Concentrates gave a value of approximately 50.

The authors wish to thank Dr. F. H. Carr for his help and advice throughout this work, and the Directors of The British Drug Houses, Ltd., for permission to publish this note.

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The Titration of Vitamin *C* in Citrus Juices

By A. H. BENNETT, B.Sc.

(*Read at the Meeting November 1st, 1933*)

The citrus fruits differ from most others in the fact that their valuable dietetic qualities are contained almost entirely in the juice; and since this juice is readily expressed and can be preserved for long periods in a palatable state, a large part of the public consumption is in this form. Of these qualities, the anti-scorbutic power is among the most important, and it is of interest, therefore, to ascertain the extent of natural variation of this power, and the degree in which it remains undiminished in the preserved juice, and to determine the conditions most favourable to its conservation.

For this purpose a titration method which can show the state of the juice from day to day is evidently the most convenient, and the method of Tillmans—titration with a solution of 2·6-dichlorophenol-indophenol—has been applied to the examination of a considerable number of samples of Sicilian lemon and orange juices, both freshly-prepared from the fruit and preserved by the usual commercial methods.

The process used and the results obtained are described elsewhere (*Biochem. Journal*, 1933, **27**, 1294; Analyst, 1934, 52), but it may be added that since that paper was submitted for publication the modified method of standardising the indicator solution, proposed by Tillmans, Hirsch and Vaubel (1933), has been employed.

Twenty-five ml. of the indicator solution (approximately $N/1000$) are titrated in presence of 5 ml. of saturated sodium oxalate solution, with a $N/500$ solution of ferrous ammonium sulphate containing $N/250$ sulphuric acid, until no colour remains.

The results agree with those obtained by the use of titanous chloride, and the process is more convenient, owing to the extreme instability of the titanous chloride solution.

The juices examined, whether extracted in the laboratory or in the factory, did not contain enough iron to affect the titrations, but it is, of course, possible that some commercial samples might contain appreciable quantities of ferrous iron, and this should be borne in mind. The iron present can be determined colorimetrically and a correction applied.

The indicator solution is itself unstable, even in the buffered solution described. If it is kept for more than a few days some change takes place which causes a red-violet tint during the titration, and makes the end-point obscure.

Natural Variation.—In earlier work on vitamin *C* by the biological method it was generally assumed that the antiscorbutic potency of freshly prepared lemon or orange juice was constant, and, in fact, the international unit is defined as being the value of 0·1 ml. of lemon juice.

Since crystallised ascorbic acid has been available for experiment, doubt has been cast on this assumption. Key and Morgan (*Biochem. J.*, 1933, **27**, 1030), in a series of experiments comparing the effects of different doses of ascorbic acid and of lemon juice, found considerable variation in the relations between the two, and their value for ascorbic acid was about half that found by Szent-Györgyi. They consider it probable that the variation was in the lemon juice used as a standard.

Nelson and Mottern (*Ind. Eng. Chem.*, 1933, **25**, 216) found that in the series of experiments there described, 1·5 ml. of the orange juice employed had given a much lower degree of protection than had been obtained in some earlier trials. These experimenters give the results of the titration of their juice with dichlorophenol-indophenol, and their figures (6·6 to 7·1 ml. of $N/1000$ indicator solution to 1 ml. of juice) are considerably lower than those found for Sicilian orange juice of the season 1932–33 (10·3 to 11·8), and appreciably below the average for lemon juice of the same period.

Conn and Johnson (*Ind. Eng. Chem.*, 1933, **25**, 218) make similar observations on the apparent variability of antiscorbutic potency.

These biological observations are, therefore, in agreement with the results of titration, which, as reported in the above-mentioned paper, show that the highest results obtained are double the lowest, and that, even excluding extremes, a variation of 25 per cent. is common.

The laboratory-pressed juice was usually made from lemons within a few hours of picking. There were some indications that the titration value of the juice was higher when the lemons were stored for some weeks before squeezing. This, however, requires confirmation with much larger numbers of fruit before any conclusions can be drawn.

PRESERVATION IN THE JUICE.—The results obtained with juices preserved in any of the usual ways showed that the effective prevention of fermentation in the juice was accompanied in all cases by a continual diminution of reducing power, ending in its total disappearance after a few weeks. These experiments were made with comparatively small volumes of juice kept at the laboratory temperature; and, since there is reason to think that the effect depends on the inhibition of a protective agency followed by atmospheric oxidation, it is probable that the rate of loss will vary considerably in different circumstances, and may very likely be lower when large volumes of juice are stored.

It was indicated in our earlier paper that, when air is excluded, the life of the reducing factor in sterilised juice is considerably prolonged. These experiments have been continued.

Lemon juice, filtered with the aid of asbestos and kieselguhr, was placed in a series of Thunberg tubes and pasteurised for an hour at 65° C. The air was exhausted by a pump and the tubes were closed.

The original titration value of the filtered juice was 6·9 ml. of indicator solution per 1 ml. of juice.

The tubes were opened at intervals, and, after 35 days, the value was 5·1 ml., whereas juice pasteurised and left exposed to air (in a tube with a stopper of cotton-wool) lost its reducing power completely in 12 days.

A similar experiment was made in which the juice, instead of being pasteurised, was treated with sulphur dioxide (0·3 grm. per litre). The original value of 7·3 was reduced to 5·0 after 56 days, and to 4·3 after 110 days, whilst, when there was access of air, the reducing power was completely lost in 26 days.

It seems, therefore, that the exclusion of air greatly retards, but does not altogether prevent, the disappearance of the reducing factor in sterilised or preserved juices when kept at room temperature. No experiments have yet been made on the effect of cold storage, which, from the biological point of view, has been investigated by several American workers.

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DISCUSSION ON THE CHEMICAL TESTS FOR VITAMINS

Dr. Leslie J. Harris said that he would like first to make two points with regard to the antimony trichloride test for vitamin *A*: (1) For an accurate quantitative estimation of the vitamin *A* content of a foodstuff by this method it was essential to saponify first, and make the test on the unsaponifiable residue. Various interfering substances were found in varying amounts in different materials, and caused seriously misleading results unless this precaution was taken to get rid of their effect. Thus the B.P. test for cod-liver oil was not an accurate quantitative method at all, but only a qualitative, or "limit" test; it enabled one to say if a given specimen was "up to scratch" or not, but one could not certify accurately from it the exact quantitative extent of the deficiency. (2) The second point was that in carrying out the antimony trichloride test it was simpler and as, or more, accurate, to take the simple *blue value* in the tintometer, rather than trouble to measure the intensity of either of the absorption bands, since these might fluctuate considerably at the expense of one another or otherwise. One further point was the manner of expressing the results. It seemed preferable to express results as so many "blue units" rather than as a "blue value," as was sometimes done. After all, the latter was only a ratio, and it was much less clumsy to express vitamin *A* results as one would any other analytical results: *i.e.* not as a ratio, but as so many units present in the sample as a whole, or per grm. of the material. This method of expressing results was the one employed by his colleagues at Cambridge, and they were glad to see that it was gradually coming into more frequent use elsewhere. Recent publications from the Nutritional Laboratory, by Dr. Thomas Moore and Mr. Alan Davies, described the technical details of the quite simple procedure adopted there, by which it was easily possible to make analyses of vitamin *A* on as many as four or five specimens per hour, in routine tests. This method was used by them for estimating the vitamin *A* reserves of humans by analysing specimens of liver, *post-mortem*, and several hundred such routine tests had been made in the course of the past year or so.

Another chemical, or physico-chemical test for vitamin *A* was the measurement of the ultra-violet absorption, but, in agreement with what Mr. Bacharach had said, he (Dr. Harris) thought that this was only suitable for use for research purposes and not as a general routine test.

For vitamin *D* (the other principal fat-soluble vitamin) no chemical test of general application to foodstuffs was yet available. For measuring the vitamin *D*

(calciferol) content of concentrated medicinal solutions, however, several possibilities offered themselves, of which the most useful and accurate perhaps seemed the measurement of ultra-violet absorption intensity at the specific band.

Coming to the water-soluble vitamins, there were no satisfactory purely chemical tests yet for vitamin B_1. He did not think there would be much delay, however, before one was elaborated, and there was already a semi-chemical test described by Peters and his co-workers, the principle of which was to measure the amount of oxidation induced in a substrate of pigeon brain upon the addition of the vitamin preparation. This was almost a pure chemical test, except for the fact that one of the "reagents" was minced brain tissue of an avitaminous pigeon. Another test of a purely chemical nature, but not truly specific for the vitamin, had been applied for some years past in determining whether a given cereal preparation, such as rice grain, had enough of the germ left in it to render it a good source of the vitamin. This was the phosphoric acid (P_2O_5) determination. This was claimed to give some rough guide to the extent of milling, and, therefore, to the vitamin value; but it was only a makeshift, and he could not claim personal experience of it himself.

For some years past they had been using at the Nutritional Laboratory a quantitative test for vitamin B_1 which, although it was in fact biological, was quite as simple as any chemical test. The principle was to determine the effect on the heart-rate of a deficient rat, and by this simple means an accurately quantitative estimate could be made in the course of a few hours.

Dr. Harris then dealt with the method of estimating vitamin *C* by titration with indophenol. Tracing the historical origin of this test, he said that it started at the Public Analyst's office at Frankfurt-on-Main, where fresh and natural fruit juices had to be distinguished from stale or artificial ones. It was found that this could be done by means of the indophenol reduction value, and after a year or two it was gradually realised that there was considerable parallelism between the reducing value of fresh foods and their vitamin *C* activity. Dr. Harris said that their own quantitative method was based on the reduction of Tillmans' indicator (2–6 dichlorophenol-indophenol), which they had applied in such a way as to make it more specific for the vitamin and suitable for application to various animal, as well as vegetable tissues. The first stage in the process was simply extraction with trichloroacetic acid; this was necessary in order to separate it from the interfering substances present in certain materials, and also to break up cell structure and so get the vitamin suitably into solution. The second stage was to run the acid extract into a measured volume of indicator solution until its colour vanished. By thus working in acid solution they ruled out the interference of such substances as glutathione, which was present in most of the animal extracts rich in vitamin *C* and would otherwise cause serious error, with readings as high as twice or three times the true value. (In a few simple cases, such as lemon juice, this preliminary addition of trichloroacetic acid was not essential, and they might even get a sharper end-point by working in somewhat less acid solution.) The sensitivity of the method was such that when working with materials of the activity, say, of orange juice, they obtained duplicate results agreeing within less than 3 per cent., and with as little as 0·03 ml. of juice for the titration. This micro amount should be compared with the quantity needed for a biological test (at least 400 ml. all told); and the chemical test took only as many seconds to carry out as the biological did days.

As to the specificity of the method, it could be said that it was accurate and quite specific when applied to foodstuffs and vitamin carriers as ordinarily met with; with certain unusual types of material, however, there were one or two special cases where interference might be found. The most important of these, perhaps, was malt extract (Bacharach), which contained a sugar-like reducing substance, and similar products were formed by boiling sugars in slightly alkaline solutions. In dealing with stale or autolysed materials a control test for free cysteine should be made by

the Sullivan colour reaction. Tumour tissues might also contain a reducing substance other than the vitamin. But, apart from these one or two special cases, the method was sufficiently specific to have proved its value in a variety of different directions. They had applied it to some forty different natural products and had obtained excellent agreement between the vitamin *C* value thereby calculated from the *ascorbic acid* content and that determined biologically.

The three principal uses of this vitamin *C* titration method seemed to be: (i) for preliminary tests, (ii) for exploratory work (detecting the vitamin in new sources), and (iii) for controlling works operations and concentration processes. In dealing with novel or unusual types of materials it was certainly always advisable to check the results by a biological test. One way, especially, in which the test had been useful was in demonstrating the presence of vitamin *C* in hitherto unsuspected sites, *e.g.* in the suprarenal medulla and cortex, in the lens of the eye, etc.; they had found that rat's suprarenal was amazingly active—about ten times as active as orange juice. All these findings, due to the chemical test, had been confirmed by subsequent biological assays.

The chemical test was also now being used for such routine "works" operations as testing the potency of lemon or orange juice and controlling the manufacture of the evaporated juice; for detecting the loss occurring on canning, preservation and storage; for checking the preparation of active vitamin concentrates; and so forth. It had had interesting results when applied to milk, the vitamin *C* content of which was found to fluctuate in a most erratic way, owing largely, it appeared, to the result of exposure to light (Kon). Again, the test had been very useful in determining the effect of pasteurisation on the antiscorbutic value of milk: traces of copper in the plant were found to have an intensely destructive action. A final illustration was the application of the test to urine. He had found that after a meal containing much vitamin *C* the amount in the urine might rise as high as ten times the normal, and this method they believed had applications, which he was now examining, for diagnostic purposes.

Professor J. C. DRUMMOND directed attention to certain defects in the B.P. procedure for the antimony trichloride test. If the influence of dilution were neglected, abnormal results were obtained. The colours should be matched at the period of maximum intensity, and trustworthy results could be obtained only with the unsaponifiable matter. There was a close correlation between the blue tintometer readings and the spectroscopic test, especially with halibut-liver oils.

Dr. R. J. MCWALTER demonstrated the construction of the Hilger "Vitameter" and the use of the $328m\mu$ spectrographic band in the estimation of vitamin *A*.

Mr. F. K. DONOVAN referred to the disappearance of vitamin *C* from citrus juices in presence of sulphurous acid. Comparative experiments were being made in Italy, and Mr. Bennett had found that not only did sulphur dioxide prevent the retention of the reducing characteristics associated with antiscorbutic activity, but also that any other preservative which was successful in preventing fermentation entailed a similar loss. Thus, anything that preserved the juice seemed to entail the more or less speedy loss of vitamin *C*.

Dr. KATHARINE COWARD said that by the use of the International Standard for vitamin *A* it was hoped to discover how nearly alike could be the estimations of the vitamin *A* content of one sample of cod-liver oil carried out in the laboratories of different workers. She hoped to publish these results in due course. She thought Mr. Bacharach had made the outlook for the chemical and physical estimations of vitamin *A* in cod-liver oil rather too rosy. She could heartily subscribe to his numerous points, and thought that any one who had followed Mr. Bacharach's analysis of the tests would be able to believe that the comparison of oils by biological tests in various laboratories agreed much better than the comparisons

of the same oils by chemical and physical methods in the same laboratories. She, therefore, still pinned her faith to the biological tests.

Mr. E. HINKS referred to the difficulty of the relation between the blue units of the Lovibond instrument and the National Physical Laboratory specification. They were informed by Tintometer Ltd. (ANALYST, 1932, **57**, 772), that the limit recommended corresponded to Lovibond blue glass No. 6·0, but it was unfortunate that the relationship was not designated in the Pharmacopoeia. With regard to vitamin *C*, he had observed, when reading Mr. Bennett's paper, that the vitamin-content of preserved lemon juice rapidly diminished: after periods of from ten days to three weeks the titration method showed no vitamin at all. This seemed to be of great significance.

Dr. KON said that he had had very little experience with the estimation of vitamin *A* in cod-liver oil, but had done some work on the vitamin *A* of butters, and had come very definitely to the conclusion that any estimation of vitamin *A* by colour tests on the butter itself was quite impossible. At Reading they had found it was only possible to test the unsaponifiable residue from the butter. They had found that the inhibitor varied according to the season, being present to a larger extent in summer than in winter; therefore, the blue colour test on the butter showed a summer vitamin *A* content lower than the winter value.

The PRESIDENT was sure that all would agree that they had been given valuable information regarding the identification of vitamins. The difficulties surrounding chemical research had been emphasised; not only were the quantities of vitamins in foods extremely small, but quantitative determinations appeared to be almost prevented by reason of other substances present. It was to be hoped that research on chemical tests would continue to be prosecuted. It had been demonstrated that a very considerable amount of research work had been and was being carried out by chemists attached to manufacturing firms, and this should be widely known.

A Modified Micro-Method of Determining Methoxyl and Ethoxyl Groups

BY HOMI RUTTONJI NANJI, PH.D., D.I.C., A.I.C.

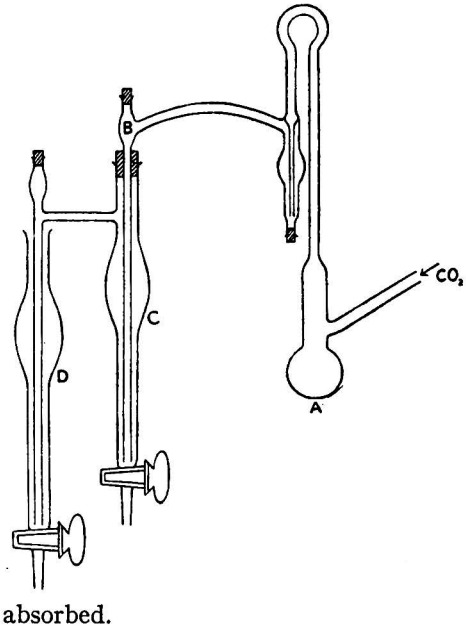
COOL (ANALYST, 1932, **57**, 585) recently worked out a method of determining small amounts of ethyl iodide as an indirect determination of cardiac output in man. It consisted in treating the ethyl iodide vapours with chlorine or bromine, whereby the iodide was immediately oxidised to iodate (*cf.* Dumas and Stas, *Ann. Chem. u. Pharm.*, 1840, **35**, 162; Dupré and Dupré, *ibid.*, 1855, **94**, 165; Friedel, *ibid.*, 1865, **135**, 126; Weszelszky, *Z. anal. Chem.*, 1900, **39**, 81). The excess of halogen was removed with phenol water, and the iodine, liberated from potassium iodide by the iodate, was determined by titration with sodium thiosulphate.

It was realised that the same principle could be applied with certain modifications in the determination of alkyloxyl groups, which are of such wide-spread occurrence in plant products.

Vieböck and Schwappach and Vieböck and Brecher (*Ber.*, 1930, **63**[B], 2818, 3207) worked out a volumetric iodimetric method on the same principle. They oxidised the methyl iodide by using a solution of bromine in acetic acid solution,

and destroyed the excess of bromine by concentrated formic acid. This method was slightly modified by Clarke (ANALYST, 1932, **57**, 402). The method afforded little saving of time compared with the old Zeisel method, the limitations of which are given in detail by both the above authors, and may be briefly stated here:—(i) It takes up a considerable time, (ii) The method has to be completely modified if sulphur or phosphorus is present. Further, the various reagents used by Vieböck and Schwappach gave a fairly high blank, and the use of formic acid for removing excess of bromine was not found satisfactory.

The above objections are overcome in the method described below. The principle is that employed by Cool (*loc. cit.*), *viz.* oxidation of alkyl iodide (obtained by heating the alkyloxyl-compound with hydriodic acid) to iodate with bromine water,* removal of excess of halogen by phenol, liberation of an equivalent of iodine from potassium iodide, and titration of the iodine with 0·01 *N* sodium thiosulphate solution.



The apparatus employed for the method is a modification of the old Zeisel apparatus. It consists of an ordinary micro-Zeisel distilling flask (A) described by Pregl (*Quantitative Organic Micro-Analysis*, p. 181 *et seq.*), to which are attached two absorption vessels (C and D) of the form shown in the figure. It is essential that the diameter of the absorption vessels should be such that the leading tube (B) of the distilling flask just goes in. By this means the bubbles of gas are flattened out and more readily absorbed.

The *reagents* required are:—(i) Hydriodic acid, sp.gr. 1·70. (ii) A suspension of purified red phosphorus. (iii) A saturated solution of bromine water; it is advisable to make a fresh solution about every fortnight. (iv) Phenol-water mixture 10 per cent.; this solution keeps indefinitely. (v) Freshly prepared 20 per cent. potassium iodide solution. (vi) A standard (*N*/100) solution of sodium thiosulphate; this may be prepared from time to time by dilution of *N*/10 standard thiosulphate.

ANALYTICAL PROCEDURE.—A few crystals of phenol and 1·5 ml. of hydriodic acid are placed in the distilling bulb, and the washer is filled with phosphorus suspension. About 3 to 4 mgrms. of the dried substance are weighed accurately in a tin-foil cup on a micro-balance and pushed into the distilling flask through the side tube, and the apparatus is connected with a Kipp generator of carbon dioxide. The receivers, containing 4 ml. of bromine water in (C) and 6 ml. in (D), are

* The following equations, given by Clarke, represent the course of the reaction:

$$CH_3I + Br_2 \rightarrow CH_3Br + IBr$$
$$IBr + 2Br_2 + 3H_2O \rightarrow HIO_3 + 5HBr$$

connected in position. A stream of carbon dioxide gas at the rate of 2 bubbles per second is passed through the apparatus. It is important to regulate the rate of flow of gas very carefully, especially at the beginning; otherwise low results may be obtained. The flask is then gently heated by a micro-burner; when the tin-foil has dissolved and the evolution of hydrogen has ceased, the hydriodic acid is heated briskly for a minute, then kept just warm for about 5 minutes, then again heated just to boiling point, and kept warm for a further 5 minutes; this operation is repeated a second time. It is quite unnecessary to keep the acid boiling the whole time. From about 50 determinations it has been observed that the reaction is complete in less than 15 minutes.*

The methyl iodide vapours are completely absorbed in the two receivers. Experiments with one receiver, even with double the column of bromine water, gave low results, showing that the methyl iodide vapours were not completely absorbed in one receiver.

The liquid in both the receivers is then run down through the tap into an Erlenmeyer flask, and the receivers are rinsed two or three times with sufficient water. Ten ml. of well-shaken phenol water† are then added all at once. After two or three minutes, 5 ml. of freshly-prepared potassium iodide solution (20 per cent.) are added, and the liberated iodine is at once titrated with $N/100$ sodium thiosulphate solution.

One ml. of 0·01 N sodium thiosulphate solution is equivalent to 0·05171 mgrm. of CH_3O.

The following table summarises the results obtained in a number of determinations:

Substance	Methoxyl found Per Cent.	Methoxyl present Per Cent.
Vanillin	20·48	
,, ...	20·09	
,, ...	19·98	20·40
,, ...	20·40	
,, ...	20·37	
Grape fruit pectin	11·58	11·6*
Commercial citrus pectin ..	7·95	8·0*
Quinine (hydrated)	8·37	8·20

* The methoxyl values for the samples of grape fruit and citrus pectins, given in the third column, represent those obtained by the old Zeisel method.

The results obtained clearly indicate the accuracy of the method, and its speed is shown by the fact that, after a little practice, four determinations can easily be carried out in two hours.

I am greatly indebted to Dr. Janet W. Brown for carrying out independent determinations to check the method, and also for some useful suggestions.

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* It is, however, to be noted that the compounds tried here are readily decomposed by hydriodic acid, and therefore it is obvious that those which are not readily decomposed would require longer heating, according to the nature of the compound.

† More phenol water should not be added, as it only increases the blank, which, however, is quite negligible with 10 ml. of the mixture. Several blank determinations with 10 ml. of bromine water and 10 ml. of 10 per cent. phenol-water gave values never exceeding 0·1 ml. of $N/100$ sodium thiosulphate solution.

A Rapid Method for the Accurate Determination of Minute Quantities of Nitrite

By G. GOPALA RAO and K. MADHUSUDANAN PANDALAI

We have made a critical examination of several of the methods for the volumetric determination of nitrites, with special reference to their accuracy for small amounts. The method proposed by Lunge (*Ber.*, 1877, **10**, 1074; *Chem.-Ztg.*, 1904, **28**, 501), which consists in the direct titration of an acidified standard solution of potassium permanganate with the nitrite solution in the burette, was found to yield an error of 0·6 to 1 per cent. when applied to $N/500$ nitrite solutions. Kubel (*J. prakt. Chem.*, 1867, **102**, 229) and Fresenius (*Quantitative Chemical Analysis*, Vol. II, p. 196) have advocated using first an excess of acid permanganate, then an excess of a reducing agent such as oxalic acid or ferrous ammonium sulphate, and finishing the titration with permanganate. Our experiments have shown that this method yielded an error of 0·8 per cent. when oxalic acid was used, and an error as high as 4 per cent. with ferrous ammonium sulphate.

Attempts to determine nitrite from the amount of iodine liberated by it from acidified potassium iodide have been made by Davisson (*J. Amer. Chem. Soc.*, 1916, **38**, 1683), Robin (*J. Pharm. Chim.*, 1898, **7**, 575) Winkler (*Chem.-Ztg.*, 1899, **23**, 454), and others. Recently Cool and Yoe (*Ind. Eng. Chem. Anal. Ed.*, 1933, **5**, 112) have reported that the iodimetric methods proposed by Davisson, Robin, Winkler, and Raschig have proved unsatisfactory. We have, therefore, thought it worth while to describe an iodimetric method which was devised by one of us (G. G. R.), and has been used by us for the last two years. This method enables small amounts of nitrite to be determined with considerable accuracy (within ±0·25 per cent.) under ordinary conditions.

The essential reaction in the iodimetric determination of nitrite is:

$$2HNO_2 + 2HI = 2H_2O + I_2 + 2NO.$$

The liberated nitric oxide tends to be oxidised to nitrogen peroxide in the presence of air; this nitrogen peroxide dissolves and liberates more iodine from the acidified potassium iodide. Hence the amount of iodine will be more than corresponds with the equation, and the results for the nitrite will be variable and rather too high. To prevent the results being vitiated by this factor, two conditions must be fulfilled:—(i) There must be no dissolved oxygen in the reaction mixture, and the atmosphere over the solution must consist of carbon dioxide. This condition is secured by causing a rapid stream of carbon dioxide to bubble through the solution for about ten minutes, so that the dissolved oxygen is driven out.

(ii) The nitric oxide must be expelled from the system as it is liberated. For this purpose some sodium bicarbonate is added to the reaction mixture (nitrite solution + potassium iodide + starch) at the outset. Then carbon dioxide is passed through it for ten minutes, after which the solution is acidified with oxygen-free sulphuric acid. The sulphuric acid liberates a large amount of carbon dioxide

from the added bicarbonate, and this removes the nitric oxide simultaneously liberated.

With these two conditions thus satisfied, the amount of iodine liberated corresponds exactly with that required by the above equation.

In making a determination, 20 ml. of the nitrite solution are placed in a 250-ml. flask, into which are then introduced about 4 grms. of sodium bicarbonate, 5 ml. of 10 per cent. potassium iodide solution, and a little starch solution. Next, a rapid stream of purified carbon dioxide is made to bubble through this reaction mixture in the flask for about ten minutes to ensure the complete removal of oxygen. The solution is then acidified with 10 ml. of 5 *N* oxygen-free sulphuric acid, and the liberated iodine is titrated with freshly prepared N/500 sodium thiosulphate solution (1 ml. of thiosulphate = 0·000028 grm. of nitrite nitrogen).

The accuracy of the method has been checked with a standard (*N*/500) solution of sodium nitrite prepared from pure silver nitrite. The method yields an average error of 0·25 per cent. With this method it is possible to determine quantities of nitrite nitrogen as small as 0·000028 grm. in 100 ml. of solution.

The following results are typical of those obtained:

Volume of standard *N*/500 sodium nitrite solution Ml.	Volume of 0·00190 *N* thiosulphate solution required Ml.	Amount of nitrite nitrogen, by experiment Grm.	Amount of nitrite nitrogen, theoretical Grm.	Error Per Cent.
20	21·10	0·0005613	0·0005601	+0·21
15	15·80	0·0004204	0·0004204	0·00
10	10·50	0·0002794	0·0002800	−0·21
5	5·30	0·0001410	0·0001400	+0·71
2	2·10	0·00005586	0·00005601	−0·25

In conclusion, one of us (K. M. P.) wishes to thank the Andhra University for giving him facilities to take part in this investigation.

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Notes

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

THE DETERMINATION OF ANTIMONY IN VISCERA AND EXCRETA

A METHOD for the determination of such small quantities of antimony as may be found in viscera and excreta was outlined by Schidrowitz and Goldsbrough (ANALYST, 1911, **36**, 101), and elaborated by Beam and Freak (ANALYST, 1919, **44**, 196). The antimony is deposited on copper, as in the Reinsch test. This deposit is removed by dissolving it in alkaline permanganate solution, the solution is filtered, acidified and treated with sulphur dioxide, any excess of which is removed by boiling, a small quantity of gum arabic solution is added, and the liquid is then diluted to a definite volume. Hydrogen sulphide is passed through the liquid, and, the orange-coloured antimony sulphide remaining in colloidal solution, the determination is made by colorimetric comparison with a standard prepared in the same way.

In expert hands this method gives consistent results, and it was successfully employed in this laboratory by Ibrahim Effendi Idris in carrying out some hundreds of determinations required in a research on the treatment of bilharziasis with antimony compounds (*Beih. Arch. für Schiffs- u. Tropen Hyg. Pathol. u. Therap. Exotisch. Krankeiten*, 1931, **35**, 12).

The technique is, however, somewhat intricate, and there are difficulties which may lead to error in the hands of a worker not thoroughly familiar with the pitfalls.

A much simplified colorimetric method has now been tried and has proved satisfactory, provided that no metal of the second analytical group, other than antimony, is present.

It is based on the process first suggested by Strzyowski* for the destruction of organic matter in preparation for the determination of arsenic, for which purpose we have found the method excellent.

A weighed sample of the tissue to be examined is cut up finely and mixed in a silica basin with sufficient magnesium oxide to give a definitely alkaline reaction. The material is then covered with a saturated solution of magnesium nitrate. In general, 35 to 40 ml. of this solution are sufficient for 100 grms. of animal matter. The mixture is then heated on a sand-bath, with frequent stirring, until the material has dried, charred and begun to whiten. The charred mass may then be crushed with a pestle and afterwards heated more strongly, if necessary over a blowpipe flame. The ash should be quite white. If it is not, it may be cooled, mixed with a concentrated solution of ammonium nitrate, and re-heated until free from nitrates.

The ash, when cold, is moistened with water, and sufficient hydrochloric acid is added to dissolve the magnesium oxide and to give a definitely acid reaction. The solution thus prepared is ready for analysis.

* "A Simple Method of Ashing for Rapid Determination of Arsenic in Foodstuffs and Animal Materials," *Pharmaz. Post*, 1906, **39**, 977 (quoted by Autenrieth, *Laboratory Manual for the Detection of Poisons and Powerful Drugs*, sixth American edition, p. 493).

The advantages of this technique are:—(i) The arsenic or antimony is converted into the non-volatile pyro-arsenate or pyro-antimonate of magnesium, which can be incinerated without appreciable loss.

(ii) Magnesium nitrate decomposes, when heated, into magnesium oxide and oxides of nitrogen, the residue being free from nitrates and nitrites.

For the determination of arsenic by the Marsh–Berzelius or the Gutzeit method no further treatment is required, except the addition of stannous chloride to reduce the arsenic to the arsenious condition.

Before the determination of antimony, however, it is necessary to remove iron. The solution is, therefore, diluted with distilled water and saturated with hydrogen sulphide. The precipitate is filtered off, washed in the usual way, and dissolved in the minimum quantity of hot concentrated hydrochloric acid. This solution is diluted with distilled water and re-filtered, if necessary, to remove fragments of filter paper, and, after the addition of gum arabic solution (1 ml. of a 5 per cent. solution being sufficient for 100 ml. of liquid), the solution is made up to a definite volume. Hydrogen sulphide is again passed through the liquid, and the colour is compared in a Duboscq colorimeter with that of a standard of approximately equal concentration.

For the preparation of the standard solution it is unnecessary to use magnesium pyro-antimonate, as was shown by the following experiment:—One ml. of a 5 per cent. solution of tartar emetic was mixed with magnesium oxide and magnesium nitrate solution, evaporated to dryness, and the residue incinerated. The ash was dissolved in the minimum quantity of hydrochloric acid, gum arabic solution was added, and the volume was made up to 1 litre with distilled water. A second solution was made by diluting 1 ml. of the 5 per cent. tartar emetic (slightly acidified and mixed with gum arabic) to 1 litre. Equal aliquot parts of the two solutions were saturated with hydrogen sulphide, and the colours were compared; they were identical.

This concentration (0·005 per cent. tartar emetic) was found to give a colour intensity quite suitable for colour comparison.

In a forensic case tartar emetic had been injected intravenously in mistake for glucose. We had no exact knowledge of the quantity injected, but it was stated that 50 ml. of a 5·6 per cent. solution had been measured out for injection by gravity. The patient almost immediately showed signs of collapse, and the hypodermic needle was withdrawn. Unfortunately the quantity of fluid not injected was unknown. The patient lived for about four hours, and, after the autopsy, samples of blood and liver were sent for analysis. Two samples of the liver, weighing, respectively, 50 grms. and 20 grms., were examined, and the results of analysis were identical, *viz.* 25 mgrms. per 100 grms., calculated as tartar emetic. A sample of blood (50 ml.) treated in the same way was found to contain 2 mgrms. The quantities estimated for 1500 grms. of liver and 5000 ml. of blood were, therefore, as follows:

Liver.—375 mgrms. (as tartar emetic) or 140 mgrms. as metallic antimony.

Blood.—200 mgrms. (as tartar emetic) or 74 mgrms. as metallic antimony.

Khalil (*loc. cit.*) states that the accumulation of 200 mgrms. of antimony in the adult human body is liable to cause death.

(*Cf.* Clarke, ANALYST, 1928, **53**, 373; 1929, **54**, 23; and Evans, *ibid.*, 1922, **47**, 1; 1927, **52**, 565.

FRANK BAMFORD

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MEDICO-LEGAL DEPARTMENT, CAIRO

THE DETECTION OF COPPER BY MEANS OF *p*-PHENYLENEDIAMINE

WHEN a few drops of a mixture of 5 ml. of potassium thiocyanate solution and 2 or 3 drops of an aqueous solution of *p*-phenylenediamine are added to a solution of a copper salt a black precipitate of copper thiocyanate phenylenediamine is produced. The test is capable of detecting 1 part of copper in 100,000.

The black copper precipitate is formed in ammoniacal or neutral solutions; it is soluble in acid, giving a black solution.

No precipitate is given by cobalt, manganese, iron, magnesium, aluminium, chromium or nickel. Cadmium and zinc form white precipitates soluble in acid and ammoniacal solutions. Mercury gives a gray precipitate soluble in ammoniacal, but insoluble in acid solutions. Silver gives a white precipitate soluble in acid, but insoluble in ammoniacal solution.

With the exception of silver, therefore, all the common metals may thus be separated from copper.

Following the general scheme of qualitative analysis, we shall have, if copper and cadmium are present, an ammoniacal solution of cadmium and copper salts in Group II. To this solution are added 2 or 3 drops of a solution of, say, 5 ml. of saturated potassium thiocyanate solution and 2 or 3 drops of an aqueous solution of *p*-phenylenediamine. A black precipitate of the copper salt is formed, but the cadmium is not precipitated. The precipitate is filtered off, and hydrogen sulphide is passed into the filtrate; a yellow precipitate indicates cadmium.

The copper compound formed is undoubtedly co-ordinated, and the diamine has been proved to be linked to the copper ion. The compound contains 25·08 per cent. of copper.

Further work is being carried out with this and similar co-ordination compounds.

R. J. McILROY

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AN INEXPENSIVE AND ECONOMICAL WARM ROOM

THE chemist in a small factory often wishes to test at different temperatures the products which he is examining, but, having no hot room in which to place any bulk of material, has to rely upon small-scale tests in the ordinary incubator. The apparatus here described is an inexpensive substitute for a hot room. The hot chamber, which has an internal capacity of about 30 cb. feet, is made of ordinary tongue-and-grooved one-inch planks and is double-walled, the space between the inner and outer walls being filled with broken cork and sawdust. The top and the door are also insulated. The heating system consists of two 250-watt tubular lamps which are fitted with a temperature-control regulator, the thermostat being at the top of the chamber. (This apparatus is shown in Gallenkamp's catalogue—B.3767.) The temperature at various parts of the chamber shows slight but uniform variations, the following figures, which were taken over a period of one month, being typical:

Position of thermometer	Maximum °F.	Minimum °F.
Top	83	78
Middle	84	79
Bottom	88	81

During the Christmas holidays the chamber remained closed for a period of 136 hours, and the temperatures at the above positions were 82° to 79°, 83° to 81°,

and 88° to 83° F., respectively. During this period the current used was only 25 units, which gives a daily consumption of about 4½ units. I have used this type of chamber at temperatures between 80° and 105° F. with complete success.

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EAST MOLESEY

G. GRINLING

A NICKEL VESSEL FOR STORING STANDARD CAUSTIC ALKALI SOLUTIONS

THE difficulty of keeping standard caustic alkali solutions is well known. Waxed vessels are often employed, but have the disadvantage that the wax peels away after prolonged contact with the solution.

I have found a vessel of heavy-gauge pure nickel to be very convenient for preserving $N/10$ caustic soda solution. The vessel, which was made to specification by a firm in the trade, has the approximate shape and dimensions of a Winchester quart bottle. The mouth is fitted with a rubber bung carrying the usual soda-lime tube and a supply tube of seamless Monel-metal for the burette.

After a period of eight months the alkali was perfectly clear and its normality was unchanged. After each titration the alkali remaining in the burette is always allowed to run back immediately into the nickel vessel.

18, DOROTHY ROAD
LONDON, S.W.11

EDWIN C. RIGHELLATO

Sub-Committee on the Determination of Unsaponifiable Matter in Oils and Fats, and Unsaponified Fat in Soaps

NOTE ON THE B.P. LIMITS FOR "FREE FAT" IN SOAPS

IN the 1932 B.P. the limit for "free fat" for Sapo Animalis, Sapo Durus and Sapo Mollis corresponds to 0·5 per cent. The method described for free fat determination returns unsaponifiable matter, as defined in the Report of the Sub-Committee for the Determination of Unsaponifiable Matter, etc., in THE ANALYST (1933, **58**, 203), as well as any free unsaponified saponifiable material. Hence, it is clear that the limit specified should be such as to include at least the maximum amount of unsaponifiable matter which is likely to be present in the oil from which it is specified that the soap should be made. The limit, however, should not greatly exceed such an amount, since the object of the test is to avoid the presence of more than a minimum of unsaponified saponifiable matter, as well as to detect any undue amount of unsaponifiable matter.

Recent determinations of the unsaponifiable matter in genuine samples of olive oil show that this oil may contain up to 1·25 per cent. of unsaponifiable matter when examined by the method given in the B.P. This quantity of unsaponifiable matter would correspond to 0·88 per cent. returned by the B.P. test as "free fat" on a soap containing 70 per cent. of fatty acids. It is recommended, therefore, that the limit of "free fat" specified in the B.P. for Sapo Durus should be altered to correspond to 1·0 per cent., and that for Sapo Mollis to 0·7 per cent., the latter being calculated on a basis of 44 per cent. of fatty acids.

Further, the unsaponifiable matter of purified solid animal fats, such as tallow, may be 0·7 per cent. or rather more. Hence, the free fat limit of 0·5 per cent., specified in the B.P. for Sapo Animalis, may not be attainable, and therefore it is suggested that the limit in this case should be fixed at 0·7–0·8 per cent.

(*Signed on behalf of the Sub-Committee*) L. V. COCKS (*Chairman*)
NORMAN EVERS (*Hon. Secretary*)

Report of the Essential Oil Sub-Committee to the Standing Committee on Uniformity of Analytical Methods

REPORT No. 11

THE DETERMINATION OF ALDEHYDES OTHER THAN CITRONELLAL*

THE investigations of the Essential Oil Sub-Committee have shown that, for the determination of aldehydes in essential oils, the hydroxylamine method offers considerable advantages over the commonly employed bisulphite and neutral sulphite methods. The results obtained by different operators are more concordant, and the method is less affected by impurities in the oils themselves. Thus, in the bisulphite and neutral sulphite methods, impurities such as water-soluble organic acids, and adulterants such as alcohol, would be recorded as aldehyde, while the addition of substances such as resin frequently results in the formation of an emulsion and renders an accurate reading of the line of demarcation between the bisulphite solution and the non-aldehyde portion of the oil a matter of considerable difficulty.

The hydroxylamine method, on the other hand, is a definite determination of aldehydic (or ketonic) substances, and is affected very little, if at all, by the presence of traces of free organic acids such as are frequently present in essential oils, as these are usually without action on the indicator used in the method.

A further advantage of the hydroxylamine method is that a small quantity only of the oil is required and the determination can be completed in a much shorter time.

Particular attention must be paid to the adjustment of the reagent and to the end-point of the reaction, and, as both these conditions depend on the change in colour of methyl orange from red to yellow, it is obvious that the operator's eyes must not be insensitive to this colour change.

The Sub-Committee recommends the following method for the determination of aldehydes in oil of cassia, oil of cinnamon, oil of lemongrass, oil of orange, oil of bitter almond, oil of cherry-laurel, oil of cummin (cumin) and terpeneless and sesquiterpeneless oils of lemon and orange.

The following solutions are required:

Indicator Solution.—A 0·2 per cent. solution of pure methyl orange in alcohol (60 per cent. v/v).

N/2 Alcoholic Potash.—Prepared with alcohol (60 per cent. v/v) and standardised against *N*/2 hydrochloric acid, using methyl orange as indicator, and running the alkali into the acid until the full yellow colour is obtained.

N/2 Hydroxylamine Hydrochloride.—Dissolve 3·475 grms. of pure hydroxylamine hydrochloride in 95 c.c. of alcohol (60 per cent. v/v), add 0·5 c.c. of the indicator solution, adjust to the full yellow colour of the indicator with the *N*/2 alcoholic potash, and make up to 100 c.c. with alcohol (60 per cent. v/v).

The alcohol used throughout must be free from aldehydes and ketones.

The full yellow colour of the indicator may be defined as that colour which is not changed by the further addition of alkali. The correct adjustment of the reagent should be confirmed in the following manner:

Place 10 c.c. in each of two tubes, and to one tube add 1 drop of *N*/2 alcoholic potash; no change in colour should be observed. To the other tube add 1 drop of *N*/2 hydrochloric acid; a slight change in colour towards orange should be produced.

* See Report No. 10.

METHOD OF DETERMINATION.—Weigh out exactly, into a glass-stoppered tube—approximately 150 mm. long by 25 mm. in diameter—a suitable quantity* of the oil, add 5 c.c. of benzene and 15 c.c. of $N/2$ hydroxylamine hydrochloride reagent. Shake vigorously and titrate with the $N/2$ alcoholic potash until the red colour changes to yellow. Continue the shaking and titrating until the full yellow colour of the indicator is permanent in the lower layer after shaking vigorously for two minutes and then allowing to stand for the liquids to separate. The reaction is slow towards the end, but should be complete in about 15 minutes.

The result should be confirmed by a second determination, and the first titration liquid *plus* a slight excess of alcoholic potash (0·5 c.c.) should be used as a colour standard for the end-point of the second titration.

The number of c.c. of $N/2$ alcoholic potash used multiplied by the correcting factor 1·008, by the factor for the appropriate aldehyde, and by 100, and divided by the weight of oil taken will give the percentage (by weight) of aldehydes, calculated as that aldehyde, present in the oil.

The following factors should be used for the aldehydes:—

Benzaldehyde*	0·053
Cinnamic aldehyde	0·066
Citral	0·076
Cuminaldehyde	0·074
Decylic aldehyde	0·078

* In the determination on benzaldehyde-containing oils, allowance should be made for the amount of acid titratable to methyl orange.

The correcting factor is necessary, owing to the fact that the end-point of the titration occurs at a p_H different from that of normal hydroxylamine hydrochloride.

The purpose of the benzene is twofold; first, it acts as a solvent for the oil and, in the subsequent shaking, aids in producing a fine dispersion of oil globules in the mixture and thus promotes surface contact between the reacting liquids; and, secondly, it is a solvent for the non-aldehyde portion of the oil and prevents the inherent colour of this from interfering with the colour of the indicator in the dilute alcoholic solution.

The Sub-Committee recommends that, in recording results, the following type of wording should be used:

Total aldehydes by the hydroxylamine method, calculated as citral (or other aldehyde) per cent. by weight.

Determinations made by members of the Sub-Committee lead us to the opinion that the maximum variation with this method should not exceed ± 1 per cent. The following tables show the results obtained by members of the Sub-Committee on samples circulated for collective testing. It should be noted that the results reported for the bisulphite method were obtained by members, each using his own technique.

RESULTS OF THE DETERMINATION OF CINNAMIC ALDEHYDE IN AN ARTIFICIALLY PREPARED MIXTURE

The cinnamic aldehyde used contained 97·6 per cent. of pure cinnamic aldehyde and 2·0 per cent. of free acid, calculated as cinnamic acid. It was completely soluble in sodium bisulphite solution. The mixture was made by diluting 60 parts by weight of the aldehyde with 40 parts by weight of copaiba oil, and thus contained 58·6 per cent. by weight of cinnamic aldehyde.

* About 1 grm. of oil of cinnamon, oil of cumin or oil of lemongrass; about 0·8 grm. of oil of cassia and about 0·6 grm. of oil of bitter almond, oil of cherry-laurel, terpeneless and sesquiterpeneless oils of lemon and orange, and from 5 to 10 grms. of oil of orange.

Sub-Committee Member	Hydroxylamine method Per cent. by weight	Bisulphite method Per cent. by volume	=	Bisulphite method Per cent. by weight
1	58·1	58·0		61·9
	58·2			
2	57·2	59·5		63·5
	58·0			
3	57·1	58·5		62·4
4	57·3	60·0		64·1
	57·4			
	58·0			
5	57·8	56·9		60·8
6	58·8	57·0		60·9
	58·8	57·0		60·9
7	57·4	58·0		61·9
	58·3			
8	57·7	56·6		60·5
9	58·0	57·0		60·9
	57·9			
10	57·6	58·0		61·9
Variation	57·1 to 58·8	56·6 to 60·0		60·5 to 64·1
Mean	57·9	57·9		61·8

CINNAMIC ALDEHYDE IN CRUDE OIL OF CASSIA

Sub-Committee Member	Hydroxylamine method Per cent by weight	Bisulphite method Per cent. by volume
1	74·8	82
2	74·9	81
	75·1	82
3	76·0	82
	75·5	82
	74·9	
4	75·4	80·5
	75·5	80·5
5	75·2	80
	75·4	
	75·3	
	75·3	
6	75·2	81
	75·1	82
	74·9	
7	75·8	80·5
	76·3	
	76·1	
8	76·2	79·5
		79·5
9	75·8	81
	76·3	82
Variation	74·8 to 76·3	79·5 to 82
Mean	75·5	81

CITRAL IN OIL OF LEMONGRASS

Sub-Committee Member	Hydroxylamine method Per cent. by weight	Bisulphite method Per cent. by volume
1	75·4	80·1
		81
2	74·7	77
	74·3	78
3	75·3	79
	74·85	78
	75·25	
	74·7	
4	74·6	73·5
	74·0	73·5
5	75·0	76·5
	75·0	(75·5 neutral)
	74·9	
	74·7	
6	74·1	74
	74·0	75
	74·2	
7	75·5	74·6
	75·0	74·6
	75·1	(75 neutral)
	75·3	
	74·7	
8	74·4	78
		78·5
9	75·4	77
	75·8	78
Variation	74·0 to 75·8	73·5 to 81
Mean	74·84	75·66

(*Signed*)
W. H. Simmons (Chairman), C. T. Bennett, S. W. Bradley, L. E. Campbell, Thos. H. Durrans, T. W. Harrison, Ernest J. Parry, C. Edward Sage, T. Tusting Cocking (Hon. Secretary).

Official Appointments

THE Minister of Health has approved the following appointments:

FREDERICK WILLIAM EDWARDS as Public Analyst for the Metropolitan Borough of Hammersmith, in place of P. A. E. Richards, resigned, December 31, 1933 (January 6th).

ERNEST VICTOR JONES as Public Analyst for the Borough of Newcastle-under-Lyme, in place of A. E. Johnson (deceased) (January 6th).

ERNEST VICTOR JONES as Public Analyst for the Borough of Stoke-on-Trent, in place of A. E. Johnson (deceased) (January 26th).

Bibliography on Heavy Metals in Food and Biological Material

(From the beginning of the year 1921 *to date)*

XI ANTIMONY

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XII CADMIUM

FAIRHALL and PRODAN. Colorimetric Determination of Traces of Cadmium in Organic Matter. *J. Amer. Chem. Soc.*, 1931, **53**, 1321; ANALYST, 1931, **56**, 412. (As colloidal cadmium sulphide.)

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XIII THALLIUM

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Ministry of Health

SALE OF FOOD AND DRUGS ACT

EXTRACTS FROM THE ANNUAL REPORT FOR 1932–1933, AND ABSTRACTS OF REPORTS OF PUBLIC ANALYSTS FOR THE YEAR 1932*

OF the 137,981 samples of food and drugs submitted to Public Analysts in 1932, 7019, or 5·1 per cent., were reported as adulterated or not up to standard. This is a slight increase as compared with 4·6 and 4·8 per cent., respectively, for the two previous years. The practice in certain areas is that all the milk samples are first examined by the sampling officers, and consequently only samples believed to be adulterated are sent to the analyst. Attention is called to the risk in these areas of failing to detect some of the less common forms of adulteration.

PRESERVATIVES.—The number of infringements of the Regulations reported was 549; in 200 cases prohibited preservatives were found in foods, such as boron in sausages, cream, margarine, caviare, and cake; formaldehyde in milk; sulphur dioxide in sweets, minced and potted meat, desiccated soups and pepper, and salicylic acid in a few samples of non-alcoholic wine, lime juice cordial, jam and mushroom ketchup. In 131 samples of such foods as raisins, sultanas, jam and raisin wine, preservative was in excess of the amount permitted. A "dusting powder," consisting of a mixture of borax and boric acid, was used for sausages.

MILK.—Of the 72,940 samples of milk examined, 5307, or 7·3 per cent., were reported against, the corresponding percentages for 1930 and 1931 being, respectively, 6·6 and 6·4. Of the 893 "appeal-to-cow" samples, 41·2 per cent. were below the presumptive standard of the Sale of Milk Regulations, 1901, but these samples were taken at farms the milk from which had previously been sampled, and found to be below standard. In several cases of adulteration with water, convictions were obtained. Visible dirt was present in 21 samples, added colouring matter in 12, and formaldehyde in 13. Of graded milk, 31 samples were deficient in fat or non-fatty solids, and 13 samples of skimmed milk in non-fatty solids.

CONDENSED AND DRIED MILK.—Of 1198 samples of condensed milk, 16 were reported against, 5 were deficient in milk solids-not-fat, 1 in milk-fat, and 2 in both.

* To be obtained from H.M. Stationery Office, Adastral House, W.C.2. Price 4d. net.

CREAM.—Forty-three of 2207 samples of cream were reported against; 24 (mostly tinned cream) were deficient in fat, 13 contained boron preservative; 4 samples sold as cream were re-constituted cream; 1 sold as dairy cream was sterilised cream, and one sample consisted mainly of vegetable oil.

BUTTER AND MARGARINE.—Of the 9707 samples of butter reported upon, 65 contained excess of water, 13 foreign fat, and 4 excess of free fatty acid. One sample sold as fresh butter contained salt, and one consisted entirely of margarine. Twenty-five of 3294 samples of margarine contained excess water; 6 boron preservative; 11 were incorrectly labelled, and one, containing only 2 per cent. of butter, was sold as "margarine butter."

LARD AND OTHER FATS.—Nine of 2727 samples of lard were reported against (4 consisting of beef or other foreign fat, 3 of partly foreign fat, 1 contained excess of free fatty acid and one sodium bicarbonate); 28 of 363 samples of suet (8 samples sold as suet contained starchy materials and 20 of shredded suet contained excess of such material or were incorrectly labelled); 7 of 628 samples of dripping contained either excess of water or fatty acid or rice flour (1 sample) or 30 per cent. pork fat (1 sample).

CHEESE.—Of the 1377 samples of cheese examined, 10 "cream cheeses" were ordinary cheeses made from whole milk, 1 was decomposed, 5 (sold as Cheshire cheese) were deficient in fat and made from partly skimmed milk. In only 2 cases was contamination by tin reported. In one of these 14 grains per lb. were present, and the vendor was prosecuted and fined.

BREAD AND FLOUR.—One sample of 227 breads examined contained 0·225 per cent. of lactic acid and was mouldy. Five samples of flour (all self-raising) of 1265 analysed, were reported against, 1 for containing calcium phosphate, 2 sulphate of lime, and 2 for being ordinary white flour. One contained weevils, caterpillars, etc.

JAMS AND MARMALADE.—Eighty-six of 1759 samples examined were reported against—53 for being deficient in fruit or other soluble solids or not being in accordance with the label. Twenty-nine samples contained an excess of preservative; 1 a prohibited preservative, and 2 marmalades, labelled free from preservative, contained preservative.

VINEGAR.—Of 1856 samples of vinegar, 114 were deficient in acetic acid; 37 samples, sold as "malt," "grape" or "table" vinegar, were wholly or partly artificial vinegars; one sample contained lead and another a trace of oil.

SPIRITS AND BEER.—Under 65 per cent. of proof spirit was reported in 7 brandies, 41 gins, and 98 whiskies of a total of 2013 samples examined, and 6 samples, sold as "wine and brandy," "wine and whisky," or "rum punch," were over 60 degrees under proof, and had misleading labels. A sample of rum was 41·5 degrees under proof. Six samples of beer (of 440 examined) contained arsenic in excess of 1/100 grain per gal.; 5 contained traces of lead, and 2 samples, sold as "special Lager mild ale" and "black malt beer," respectively, contained so little alcohol as to be practically non-alcoholic drinks.

MISCELLANEOUS ARTICLES OF FOOD.—Some 30,000 other samples of food were examined. Sugar was found adulterated with salt, ground rice, desiccated coconut and lard; one sample contained 33·66 per cent. of sodium thiosulphate; 23 samples of cinnamon and mixed spice were adulterated with silicious matter. Mustard was reported to be adulterated with flour and the ground skins of mustard seed, and samples of pepper contained 75 per cent. of rice starch. Contamination with arsenic was reported in cocoa, potted shrimps, apples, sweets, black beer and baking powder; tinned sardines and cocoa were contaminated with lead; cider with lead and copper; dried peas, mincemeat, mushrooms-in-butter, and Worcester sauce with copper, and tin was found in various tinned foods. "Pure chocolate

cream Easter eggs" consisted of a filling of sugar and glucose and gelatin covered with chocolate, itself consisting of hardened vegetable oil (probably coconut), cornflour and cocoa husk. "Honey and butter rock" was reported to contain no honey.

DRUGS.—Of 5606 samples of drugs, 209 were adulterated or below standard and included ground ginger, camphorated oil, lozenges, medicated tablets, ointment, liquorice powder and iodine. Sixteen samples of camphorated oil were deficient in camphor up to 48·5 per cent., and one sample was stated to contain 61 per cent. of synthetic camphor. Bismuth lozenges contained no calcium carbonate or contained French chalk; aspirin tablets were deficient in aspirin, lime water in lime, borax and honey was devoid of borax, and creosote ointment was deficient in creosote. A cream of tartar was 100 per cent. arrowroot. Nerve and digestive tablets were 100 per cent. deficient in potassium iodide, samples of cod-liver oil emulsion with hypophosphites were found to be deficient in hypophosphites and in oil, and Seidlitz powders contained less tartaric acid than is prescribed in the British Pharmacopoeia. Excess of lead was present in Epsom salts and tartaric acid, and excess of arsenic in borax; peach or apricot kernel oil was supplied for almond oil, and ground ginger contained preservative.

D. G. H.

Department of Scientific and Industrial Research

THE MEASUREMENT OF HUMIDITY IN CLOSED SPACES *

THIS is a new edition of a Report, originally published in 1925, and now revised and much extended by Dr. Ezer Griffiths, incorporating the results of further experiments carried out by himself and Mr. J. H. Awbery. It is introduced by a prefatory note by Sir J. A. Ewing explaining its scope.

MEASUREMENT OF HUMIDITY.—The Report points out that there are three methods in common use—the wet-and-dry bulb hygrometer invented in 1813; the dew-point apparatus dating from 1827; and the hair hygrometer used as far back as 1783—and states that "no fundamentally new method of hygrometry has been introduced, in spite of the immense amount of attention which has been given to the subject, during the past one hundred years." While the report deals mainly with the three classical methods, it indicates how much has been done towards improving the accuracy of these methods and in devising automatic, distant-reading and recording apparatus for use in special industrial applications, such as the determination of humidity in a closed store, in a stack of fruit, or in other inaccessible places.

WHIRLING THERMOMETERS.—With the wet-and-dry bulb hygrometer, or psychrometer as this instrument is sometimes called, the humidity is determined from the difference in the reading of two thermometers, the bulb of one of which is covered with a wet muslin sheath. The report lays stress on the importance of keeping the air moving past the thermometers. This may be done either by whirling the thermometers or by drawing air through a tube in which they are placed. To make the instrument distant-reading, the ordinary mercury thermometers may be replaced by electrical thermo-couples.

THE DEW-POINT HYGROMETER.—The dew-point apparatus depends on finding to what temperature a surface must be cooled for dew to be deposited on it. In

* Food Investigation Special Report No. 8. (Revised Edition, 1933.) Pp. viii+70, with 49 figures. H.M. Stationery Office, Adastral House, Kingsway, London, W.C.2.

its simple form, therefore, the method depends primarily upon the observer's visual acuteness in detecting a trace of dew. In distant-reading instruments some other method of detecting the presence of dew is necessary. In one instrument, radiation reflected by a polished surface is arranged to produce an electric current by falling on a thermo-couple. The formation of dew changes the reflecting power of the surface and thus alters the current. The observer, therefore, instead of watching for the dew to form, watches for the movement of the pointer of a sensitive galvanometer. Another instrument uses a photo-electric cell. Light is reflected from the cooled surface, and the photo-electric cell is so placed that some of the light scattered from this surface when dew begins to form on it falls on the cell and reduces its resistance. An electric circuit connected with the cell is so arranged that clicks are produced in a telephone in the circuit when there is no dew, but cease when dew is formed.

THE HAIR HYGROMETER.—The hair hygrometer depends on the fact that hair, and some other organic materials, lengthen and shorten as the humidity of the surrounding atmosphere increases and decreases. These changes of length may be communicated to a pointer moving over a scale or over a recording drum. The materials studied for humidity work include specially prepared bundles of human hair, single horse-hairs, fibres of raw silk and of artificial silk, strips of goldbeaters' skin, jewellers' tissue paper and mohair. The Report states that a single horse-hair is remarkable for the closeness with which its indications are repeated when it is taken through several cycles of humidity, and that goldbeaters' skin is remarkable for the rapidity with which it reaches equilibrium when the humidity is rapidly changed.

OTHER FORMS OF HYGROMETER.—A further method of measuring humidity that has been tried consisted in finding the variation with humidity of the refractive index of a prism to the surface of which a film of glycerin had been applied. In another method the effect of humidity in changing the temperature, and therefore the resistance, of cotton-covered nickel wire was used, and another interesting instrument, termed the fog-formation hygrometer, was constructed as follows:— Some of the air under examination was drawn into a vessel and suddenly expanded in another vessel at a lower pressure. Ordinarily the water vapour in the air produces a dense fog as the result of the cooling of the air by expansion. The pressure of the vessel into which the air expanded was adjusted, however, until the fog was either just visible or just invisible. This pressure is directly related to the humidity.

Yet another method considered in the report depends on the fact that the loss of heat from a hot wire depends on the conductivity of heat of the surrounding atmosphere. The thermal conductivity of hydrogen is, for example, six times that of air. Accordingly, this method has had an extensive application in recorders of the amount of hydrogen and carbon dioxide present in a particular atmosphere. The report refers to the fact that Koepset claimed for a German apparatus made on this principle that it can easily detect the presence of 0·001 per cent. of hydrogen (*Ber. physik. Ges.*, 1908, **10**, 814); a simple British instrument on these lines was developed during the war for measuring the permeability of balloon fabrics to hydrogen (Shakespear, *Proc. Phys. Soc.*, 1921, **33**, 168). Unfortunately, the change in thermal conductivity in air, due to the presence of water vapour, is not sufficiently marked to make this generally as accurate as the ordinary methods for measuring humidity. The Report adds, however, that the method may prove of great service for measurements of humidity at temperatures beyond the range of the classical methods.

BIBLIOGRAPHY.—The classified bibliography, occupying 5 pages, contains 106 references and includes most of the important papers bearing on the present Report.

The Pharmaceutical Society of Great Britain Codex Revision Committee

THE following reports have been published by direction of the Council of the Pharmaceutical Society.* In each instance the Editor of the *British Pharmaceutical Codex* (17, Bloomsbury Square, W.C.1), will be pleased to receive any observations on points relating to them.

REPORT OF PHARMACOGNOSY SUB-COMMITTEE.—This gives a summary of the principal standards for Crude Vegetable Drugs recommended by the Sub-Committee and accepted provisionally for inclusion in the British Pharmaceutical Codex, 1934. It comprises 100 crude drugs arranged in alphabetical order. It is published at 2s.

REPORT OF "ACTION AND USES" SUB-COMMITTEE.—In addition to the revision of the "action and uses" sections of about one thousand monographs, the Sub-Committee, assisted by a number of specialists, has supplied the descriptive portions for about fifty drugs, mostly of animal origin, including antitoxins, toxins, and gland products.

In this summary are given certain sections from the descriptions recommended and the standards proposed for the more important of these substances, in the hope that they may be of interest to manufacturers and others. For the substances which are not included in the British Pharmacopoeia, the B.P.C. standard for substances controlled by the Therapeutic Substances Act will be the same as the standard laid down by the regulations made under that Act. The Report is published at 1s. 6d.

REPORT OF PHARMACEUTICAL CHEMISTRY SUB-COMMITTEE.—The chemical substances for inclusion in the next issue of the British Pharmaceutical Codex fall into three groups. First, those for which a standard is laid down in the British Pharmacopoeia and for which the Sub-Committee has provided only additional and useful descriptive matter. Secondly, those for which the Sub-Committee has worked out a series of tests to form a British Pharmaceutical Codex standard for the substance. Thirdly, those for which the Sub-Committee has not recommended a particular standard and which are included to provide necessary and useful information. It is to the more important substances of the second group that this summary relates.

The Sub-Committee recommends that the methods of procedure for limit tests and other tests which form tests of purity, unless directed otherwise, shall be the same as those of the corresponding tests of the British Pharmacopoeia.

REPORT OF PHARMACY SUB-COMMITTEE.—This contains a summary of the principal new or revised formulae recommended by the Sub-Committee for inclusion in the British Pharmaceutical Codex, 1934. For many of the preparations the Sub-Committee has worked out tests, and has recommended that these should be included to form a B.P. Codex standard for the preparation. The inclusion of alcohol limits for the concentrated infusions, spirits and tinctures is also recommended. The Sub-Committee hopes that it will be possible to obtain the permission of the Board of Customs and Excise to use methylated spirit in making preparations which are not to be taken internally.

The formulae are expressed in both metric and Imperial systems. The Report is published at 2s. 6d.

* Published by the Pharmaceutical Press, 23, Bloomsbury Square, London, W.C.1.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs Analysis

Chemical Study of Tomato Juice. C. F. Poe, A. P. Wyss, and T. G. McEver. (*J.A.O.A.C.*, 1933, **16**, 624–627.)—Raw and cooked tomato (Colorado) juices, containing practically all the liquid and pulp, show virtually no differences in composition, refractivity, or specific gravity, if prepared from the same sample. Analysis of 8 samples of home-canned tomato juices, containing no added salt, gave the following minimum, average, and maximum percentage results: Total solids, 5·02, 5·71, 6·48; soluble solids, 4·66, 5·31, 6·10; insoluble solids, 0·32, 0·43, 0·55; per cent. of total "serum" solids soluble in 95 per cent. alcohol, 53·98, 59·47, 66·12; ash, 0·41, 0·47, 0·51; water-soluble ash, 0·39, 0·44, 0·47; alkalinity of water-soluble ash in c.c. of *N* hydrochloric acid per 100 grms. of juice, 4·65, 5·90, 6·53; salt, 0·06, 0·09, 0·12; acetic acid as ml. of 0·1 *N* sodium hydroxide per 100 grms. of juice, 0·61, 0·71, 0·77; fixed acid, expressed similarly, 44·1, 63·8, 79·1; fixed acid as malic acid, 0·30, 0·43, 0·53; reducing sugar after inversion, 2·44, 3·08, 3·38; n_D at 20° C., 1·3400, 1·3412, 1·3424; immersion refractometer reading at 20° C., 32·8, 35·9, 39·1; sp.gr. at 20° C., 1·0209, 1·0232, 1·0267.

The corresponding results for 8 samples of home-canned juices containing salt were: Total solids, 6·37, 7·24, 7·90; total solids, salt-free, 5·22, 5·91, 6·42; soluble solids, 5·99, 6·82, 7·44; insoluble solids, 0·33, 0·45, 0·55; per cent. of total "serum" solids soluble in 95 per cent. alcohol, 54·93, 58·27, 64·12; salt-free ash, 0·34, 0·42, 0·50; salt, 0·62, 1·33, 1·74; acetic acid, as above, 0·58, 0·75, 0·83; fixed acid, as above, 55·0, 69·3, 81·2; fixed acid as malic acid, 0·37, 0·46, 0·54; reducing sugars after inversion, 2·26, 2·90, 3·28; n_D at 20° C. (or corrected for salt), 1·3421 (1·3403), 1·3436 (1·3414), 1·3448 (1·3421); immersion refractometer reading at 20° C. (or corrected for salt), 39·0 (33·2), 42·4 (36·3), 45·3 (38·4); sp.gr. at 20° C. (or corrected for salt), 1·0278 (1·0204), 1·0328 (1·0235), 1·0356 (1·0255).

Indications of adulteration may be obtained from determinations of: Total solids and insoluble solids, on the whole juice; solids, water-soluble solids, ash, alkalinity of the ash, immersion refractometer reading, volatile acids, and reducing sugars, all on the "serum." T. H. P.

Composition of the Egg Plant Fruit at Different Stages of Maturity. C. W. Culpepper and H. H. Moon. (*J. Agric. Res.*, 1933, **47**, 705–717.)—The fruit of the egg plant (*Solanum melongea*) is always eaten in the immature state. A study of the composition of the fruit from some 10 varieties of egg plant at different stages of maturity showed that the total solids generally ranged from 7 to 9 per cent. of the fresh green weight, and were highest at the time of flowering; decreased until the fruits were 15 to 20 days old, and, after that, changed little. Moisture-content was high. Total sugars (2·0 to 3·5 per cent. of the fresh fruit) varied little, but appeared to be highest in fruit 40 days old; total nitrogen was low, but a small amount of nitrate nitrogen was present at all stages except the

very earliest. The sp.gr. of the fruit flesh was low (0·560–0·776), and the difference between that of the intact tissues and the expressed juice indicated the presence of 25 to 44 per cent. by volume of air in the tissues. Titratable acidity (as citric acid) was consistently low (0·1 to 0·2 per cent. at 15 days); total astringency (determined by a modification of the Procter–Loewenthal method) ranged from 0·15 to 0·35 per cent., and was highest at the time of flowering, thereafter continually decreasing. The failure of the material to retain its form during cooking is ascribed to the high moisture content associated with a change of much of the protopectin to pectin, and expulsion of air. Darkening on cooking appeared to be due to tannin-like substances and low acidity. When canned, the material turned dark, became soft and lost its form, possibly owing to the presence of oxygen and nitrates. The following results are typical of those obtained:

Variety	Age in days	Soluble solids Per Cent.	Insol. solids Per Cent.	Total solids Per Cent.	Reducing sugars Per Cent.	Sucrose Per Cent.	Total sugars Per Cent.	Titratable acidity Per Cent.	Total astringency Per Cent.	Substances hydrolysable by acid Per Cent.	Nitrate nitrogen Per Cent.	Total nitrogen Per Cent.
Florida Highbush	15	4·13	4·76	8·89	1·50	0·42	1·92	0·24	0·52	1·31	0·00014	0·26
New York Improved	15	3·78	4·58	8·36	0·96	0·40	1·36	0·27	0·47	0·92	0·00040	0·41
Black Beauty	15	3·73	4·72	8·45	0·99	0·45	1·44	0·26	0·43	1·04	0·00040	—
Chinese Giant	15	4·26	3·81	8·07	2·10	0·50	2·60	0·17	0·20	0·98	0·00078	—
Long Purple Japanese	20	4·06	4·06	8·12	2·01	0·69	2·70	0·21	0·12	1·16	0·00097	—
Long Purple Ovigerum	40	5·34	2·95	8·29	3·31	0·59	3·90	0·13	0·14	0·90	0·00095	0·72
	60	3·00	5·25	8·25	1·17	0·28	1·45	0·30	0·12	1·31	0·00115	—

D. G. H.

Determination of Iron in Beer by means of $\alpha\alpha'$-Dipyridyl. G. Bode. (*Woch. Brau.*, 1933, **50**, 321–323; *J. Inst. Brewing*, 1933, **39**, 658–659.)—Since direct qualitative tests for iron in beer with potassium thiocyanate are unreliable, and since quantitative determinations on the ash are uncertain owing to the presence of phosphates, the author has modified Blau's reaction (*Monatsh. Chem.*, 1898, **19**, 647), in which an intense red, soluble complex cation, stable to acids and alkalis, is produced by the interaction of ferrous ions and $\alpha\alpha'$-dipyridyl ($C_{10}H_8N_2$) in an acid medium. A mixture of 1 ml. of the sample, 0·5 ml. of concentrated sulphuric acid and 1 ml. of perhydrol is boiled gently until white fumes are evolved, more perhydrol being then added until all colour is destroyed. The iron is then reduced by addition of 0·5 ml. of a solution of sodium sulphite containing a slight excess of sulphur dioxide, and heating is renewed until white fumes again appear. One or two drops of a 1 per cent. solution of *p*-nitrophenol in alcohol are then added to the cooled liquid, followed by ammonia in very slight excess of the amount required for neutralisation, and 0·005 *N* sulphuric acid to neutralise this excess, as shown by the disappearance of the yellow colour. The mixture is then washed into a 10-ml. cylinder with three 2-ml. portions of the 0·005 *N* acid, the final volume being made up to 8·5 ml. with the acid. The resulting solution is mixed well with 0·5 ml. of the sodium sulphite solution and 1 ml. of a 0·5 per cent. solution of $\alpha\alpha'$-dipyridyl, and, when the colour is no longer increasing, its intensity is matched

against the colours produced by addition, to 8·5 ml. of the acid containing the equivalent of 0·001 to 0·021 mgrm. of iron, of 0·5 ml. of sodium sulphite solution and 1 ml. of reagent. The standards are stable for some months in filled sealed test-tubes; allowance should be made for any blank on the reagents. Beer normally contains about 1 mgrm. of iron per litre, but 13 mgrms. per litre were found in a sample which developed iron-protein cloudiness; this was traced to imperfect removal of iron during the purification process. When 19 sq.cm. of sheet iron were suspended in 330 ml. of beer, the iron-content rose from 1 mgrm. per litre to 11, 40 and 135 mgrms. per litre after 3, 40 and 144 hours, respectively. J. G.

Distinction of Wine Vinegar from other Vinegars. J. Pritzker. (*Chem.-Ztg.*, 1933, **57**, 927.)—The work of A. Patzauer (ANALYST, 1933, **58**, 700) is criticised. In using Bonnstadt's *l*-tartaric acid method for the detection of tartaric acid the author shakes 10 ml. of vinegar with 1 ml. of a 20 per cent. solution of calcium acetate (not potassium acetate as erroneously given by Patzauer), then immediately adds 0·5 ml. of a 0·2 per cent. solution of *l*-tartaric acid, shakes again, and allows the mixture to stand for 12 hours. In the author's opinion there is no evidence that during the acetification of wine the tartaric acid present undergoes any change. It has also to be borne in mind that diseased and defective wines are converted into wine vinegar, and that in some of these the tartaric acid may be completely decomposed by bacteria. Tests on 5 genuine wine-vinegars gave only crystals of calcium tartrate, not the S-shaped crystals (more accurately termed propeller-shaped) described by Patzauer. These are obtained only in very dilute solutions of tartaric acid, and their formation bears no relation to the degree of acetic fermentation. Thus, *e.g.*, the "S" type was obtained with 0·002 to 0·04 per cent. of pure *d*-tartaric acid, but the crystals appeared longer and thicker with 0·06 per cent.; with 0·08 to 0·14 per cent. they became fewer in number. From solutions of 0·08 per cent. upwards calcium tartrate began to appear in increasing quantities, and with 0·2 per cent. no "S" crystals were to be seen. It is concluded that when the concentration of tartaric acid is less than 0·07 per cent. the calcium salts of *d*- and *l*-tartaric acids and the S-shaped crystals of racemic acid are all produced, but that the last only is insoluble. J. G.

Soya Bean Lecithin. F. Rothéa and F. Nielloux. (*J. Pharm. Chim.*, 1933, **125**, 443–445.)—Soya bean lecithin may be added to chocolate mass in small quantities (some 0·3 grm. per cent.) to facilitate working and milling. The crude commercial lecithin is of a brownish-yellow colour, and a yellow oily layer separates on standing. The material is soluble in chloroform and ether, and partly soluble in acetone; the insoluble part consists of true lecithin, which may be separated from mineral matter by dissolving in chloroform the residue insoluble in acetone, filtering, and evaporating the chloroform. The lecithin may also be obtained by dissolving the chocolate mass in chloroform to eliminate the mineral matter, and precipitating it with at least 5 times its volume of acetone. As obtained by the first method the lecithin consists of a soft, orange-brown, gummy, elastic and translucent substance, which is greasy to the touch, and has a slight odour. The lecithin separated by the second method is in small granules, which gradually assume the gummy translucent state. This lecithin, although very soluble

in chloroform and ether, differs from animal lecithin in being practically insoluble in ethyl alcohol. A specimen of the purified lecithin contained 2·76 per cent. of total phosphorus and 1·37 per cent. of total nitrogen, giving a ratio of P/N of 2·015, as compared with 2·07 for egg lecithin. If a weighed quantity of chocolate is extracted with chloroform or ether, the solvent evaporated, and the residue taken up with acetone, the lecithin will be left, and its percentage may be determined. D. G. H.

Comparative Action of Periodic Acid on α- and β-Glycerophosphoric Acids. New Method of Determining α-Glycerophosphates. P. Fleury and R. Paris. (*J. Pharm. Chim.*, 1933, **125**, 470–481.)—In agreement with the earlier observation that only those polyhydroxy compounds with an α-glycol function are attacked by periodic acid, it is found that this acid acts on α-glycerophosphoric acid, but not on its β-isomeride. For each molecule of the α-acid this reaction consumes one atom of oxygen, the molecule being split into one molecule of formaldehyde and one molecule of a compound which exhibits the properties of a phosphoric ether of glycollaldehyde. The reaction allows of the determination of α-glycerophosphoric acid in presence of the β-acid, the amount of periodic acid used up being readily determinable. Details of the procedure are given. T. H. P.

Colour Reaction for Glycerin. K. Täufel and H. Thaler. (*Z. anal. Chem.*, 1933, **95**, 235–239.)—Glycerin in small amount (100γ or, in some instances, 50γ) may be detected by oxidising it to acraldehyde and oxidising this further to epihydrin aldehyde, which gives a red coloration with phloroglucinol. A few ml. (0·5 to 2 for wine, 2 to 5 for fruit wine, 5 to 10 for fermentation vinegar) of the liquid to be tested are evaporated to dryness in a porcelain dish on a water-bath, and the residue is mixed with 2 grms. of 40 per cent. lime paste and again evaporated to dryness; substances like tooth-paste, fats, etc., are treated at once with the lime paste. The dry residue is moistened with water and again evaporated, this operation being repeated. The dry mass, preferably after being moistened with absolute alcohol, is powdered with the help of a glass rod and then extracted with three quantities (3 to 4 ml. each) of absolute alcohol, the total extract being evaporated to dryness on a water-bath. The very small residue is taken up in 3 ml. of an absolute alcohol-ether mixture (1:1) and the solution is filtered, 3 ml. of the same mixture being used for washing. The filtrate is evaporated in a small distilling tube closed by a rubber stopper covered with tinfoil and having the condenser jacket fitted directly on the side-tube. The residue, after addition of a little pumice powder and 15 drops of glacial phosphoric acid, is carefully heated by fanning it with a small flame; the distillation is continued until boiling almost ceases. The cooled distillate, collected in a small test-tube (100 × 13 mm.), is treated with one drop of 3 per cent. hydrogen peroxide solution, and 1 ml. of concentrated hydrochloric acid, cooled under the tap, and shaken vigorously for a minute; 1 drop of 10 per cent. potassium iodide solution is added to destroy the excess of the peroxide and just sufficient 10 per cent. sodium thiosulphate solution to react with the separated iodine. The solution thus obtained is shaken with 0·5 ml. of a 0·15 per cent. solution of phloroglucinol in ether. Within

30 minutes a red to violet-red coloration appears in presence of epihydrin aldehyde. Occasionally the reaction mixture turns yellow, observation of the red colour thus becoming difficult; in such case the liquid is diluted with a little water, which destroys the yellow colour and at the same time dissolves any separated sodium chloride. Sucrose, which readily yields methoxyfurfuraldehyde, interferes with the test, and must be inverted. To this end, the initial residue in the porcelain dish is heated for 10 minutes on a boiling water-bath with about 5 ml. of 4 per cent sulphuric acid, the water being replaced meanwhile. Three grms. of the 40 per cent. lime paste are then added and the test is completed as described above.

From 2 to 3 mgrms. of glycerin are readily detectable when mixed with glucose, lactose, maltose, sucrose, starch, glycol, mannitol or hens' egg albumin, these substances alone giving no red colour when subjected to the above procedure. The method is applicable also to fats, fatty acids, and therapeutic and cosmetic preparations. Sweet apple juice is found to be free from glycerin, fruit wines contain appreciably less than grape wines, and vinegars of different origins are distinguished by different glycerin-contents. Blood and other body fluids are under examination. T. H. P.

Determination of the Smouldering Capacity of Tobacco. V. L. Nagy. (*Chem.-Ztg.*, **1933**, **57**, 971–972.)—The use of powdered tobacco, rather than leaf or cut tobacco, is recommended for this test, and a device is described for measuring the speed of smouldering and the length of the layer of powder which burns. T. H. P.

Biochemical

Nutritive Value of Pure Fatty Acid Esters. N. M. Cox, Jr. (*J. Biol. Chem.*, **1933**, **103**, 777–790.)—The author has fractionated a natural fat (coconut oil) and has studied its various components by feeding experiments upon rats of the Wisconsin inbred strain. A brief summary is given of previously reported results of investigations of certain fatty glycerides; it is evident that the previous workers have used different species of experimental animals receiving diets of varying composition, and that relatively small amounts of the purified fat constituent were given. Feeding experiments have now been carried out on white rats with esters fractionated from coconut oil, and the series of saturated even-carbon fatty acids between 2 and 18 carbon atoms has been studied. It is shown that in the rat mixed ethyl esters permit of practically as good growth as do mixed triglycerides. The fat is well split, in contrast to the findings of others on dogs. When individual saturated fatty acid esters supply 77 per cent. of the caloric intake, nutrition, as measured by growth, *is in no case equal* to that obtained with mixtures of esters. Three portions of the saturated series resulted in death. Death occurred when ethyl butyrate and ethyl caproate were included in the diet because the animals usually refused to eat them. Ethyl palmitate and ethyl stearate did not support life because of inadequate absorption. With ethyl caprate, but more regularly with ethyl laurate, the rats died suddenly within 2 weeks. The toxicity is a function of the weight of the animal and of the level

at which the fat is given. No characteristic chemical, bacteriological, or pathological changes were demonstrable. It is shown that saturated fatty acids with chains shorter than 10 carbon atoms, when given to rats, fail to appear to any conspicuous extent in the body-fat of the animal, in contrast with the longer-chain acids, which appear regularly. P. H. P.

Metabolism of Azelaic Acid. H. G. Smith. (*J. Biol. Chem.*, 1933, **103**, 531–535.)—Azelaic acid, a dibasic acid formed by *in vitro* oxidation of most naturally occurring unsaturated fatty acids, was given to dogs in amounts smaller than would be produced normally in the body if oxidation took place at the unsaturated linkages. The experiments described show that the acid was but little utilised by the animals; an average of 60 per cent. of the ingested acid was recovered from the urine. The acid was not excreted in the faeces. The accumulating evidence thus seems to indicate that the metabolic oxidation of fatty acids proceeds in a manner independent of the presence or position of a double bond. P. H. P.

Stability of Carotene in Ethyl Esters of Fatty Acids, and in Liver and Vegetable Oils. F. G. McDonald. (*J. Biol. Chem.*, 1933, **103**, 455–460.)—Two years ago it was recommended that carotene should be adopted as the provisional standard for vitamin *A*. In the preparation of carotene solutions for administration to animals, the solvent used is important because of the unstable character of carotene. A study of the stability of carotene in various solvents and under different conditions was suggested by this instability. The following solvents were selected: three highly-purified esters (ethyl butyrate, ethyl laurate and ethyl palmitate), one liver oil (medicinal cod-liver oil), and three refined vegetable oils [maize oil, arachis oil and Wesson (cottonseed) oil]. Solutions of 0·05 per cent. of carotene in these solvents were prepared by warming the mixtures to about 30° C. for a few minutes, and were stored in partly filled, tightly-stoppered brown bottles at 37° C., 24° C. and 5° C. Similar solutions were made up in tubes, which were evacuated, sealed and stored at 37° C. The carotene used was a mixture of the α- and β-isomers which melted at about 172° C. At the end of 1, 2, 4 and 8 weeks the solutions were sampled for carotene determinations, care being taken to avoid excessive exposure. The determinations were carried out with the aid of a spectrograph and sector photometer by suitably diluting the solutions in benzene and making a series of sector photometer spectrograms. From the observed extinction limits of the $462m\mu$ carotene absorption band the quantity of carotene was calculated. From the results, shown graphically, it is seen that solutions of carotene in ethyl butyrate, ethyl laurate, ethyl palmitate and arachis oil stored in partly filled, tightly-stoppered brown bottles at 37° C., 24° C. and 5° C., and in evacuated sealed tubes at 37° C. decomposed very rapidly. The loss of carotene in cod-liver oil under similar conditions was also rapid at 37° C. and 24° C., but it was considerably retarded at 5° C. and, in the absence of oxygen, at 37° C. Solutions in maize oil and Wesson oil in stoppered bottles at 37° C. and 24° C. were unstable, but at 5° C. they kept well for 4 weeks, and showed a small loss in 8 weeks. In evacuated sealed tubes at 37° C. they showed a loss of carotene of 15 per cent. in 8 weeks. Therefore, solutions of known concentration can be made up in maize oil and Wesson oil, stored in bottles at 5° C., and can be

kept for as long as 4 weeks. It is evident that a combination of the favourable factors—absence of oxygen, low temperature, and the proper vegetable oil—constitutes the ideal condition for the storage of carotene solutions. P. H. P.

Micro-organisms and the Synthesis of Carotene and Vitamin *A*. C. A. Baumann, H. Steenbock, M. A. Ingraham and E. B. Fred. (*J. Biol. Chem.*, 1933, **103**, 339–351.)—Skinner and Gunderson (*J. Biol. Chem.*, 1932, **97**, 53) showed that a certain yellow *Corynebacterium* could synthesise a substance active in vitamin *A* from an inactive substrate, but did not determine whether vitamin *A* itself, its precursor carotene, or some other substance capable of functioning as vitamin *A* in the rat was produced. As vitamin *A* itself has not been found except in materials of animal origin, the authors have further examined this *Corynebacterium* to determine the nature of the biologically active material; they have also analysed cultures of other yellow organisms for carotene, and have made confirmatory tests by feeding rats which showed symptoms of vitamin *A* deficiency with the dried crude cultures of four different strains of these organisms. The results show that certain micro-organisms can synthesise carotene. Whenever an organism showed vitamin *A* activity, as determined by feeding experiments, enough carotene was found to be present to account for that activity. The vitamin *A* activity of micro-organisms did not appear to be affected by the presence of yellow pigments other than carotene. Since spectrographic determination failed to reveal an absorption band at 328$m\mu$, it is exceedingly improbable that vitamin *A* as such is generally present in bacteria. Attempts to effect the transformation of carotene into vitamin *A* by micro-organisms failed; the organisms used included various bacteria, yeasts, moulds, and mixed cultures from soil, manure, air, water, milk, sewage, and peat. If the evidence is accepted that carotene is actually synthesised by the organisms in question, then in this respect these organisms resemble plants more closely than animals, since carotene, when present in animals, is of exogenous origin. The absence of vitamin *A* from micro-organisms and their failure to transform carotene into vitamin *A* likewise associate these organisms with plants, since vitamin *A*, as such, has not been demonstrated in a plant material. The similarity in structure of the carotene and the phytol molecules, as well as the fact that carotene always accompanies chlorophyll in plants, has led to the belief that carotene synthesis is in some way associated with chlorophyll activity. Micro-organisms, however, illustrate the fact that the formation of carotene is not dependent upon the presence of chlorophyll.

P. H. P.

Vitamin *A* Content of Barley. E. H. Hughes. (*J. Agric. Res.*, 1933, **47**, 487–494.)—The vitamin *A* content of California barley was determined and compared with that of yellow maize and white maize by measuring the increase of body weight in rats and noting external evidence; by observations of the effect on the oestrous cycle of the rat, assuming the truth of the conclusion of Evans and Bishop (*Anat. Rec.*, 1922, 17–18), that the constant appearance of cornified cells in the vaginal smear is a characteristic test for deficiency of vitamin in the diet; and by the quantitative method of Smith (*J. Agric. Res.*, 1930, 1147). Barley was found to be low in vitamin *A* content, and as the only source of

vitamin *A* it did not produce normal growth in rats; in the sexually immature rat addition of vitamin *A* to a barley diet resulted in a decided increase in growth and a normal oestrous cycle. All the experiments showed that barley contains less vitamin *A* than yellow maize, and more than white maize. The amount appears to be less than one-sixth the amount in yellow maize. D. G. H.

Vitamin *A* Content of Pimiento Pepper. L. Ascham. (*Georgia Experiment Station Bull.*, No. 177, 1933.)—Pimiento pepper has been found to be rich in vitamin *A*, 2 mgrms. of dried pod giving a growth response in rats above that of the Sherman unit (*cf.* ANALYST, 1931, **56**, 492). The pepper contained 0·33 grm. of carotene per kilo. of dried material, as determined by the method of Zechmeister and von Cholnoky (*Ann. Chim.*, 1927, **70**, 455). It is computed that "a unit" of carotene, as determined by the results of pepper feeding, may conceivably be as low as, if not lower than, 0·0005 mgrm. D. G. H.

Losses of Vitamin *A* on Drying Fresh Raw Carrots, Sweet Potatoes and Canned Spinach. G. S. Fraps and R. Treichler. (*J. Agric. Res.*, 1933, **47**, 539–541.)—The loss in vitamin *A* on drying raw carrots, spinach, and sweet potatoes was determined, and the units of vitamin *A* were estimated by the Sherman and Munsell unit method. Raw yellow carrots contained approximately 43 units of vitamin *A* per grm. of fresh material or 377 units per grm. of dry material. Vacuum-dried carrots contained 77 units per grm., the carrots losing 80 per cent. of vitamin *A* when dried. Similarly, canned spinach after vacuum-drying lost 65 per cent. of its vitamin *A* (952 units per grm. of dry material being reduced to 333 units) and yellow Puerto Rico sweet potatoes lost 29 per cent. (70 units per grm. of dry material falling to 50 units). Fresh green vegetables may contain much higher amounts of vitamin than might be expected from experiments with the dried material. D. G. H.

Distribution of Vitamin *C* in Plant and Animal Tissues, and its Determination. O. A. Bessey and C. G. King. (*J. Biol. Chem.*, 1933, **103**, 687–698.)—The use of 2·6-dichlorophenol-indophenol has been found relatively satisfactory for the direct titration of vitamin *C* in animal and plant tissues. The authors describe various minor deviations from the method proposed by Tillmans (ANALYST, 1932, **57**, 260, 397). The titration of lemon juice with the dye and then with standard iodine solution has proved a rapid and satisfactory procedure for standardisation of the dye indicator, 2, 6-dichlorophenolindophenol. Acetic acid (8 per cent.) has been found preferable as an extracting and titrating medium with most plant tissues, whilst trichloroacetic acid (8 per cent.) is preferable with most animal tissues. Cysteine, glucic acid and heated sugar solutions may interfere seriously if present; glutathione and various other substances may interfere unless special precautions are taken. In interpreting the results it is necessary to take into consideration possible animal assays and the effect of interfering substances. Tables show comparisons of animal assays with dye titrations, and the amounts of vitamin *C* found in tissues of various animals. The adrenals and corpus luteum contain nearly the same amount of vitamin *C* (approximately 1·4 to 2·3 mgrms. per grm.); the brain, liver, testes, ovaries, and other glandular

tissues comprise a second group, with considerably lower range (approximately 0·1 to 0·4 mgrm. per grm.); the more active muscular tissues, such as the heart, comprise an intermediate group (*e.g.* 0·05 to 0·15 mgrm. per grm.), whilst lean muscle contains only about 0·04 mgrm. per grm. There is a close relationship with the complex lipid content of animal tissues and also with the general rate of metabolism or respiration in the individual tissues. In younger animals the vitamin *C* content of the tissues tends to be higher than in older animals. In human tissues the distribution of vitamin *C* is similar to that in experimental animals. For those animals that require a direct dietary source of vitamin *C* the time required for marked general tissue depletion is apparently much shorter than the time required for symptoms of scurvy to appear. P. H. P.

Bacteriological

Reddening of Salted Hides. L. S. Stuart, R. W. Frey and L. H. James. (*U.S. Dept. of Agric., Tech. Bull.* No. 383, 1933.)—"Red heat" in salted hides is associated with the type of curing salt used. Red chromogenic organisms have been found in 34 out of 35 samples of crude solar-evaporated salts, and in 25 out of 39 open-pan evaporated granular salts. All the kiln-dried solar-evaporated salts (12 samples), all the vacuum salts (17 samples), and all the rock salts (62 samples) were found to be free from these organisms. The culture medium was a modification of Clayton Gibbs fish-salt-rice medium, in which the fish broth was replaced by hide broth. Growth could also be induced by inoculating salted calf skin and incubating at temperatures from 20° to 55° C. Chromogenic growths on salted hides are usually of a highly mixed flora, but by employing a large number of dilutions on dextrose agar, apparently pure cultures of red organisms can be obtained, and these under specified conditions will produce a reddening of the flesh of salted hides and skins. Repeated propagation on media of high salt-content markedly increases the salt tolerance of the organisms. They are identified provisionally as belonging to the *Thiobacteriales* or the *Myxobacteriales*. A very complete bibliography is appended, and is fully reviewed in the text. R. F. I.

Mould-growth Test for Minute Amounts of Arsenic. H. R. Smith and E. J. Cameron. (*Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 400–401.)—The authors have utilised, as a test for arsenic, the property of certain mould growths of liberating arsenic as a gas with a garlic-like odour, found by Challenger and his co-workers to be trimethylarsine (ANALYST, 1933, **58**, 235; *J. Chem. Soc.*, 1933, 95). It is claimed that 1 part per million of arsenic may be readily detected in a sample of 1 grm. or less. The mould *Scopulariopsis brevicaulis* (*Sacc.*) *Bainier*, as described in detail by Thom (*The Penicillia*, 1930), was used, with Czapek's* mixture as the medium for spore-growth. The following method was adopted:—A thin layer of melted Czapek's medium is placed in a Petri dish under sterile conditions, allowed to harden, and inoculated with 3 or 4 drops of

* Czapek's medium contains 1000 ml. of water, 3 grms. of sodium nitrate, 1 grm. of potassium monohydrogen phosphate, 0·5 grm. of magnesium sulphate, 0·5 grm. of potassium chloride, 30 grms. of sucrose, and 15 grms. of agar-agar.

aqueous spore suspension, which are made to cover the surface of the medium as completely as possible, and the whole is incubated for 48 to 72 hours at 30° C. until an obviously active growth develops. The sample (see below), about 1 grm., is distributed over the surface of the mould growth; the dish is covered and is allowed to remain at room temperature, and is tested for the characteristic odour by gently lifting the cover and smelling after periods of one-half, 1, 2 and 5 hours. If small amounts of arsenic (10^{-6} grm.) are present, the odour should be apparent in 2 to 5 hours, and sooner with larger amounts. The sample, if solid, should preferably be dry and ground to a coarse powder; if it is very fine, *e.g.* flour, it should be mixed with sodium carbonate, completely charred, and an extract of the residue in dilute hydrochloric acid used for the test; strongly smelling samples should likewise be charred and the extract used; oily constituents should be removed by previous extraction with a solvent. Liquid samples should first be rendered very slightly acid to litmus, and concentrated by evaporation, if necessary. Appreciable amounts of antimony do not interfere, but inorganic mercury compounds inhibit the test. Selenium and tellurium give odours similar to that of arsenic. S. G. C.

Toxicological and Forensic

Silicosis. W. R. Jones. (*J. Chem. Met. and Mining Soc., S. Africa*, 1933, **34**, 99–123.)—The author has previously described (*J. Hyg.*, 1933, **33**, No. 3) a technique for the examination of silicotic lungs, whereby the dust is left in a form in which it can be analysed chemically. For examination under the petrological microscope the dust is mounted in Canada balsam in the usual way. In all the residues of silicotic lungs examined there were countless mineral fibres, which, from their form, cleavage, sp.gr., birefringence and optical orientation, were identified as fibres of sericite. Most of the acicular, flexible fibres in lung residues range from 0·5 to 2μ in length, and from 0·1 to 0·5μ in thickness; many of them are bent towards their sharper ends.

Sericite, also known as "secondary white mica," is a hydrated silicate of aluminium and potassium. A specially pure specimen contained: silica, 46·58; alumina, 37·46; ferric oxide, 0·80; magnesia, 1·16; potash, 6·38; soda, 0·64; and water, above 110° C., 6·06; below 110° C., 0·30 per cent. (Shannon, *U.S.A. Nat. Mus. Bull.*, No. 131, p. 372). Certain minerals, notably potash felspars, under certain conditions of pressure, temperature, etc., become "sericitised," *i.e.* changed into minute scales and aggregates of acicular fibres of sericite. These fibres are held only loosely in the body of the rock, so that during the impact of drilling or blasting they are readily disseminated into the surrounding atmosphere. Sericite is most common in rocks that contain a high percentage of free silica in the form of quartz; it is not present, however, in all rocks that contain quartz. It is the dust from those rocks which contain much sericite that has been proved to cause silicosis. The dust from rocks containing little, if any, sericite, but containing a very high percentage of quartz, higher even than in rocks that have proved dangerous, has never given rise to a single authenticated case of silicosis.

Analyses of Mineral Residues from Silicotic Lungs.—Complete chemical

analyses of three mineral residues were made by A. W. Groves at the Imperial College of Science and Technology, with the following percentage results:

Mineral residue from	SiO_2	Al_2O_3	Fe_2O_3	FeO	CaO	MgO	K_2O	Na_2O	TiO_2	P_2O_5	MnO	Water			Total
												Below 110°C.	110°C. to 350°	Above 350°	
Lung A	49·52	21·99	3·55	—	—	1·73	2·47	0·78	0·08	2·55	—	2·18	0·51	0·33	97·49
Lung B	62·16	19·85	—	0·19	0·90	0·84	3·83	0·52	0·64	5·77	0·01	10·37	0·20	0·26	100·11
Lung C	59·23	26·38	—	0·11	0·45	0·62	0·78	0·64	0·78	2·68	0·01	2·27	1·39	0·56	99·57

Lung A was that of a man, 66 years of age, who had been engaged in the pottery industry for 50 years as earthenware hollow-ware jigger. Certified silicosis.

Lung B was that of an underground colliery worker, Swansea district, employed as a hard-heading worker. Certified silico-tuberculosis.

Lung C was that of a collier at Pontycymmer, S. Wales, aged 51 years, who had also worked as a quarryman in N. Wales. Certified silico-tuberculosis.

Under the petrological microscope these three residues were found to consist mainly of minute acicular fibres of sericite, but in the potter's lung there was also a little clay. Some of the alumina in this residue should, therefore, be allocated to kaolin (SiO_2, 43·5; alumina, 36·9; water, 19·6 per cent.), but, as the ratio of alumina to silica in kaolin is not appreciably different from the ratio in sericite, any correction would influence the results only to a minor extent. The presence of this aluminium silicate explains, however, the lower potash-content in relation to alumina in A than in B and C. The water determinations proved that the fibrous mineral was not one of certain mineral constituents of clay that lose all their water at temperatures well below 350° C. If the alumina in these three residues is allocated to the amounts of silica, potash and water required to form sericite, the following results are obtained:

	A Per Cent.	B Per Cent.	C Per Cent.
Sericite ..	55·83	51·01	59·48
Free silica ..	22·04	37·35	19·66

It is important to note that the few large grains of quartz in these residues contribute their quota of free silica in the analyses out of all proportion to their probable silicotic effect in the lung. It is mainly the presence in the rocks of fibrous minerals, such as sericite, sillimanite, tremolite, etc. (or a fibrous form of free silica as in chert, or a fibrous rock as in pumice) in aggregates which are liberated during drilling, etc., into the atmosphere as individual fibres, that enables sufficient material in course of time to enter the lungs to cause silicosis. It is not contended that sufficient minute particles of quartz could not, under any conditions, enter the lungs to cause silicosis, although the cases investigated appear to show conclusively that they have not done so; but it is maintained that the fibrous minerals hasten the process so greatly that their presence in the exploited rocks and materials is of far greater importance than the presence of quartz in causing the disease. (*Cf.* ANALYST, 1933, **58**, 775.)

Spectro-photometric Study of Fluoro-Methaemoglobin for Detecting Methaemoglobin and for Determining Fluorides. R. Fabre and (Mlle) S. Bazille. (*J. Pharm. Chim.*, 1933, **125**, 465–470.)—In presence of a small quantity of sodium fluoride, acid methaemoglobin exhibits a spectrum composed of two bands—one very intense—in place of the four bands shown in absence of

fluoride. By spectro-photometric examination of blood containing fluoride, it is possible to detect one part of methaemoglobin in the presence of about nine parts of haemoglobin. For the zone $\lambda = 6100$ to 6200 Å.U., the position of maximum absorption varies little with methaemoglobin solutions containing from 0·1 to 2 mgrms. of sodium fluoride per 10 ml., and within these limits of concentration it is possible, by measuring the optical density at the maximum absorption, to determine the fluoride-content with satisfactory accuracy. T. H. P.

Organic Chemistry

Indicator Properties of *p*-Nitrophenyl-acetyl-hydrazine, 2, 4-Dinitrophenyl-acetyl-hydrazine, and 2, 4, 6-Trinitrophenyl-acetyl-hydrazine. A. Bloom and A. Osol. (*Amer. J. Pharm.*, 1933, **105**, 551–553.)—The nitrophenyl-acetyl-hydrazines were prepared by acetylisation of the corresponding nitrophenyl hydrazines with an equivalent weight of acetic anhydride, glacial acetic acid being used as solvent, and the mixture boiled for several hours under a reflux condenser. The precipitated nitrophenyl-acetyl-hydrazines were filtered off and recrystallised from alcohol. The three products had the following melting points: (1) *p*-nitrophenyl-acetyl-hydrazine, 196·8° C.; (ii) 2, 4-dinitro-phenyl-acetyl-hydrazine, 209–210° C.; (iii) 2, 4, 6-trinitrophenyl-acetyl-hydrazine, 222–223° C. Their indicator properties were compared with those of methyl red. All three were satisfactory for the titration of strong acids, the acid colours in each case being pale green, and the alkaline (i) brown, (ii) reddish-brown, and (iii) orange-brown, respectively. These indicators are not suitable for the titration of alkaline solutions, since they gradually fade, and the colour-changes are indistinct. D. G. H.

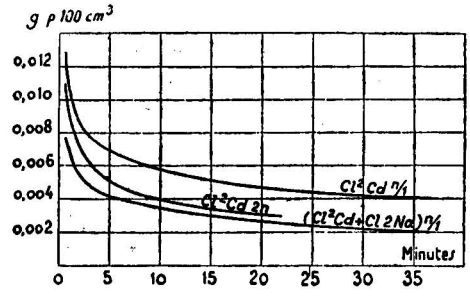
Determination of *o*-Toluidine. S. Ueno and H. Sekiguchi. (*J. Soc. Chem. Ind., Japan*, 1933, **36**, 613B.)—About 1 grm. of the *o*-toluidine is converted into the hydrochloride by adding 1 ml. of concentrated hydrochloric acid. After being well mixed a small portion is spread on a watch-glass and left overnight in a desiccator containing sulphuric acid, after which its m.pt. is determined.

The m.pts. of anhydrous *o*-toluidine hydrochloride in the absence and presence of the *p*- and *m*-isomers were as follows, and from these results the degree of purity of a sample may be deduced.

o-Toluidine Per Cent.	*p*-Toluidine Per Cent.	*m*-Toluidine Per Cent.	M.pt. of hydrochloride observed. °C.
100	—	—	216
98	2	—	213·8
96	4	—	211·6
94	6	—	209·7
92	8	—	207·4
90	10	—	205·4
98	—	2	213·8
96	2	2	211·9
96	—	4	212·1
94	4	2	210·0

Since the hydrochloride is very hygroscopic, it is recommended that in very rainy weather the m.pt. of the hydrobromide should be taken. R. F. I.

Determination of Pyridine in Dilute Aqueous Solution. V. Ionescu and H. Slusanchi. (*Bull. Soc. Chim.*, 1933, **53**, 1087–1096.)—The addition of cadmium chloride to sufficiently concentrated aqueous pyridine results in the formation of a crystalline precipitate of cadmium pyridine chloride. The corresponding reaction with mercuric chloride is more sensitive. The sensitiveness of both reactions increases with increased addition of cadmium or mercuric chloride, more particularly if sodium chloride is also present. Under specified conditions, as selected for the method below, relations have been found to exist between the time required for the appearance of the precipitate and the concentration of pyridine, when either *N* cadmium chloride-sodium chloride solution or *N* mercuric chloride-sodium chloride solution are employed as the reagent; the relations are shown as curves in the figure, in which the ordinates show the concentration of pyridine (grm. per 100 ml.) in the final solution *after* the addition of 50 per cent. by volume of reagent, and the abscissae show the time in minutes for the appearance of the precipitate. The method proposed for the determination of pyridine is as follows:



To a sample of the solution contained in a test-tube is added 50 per cent. by volume of the reagent. The mouth of the test-tube is closed with the thumb, and it is inverted 2 or 3 times to mix the contents. The liquid is kept under observation with the aid of a lens, and the period of time up to the appearance of the first signs of the precipitation of acicular crystals is noted. This time, multiplied by 1·5, gives the time from which the corresponding concentration of pyridine can be read from the curves. The temperature of the solution should be 16 to 18° C. Some preliminary trials may be required to find the appropriate dilution of the sample solution to be used in the method, such that the time determined shall fall within the limits of the curves. The limits of sensitiveness of these reactions for pyridine are 3 parts in 10,000 parts with the cadmium reagent, and 0·45 part in 10,000 parts with the mercury reagent; the mercury reagent should thus be used for the weaker solutions of pyridine. S. G. C.

Determination of Sulphur in Benzene. H. A. J. Pieters, J. Van Iterson and S. J. H. Spronck. (*Chem. Weekblad*, 1933, **30**, 756–762.)—*Free Sulphur.*—Existing methods are reviewed. In that of the *Benzol Verband* (*ibid.*, 1933, **30**, 675) the benzene is evaporated, the residue is heated with sodium hydroxide solution and hydrogen peroxide, and the resulting sulphate is determined in the usual way. This is supposed to give "α-sulphur," which is held to be responsible for the corrosion of copper; actually, complete recovery of added sulphur (5 to 40 mgrms. per litre) was obtained. The copper stain-test (*cf.* Ormandy and Craven, *J. Inst. Pet. Tech.*, 1923, **9**; 1925, **11**) will detect over 5 mgrms. per litre, but is not quantitative, and the following method, adapted from that of E. Dittrich (*Brenstoff-chem.*, 1933, **14**, 282), gives results as accurate as the first method (for 1·7 to 25·3 mgrms. of sulphur per litre), but in much less time:—100 to 1000 ml. of the

sample are shaken for 30 minutes with 2 ml. of pure mercury, and filtered, and the residue is washed first with pure benzene and then with a little warm water. The filter and its contents (sulphur as mercuric sulphide), together with 2 grms. of aluminium turnings, are placed in a distillation flask (a special apparatus is described), which is then closed, and the air is displaced by means of a stream of carbon dioxide. Concentrated hydrochloric acid (50 ml.) is then added through a funnel attached to the flask, a little at a time, and the gas evolved is washed first in 50 ml. of water and then in two wash-bottles, each containing 50 ml. of a mixture prepared by diluting a solution of 25 grms. of cadmium acetate in 200 ml. of glacial acetic acid to 1 litre; the second serves as a control. The flask is warmed towards the end of the reaction, which is complete after 20 to 30 minutes, and the contents of the cadmium acetate wash-bottles are then transferred to an Erlenmeyer flask and are mixed with 10 ml. of 0·1 *N* iodine solution and 5 ml. of 4 *N* hydrochloric acid. The flask is stoppered for 2 minutes, and the excess of iodine is then titrated with 0·05 *N* sodium thiosulphate solution. *Carbon disulphide.*—If this is less than 500 mgrms. per litre the golden-brown colour produced on mixing, in the order given, 1 ml. of sample, 1 ml. of a 1 per cent. solution of diethylamine in pure benzene, 1 ml. of a 0·03 per cent. solution of crystalline copper acetate in cold alcohol, and 7 ml. of alcohol, is matched within 20 minutes against the colour produced from standards containing 1 to 0·01 per cent. of carbon disulphide in pure benzene (*cf.* Callan, Henderson and Strafford, ANALYST, 1932, **57**, 590). For larger quantities, 20 to 100 ml. of sample are evaporated with 10 ml. of a solution prepared by dissolving potassium hydroxide in the minimum amount of water and subsequently diluting with 96 per cent. alcohol until the strength is 6 per cent., and filtering on the following day. The residue is warmed with 30 ml. of 3 per cent. hydrogen peroxide for 20 minutes, after which it is cooled, and the excess of alkali over the sulphuric acid so produced is back-titrated with 0·1 to 0·5 *N* sulphuric acid ($1H_2SO_4 \equiv \frac{1}{2}CS_2$) with bromphenol blue as indicator (*cf.* Hoffert and Claxton, *Motor Benzole*, 1931). *Thiophen* (after Schwalbe).—The blue colour produced from 1 ml. of sample and 25 ml. of a 0·05 per cent. solution of isatin in concentrated sulphuric acid is matched, using a series of standards containing from 1 to 0·01 grm. of thiophen per litre. *Total Sulphur.*—A modification of Davidson's procedure (*Gas. J.*, 1920, **185**, 95; Hoffert and Claxton, *loc. cit.*) is preferred to the B.E.S.A. (1921, No. 35) method. An apparatus is pictured and described in which the sample is diluted with 4 volumes of 96 per cent. alcohol and is fed by gravity, with sulphur-free air, into a special burner placed in the wide limb of a U-tube, the other limb being a measuring burette connected to it by means of a capillary tube. The lamp is enclosed, by means of a mercury-seal, in a chimney which forms the wide limb of the tube and conveys the products of combustion into wash bottles, where sulphur compounds are oxidised to sulphuric acid by means of 1·5 per cent. hydrogen peroxide neutralised with potassium hydroxide solution, the sulphuric acid being then precipitated or titrated. J. G.

Chemistry of Australian Timbers. Part 3. Chemical Composition of Four Pale-Coloured Woods of the Genus *Eucalyptus*. W. E. Cohen, A. G. Charles and A. B. Jamieson. (*Commonwealth of Australia, Council of*

Sci. and Indust. Res., Pamphlet No. 44, Aug. 1933.)—The Table shows the average values for 4 woods of the Genus *Eucalyptus* ("Ash" group) as determined by the methods previously described (*cf.* Cohen and others, ANALYST, 1932, **57**, 101;

Species	Number of Samples	Ash	Alkalinity of ash (ml. of 0·1*N* H_2SO_4 per grm.)	Solubility in: Hot Water	Cold Water	Alcohol	Ether	*N*/8 Sodium hydroxide Sol.	Benzene Alcohol (2:1)	Pentosans: Total	Pentosans: Cellulosic	Lignin	Cellulose
		Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.
E. gigantea ..	6	0·06	0·09	4·0	1·9	4·3	1·1	16·9	1·5	20·8	10·9	19·9	56·9
E. obliqua ..	20	0·05	0·03	14·3	9·5	15·1	1·3	26·6	7·7	15·0	7·5	21·1	46·6
E. regnans ..	13	0·07	0·06	10·2	5·0	6·7	0·5	20·1	3·2	17·6	8·5	20·4	49·6
E. sieberiana ..	10	0·06	0·09	9·2	6·4	10·1	1·4	23·7	6·4	17·0	9·5	19·9	50·0

1933, **58**, 635, 636), and expressed as percentages on the wood dried at 105° C. These woods vary from white to pale brown in colour, and are of open texture, low density and moderate durability. They occur in the east of Australia and in Tasmania and are used for constructional work, furniture and pulping. Identification by physical characteristics is difficult; the principal chemical features are the relatively low ash and extractive contents, and high cellulose values. Except for *E. obliqua*, which contained variable amounts of extraneous matters, the values of all determinations for each species were uniform. Apart from certain chemical points of resemblance in the case of *E. gigantea*, there are few similarities between these woods and the North American hardwoods, the cellulose, ash and total pentosan contents in particular being lower in the former case; mesquite is excluded from the latter, since it resembles *E. obliqua*, although only in chemical composition. Distinction of *E. gigantea* and *E. regnans*, and in some cases of *E. obliqua*, is difficult by macroscopic and microscopic methods, but the ratios of solubility in *N*/8 sodium hydroxide solution to solubility in (*a*) hot water, (*b*) alcohol, and (*c*) in the mixture of benzene and alcohol, are useful diagnostic characters, maximum and minimum values being, respectively:—*E. gigantea*, (*a*) 6·3, 3·3; (*b*) 9·4, 3·2; (*c*) 12·7, 7·3. *E. obliqua*, (*a*) 2·7, 1·3; (*b*) 2·3, 1·4; (*c*) 6·2, 1·9; *E. regnans*, (*a*) 3·5, 1·3; (*b*) 11·5, 1·8; (*c*) 25·4, 3·6; *E. sieberiana*, (*a*) 6·6, 1·9; (*b*) 4·7, 1·6; (*c*) 5·7, 2·5. A diagrammatic scheme of chemical analysis has been evolved, the value of which, however, is limited by the facts that the samples of *E. gigantea* were all from one locality, and that there are no chemical values that can be used in all cases to separate definitely one species from all the others. J. G.

Inorganic Chemistry

Determination of Nitrogen by Combustion in the Electric Arc. W. D. Treadwell and T. Zürrer. (*Helv. Chim. Acta*, 1933, **16**, 1180–1187.)—Nitrogen in noble gases is determined either by absorption over lithium or by combustion with excess of oxygen, with the aid of an electric spark (Cavendish). The latter procedure is very slow, which is ascribed to lack of movement in the gas. The authors expedite the combustion by rapid circulation of the gases. The circuit includes a flask containing dilute caustic soda solution, the progress of the neutralisation of which by the nitric oxides is observed conductometrically. The decrease in conductivity is proportional to the nitrogen. The paper contains a description

with illustrations of the apparatus and directions for its use. The speed of combustion of the nitrogen is given as 0·2 to 0·3 ml. per minute with an arc of about 20 watt. The last of the nitrogen combines at a lower speed (about 0·1 ml. per minute), the determination requiring 15 to 120 minutes according to quantity.

W. R. S.

Determination of Lead by means of Picrolonic Acid. F. Hecht, W. Reich-Rohrwig and H. Brantner. (*Z. anal. Chem.*, 1933, **95**, 152–163.)—The determination is analogous to that of calcium with the same reagent (ANALYST, **1931, 56**, 832). The lead precipitate forms pale greenish-yellow crystals of the composition $Pb(C_{10}H_7N_4O_5)_2.1{\cdot}5H_2O$ (Pb factor, 0·2725). The solubility of the compound is equivalent to 6·61 mgrms. of lead per litre. The neutral nitrate solution, as free as possible from alkali and ammonium salts, and containing 0·1 grm. of lead in about 50 ml., is heated to incipient boiling, and precipitated, drop by drop, during agitation, with the required quantity of the reagent (100 ml.), *i.e.* a 0·01 *N* solution containing 2·64 grms. per litre. A further addition of half the quantity of reagent previously used is then made all at once. The liquid is cooled in an ice-chest, filtered through a porous crucible, and the precipitate is washed with a minimum of ice-cold water until the washings are colourless. Constancy of weight is reached by drying at 130 to 140° C. for 1 to 1½ hours. The procedure was also found suitable for micro-work, the technique for which is described.

W. R. S.

Use of Amyl Alcohol in the Sodium Diethyl Dithiocarbamate Method for the Determination of Copper. R. W. Thatcher. (*J. Amer. Chem. Soc.*, **1933, 55**, 4524.)—A difficulty is sometimes found in securing a sharp separation of the amyl alcohol layer in the MacFarlane method for determining the presence of copper in organic tissues (*J. Biol. Chem.*, 1932, **26**, 1022). The difficulty may be overcome by the use of isoamyl alcohol instead of normal amyl alcohol. This difference in behaviour is possibly due to the lesser solubility in water of the iso compound.

R. F. I.

Osmium Tetroxide as Catalyst for the Oxidation of Arsenious Acid. K. Gleu. (*Z. anal. Chem.*, 1933, **95**, 305–310.)—In 0·5 *N* sulphuric acid solution, the oxidation of arsenious acid by means of permanganate or ceric sulphate proceeds stoichiometrically after addition of 3 drops of a 0·01 *m* solution of osmium tetroxide in 0·1 *N* sulphuric acid (bulk, 100 to 200 ml.). The oxidation proceeds quite smoothly to a sharp end-point, provided that the titration is not hurried. Ceric sulphate, which has no action on arsenious acid in sulphuric acid solution, acts at once in presence of a little osmium tetroxide. The indication of the end-point is given by ferro-*o*-phenantrolin, which is decolorised by oxidation after complete oxidation of the arsenious acid.

W. R. S.

Separation of Zinc from Aluminium. J. N. Frers. (*Z. anal. Chem.*, 1933, **95**, 1–36, 113–142.)—The paper contains a detailed examination of the subject and an investigation of the method adopted, *viz.* precipitation of zinc sulphide from feebly acid solution. The procedure is as follows: The solution of 0·2 grm. Zn (or less) [containing not more chloride than is equivalent to the (Al+Zn), otherwise

evaporation with sulphuric acid is necessary] is treated with enough ammonium sulphate to provide a 4 per cent. solution after dilution to about 400 ml., and 20 drops of tropaeolin OO indicator, and is neutralised with pure ammonia until the tint matches that of the indicator in the washing liquor (4 per cent. ammonium sulphate) under the same conditions. After addition of filter pulp (half an 11-cm. paper) the solution is boiled and submitted, while cooling, to a vigorous current of hydrogen sulphide for ten minutes, then to a moderate current for half an hour. The precipitate is collected and washed with the hot wash-liquor saturated with hydrogen sulphide. The paper may be kept filled with distilled water in the funnel for an hour before use (method not stated); this prevents any cloudiness of filtrate. Filter and precipitate are ignited in a glazed porcelain crucible with liberal admission of air; the oxide is then treated for 10 minutes with a current of hydrogen sulphide while the bottom of the crucible is heated to incipient red heat. After cooling for 5 minutes in the gas stream, the crucible may be removed. The precipitate is weighed as ZnS. The aluminium in the filtrate from the zinc sulphide is determined in the usual manner by neutralisation with ammonia or by boiling with sodium thiosulphate. W. R. S.

Antimony as Indicator Electrode in the Potentiometric Titration of Iron and Aluminium. E. W. Kanning and F. H. Kratli. (*Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 381–383.)—The titration of ferric and aluminium chlorides with sodium hydroxide solution has been followed potentiometrically, an antimony indicator electrode being employed and its potential being measured against a saturated calomel electrode. With ferric chloride solutions a jump in the potential occurred at the point of precipitation of ferric hydroxide; with aluminium chloride a jump at a higher value of potential occurred, corresponding with the precipitation of aluminium hydroxide, followed by a less distinct jump when the aluminium hydroxide re-dissolved, forming sodium aluminate. The potential jumps enabled the end-points to be located accurately with ferric and aluminium chlorides separately, but with mixtures the potential changes were less pronounced, and the "end-point" for the equivalence point of ferric hydroxide was too early, and that for aluminium hydroxide was too late, rendering the method only an approximate one. The titrations were less precise with 0·1 *N* than with *N* sodium hydroxide solution. The presence of alkali sulphate caused more or less considerable errors. The hydrogen electrode, owing to the slowness with which equilibrium values of potential were reached, was found less satisfactory than the antimony electrode in the above titrations. S. G. C.

Indirect Volumetric Determination of Chromium. A. Ionesco-Matiu and S. Herscovici. (*Bull. Soc. Chim.*, 1933, **53**, 1032–1038.)—The process is an application of a general "mercurimetric" method, due to the authors, in which mercuric sulphate is titrated with sodium chloride in the presence of sodium nitroprusside, which acts as an indicator by forming a turbidity of mercuric nitroprusside which dissolves to a clear solution when sufficient chloride has been added to convert the mercury into mercuric chloride. The scheme adopted is to precipitate the chromium, which must be present as chromate, as mercurous chromate, and to dissolve this precipitate, after centrifuging and washing, in

dilute sulphuric acid containing potassium permanganate, thus obtaining mercuric sulphate, which is titrated as indicated. An empirical relation between the mercury and the chromate has had to be worked out, since the composition of the mercurous chromate as precipitated lies between those of normal and basic mercurous chromate. For further details the original paper should be consulted.

S. G. C.

Determination of Alkalis as Silicofluorides. W. D. Treadwell and W. König. (*Helv. Chim. Acta*, 1933, **16**, 1201–1208.)—When alkali chloride, nitrate, or fluoride is evaporated with a sufficiency of hydrated silicic acid and excess of hydrofluoric acid, it is converted into pure silicofluoride, which can be heated until constant in weight. The solution is treated in a small platinum basin with 3 times the theoretical amount of silicic acid and 1 to 2 ml. of re-distilled 40 per cent. hydrofluoric acid per 0·1 grm. of added silica. The liquid is evaporated to dryness, and the residue is heated for 1 to 2 hours in an oven at 120° C., after which it can be weighed in the open basin. The silica is prepared from waterglass and hydrochloric acid, the jelly being repeatedly dissolved in pure caustic soda solution, and re-precipitated; after being freed from chloride by washing, it is dried on the water-bath. The alkali silicofluoride may also be titrated after solution in 10 ml. of water, addition of 10 to 25 ml. of strictly neutral 4 *N* calcium chloride solution, and short heating: $Na_2SiF_6+3CaCl_2+3H_2O=3CaF_2+H_2SiO_3+4HCl+2NaCl$. The liberated acid is titrated against bromcresol purple. Titration of the weighed mixed silicofluorides permits of the indirect determination of two alkalis. Very good results are obtained with lithia. The separation from magnesium is best effected by means of *o*-hydroxyquinoline (ANALYST, 1927, **52**, 431), especially if lithium is present; the filtrate containing the alkalis is evaporated to dryness, and the residue is cautiously ignited, and dissolved in hydrochloric acid, and the carbonaceous particles are filtered off. The filtrate is evaporated, and the residue is converted into silicofluoride as described above. W. R. S.

Determination of Small Quantities of Fluorine by the Steiger-Merwin Reaction. I. Optimum Conditions, and Interference. II. Details of Procedure. H. J. Wichmann and D. Dahle. (*J.A.O.A.C.*, 1933, **16**, 612–619, 619–624.)—The conditions governing the bleaching effect of the fluorine ion on the colour of a peroxidised titanium solution (*cf.* Steiger, ANALYST, 1908, **33**, 139; Merwin, *Amer. J. Sci.*, 1909, **28**, 119) have been investigated. This effect increases as the p_H value of the solution increases to 1·5, and subsequently decreases to practically zero at p_H about 2·5. Moreover, the bleaching action per unit of fluorine increases as the concentration of fluoride increases and as that of titanium diminishes, and, as far as a certain limit, it is proportional to the quantity of fluorine present. Interference with the effect is caused by a number of substances, including the colouring matter of fruit, phosphates, aluminium, iron, and sulphates.

The results of this investigation form the basis of the following procedure, which gives satisfactory results when applied to the determination of fluoride in apple peelings, cabbage, and rat feed containing known amounts of fluorides. The sample is ashed, and the ash is distilled at 135° C. with sulphuric or perchloric acid. Of the distillate, filtered if necessary, an aliquot part estimated to contain

between 0·01 and 0·05 mgrm. of fluorine is placed in a Nessler tube, together with 1 ml. of titanium chloride solution (2 ml. of a 20 per cent. solution and 20 ml. of hydrochloric acid, made up to 1 litre), and 1·3 ml. of hydrochloric acid (1+9). The liquid is then made up nearly to the 50-ml. mark with water, 2 ml. of hydrogen peroxide solution (5 ml. of the 30 per cent. solution made up to 100 ml.) being added and the tube filled to 50 ml. and inverted to mix. The p_H value is determined and adjusted to 1·5 ± 0·02. Samples containing appreciable amounts of chloride or nitrate give distillates containing mineral acid, and experimental adjustment of the p_H is then necessary.

When the necessary amount of acid is ascertained, a second sample tube is prepared, the volume of hydrochloric acid added being adjusted as found necessary. This tube is compared with the standard tubes. These are made by adding, to six of the Nessler tubes, 1 ml. of the titanium reagent and sufficient of the dilute hydrochloric acid (about 1·3 ml.) to give the p_H value 1·5 ± 0·02. Next are added to the six tubes 0, 1, 2, 3, 4, and 5 ml., respectively, of sodium fluoride solution (containing 0·01 mgrm. of fluorine per ml.); 40 ml. of water are added to each, then 2 ml. of the peroxide solution, and sufficient water to make 50 ml. The tubes are inverted to mix, and the p_H value of each is determined to see that this is 1·5 ± 0·02 in all cases. If the colour of the sample tube does not fall within those of the 0·01 and 0·05 mgrm. standard tubes, another aliquot portion of the distillate must be used, and the amount of acid necessary to give the p_H value 1·5 determined anew. If the fluoride-content of the sample is very small, it may be necessary to concentrate the distillate from the ash—after making it distinctly alkaline—before proceding with the test. Four samples of Arizona drinking water showed from 0·45 to 3·92 parts of fluorine per million. Sprayed apples showed 2 parts; sprayed cabbage, 33·6 for the outside leaves, and 2·7 for the remainder of the head; sprayed celery (2 samples), 7·6 and 3·6 in the petioles, and 77·1 and 135·3 in the leaves. Strawberry juice, preserved with fluoride, gave 141 parts of fluorine per million.

T. H. P.

Volumetric Determination of Small Quantities of Iodine. J. F. Sadusk and E. G. Ball. (*Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 386–389.)—Some factors affecting the sensitiveness and accuracy of the volumetric method which involves oxidation of iodine to iodate and subsequent titration with thiosulphate have been investigated, and the following modified method was developed:—To the neutral iodide solution (50 ml.), contained in a 125-ml. Erlenmeyer flask, are added 1 ml. of 2 *N* sulphuric acid and sufficient bromine to colour the liquid yellow; the bromine is added as vapour, obtained by passing air through liquid bromine contained in a gas-washing bottle, and delivered by a jet just above the surface of the iodide solution, which is swirled gently until sufficient bromine is absorbed. A few glass beads are placed in the flask, and the bromine is dispelled by boiling, which is prolonged for 2 minutes after the bromine colour has disappeared. The solution is cooled, water is added to replace that lost in the boiling; 1 ml. of potassium iodide solution (10 per cent.) is added, and the liberated iodine is titrated with standard thiosulphate solution, starch being used as indicator. Results accurate to within a few units per cent. of the total were obtained with amounts of iodine from

1 mgrm. down to 0·02 mgrm., 0·005 *N* thiosulphate solution being used for titrating the larger amounts and 0·001 *N* for the smaller amounts. Under the conditions employed, the smallest quantity of iodine required to give a blue colour with starch was found to vary with the temperature of the solution; thus at 31° C. the quantity was 0·024 mgrm., while at 17° C. it was 0·012 mgrm. The p_H value of the solution must be maintained between 1·0 and 2·0, since at higher p_H values the interaction of iodine and thiosulphate proceeds slowly, while at lower p_H values the rate of oxidation of iodide by air becomes marked, but it is slower the lower the temperature; solutions of potassium iodide at p_H 1–2 showed no appreciable oxidation by air in half-an-hour. The practice of some workers of adding phenol or salicylic acid to destroy any residual bromine after the oxidation process is not only unnecessary, but tends to cause low results; a small amount of salicylic acid used as a preservative for the starch indicator is, however, harmless. Iron was found to interfere when more than 0·1 mgrm. was present; bromide in small amount was without effect, but when present in larger amount, *e.g.* more than a 20-fold excess over the iodine present, it prevented quantitative oxidation of the iodine by bromine.

Determination of Iodine in "Iodised Salt."—A 5-grm. sample is dissolved in 100 ml. of water, neutralised to methyl orange indicator with sulphuric acid, and acidified with 2 ml. of 2 *N* sulphuric acid; the oxidation and subsequent titration are carried out as in the above method. S. G. C.

Direct Titration of Sulphate. W. C. Schroeder. (*Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 403–406.)—The titration of sulphate with barium chloride can be accomplished with the aid of tetrahydroxyquinone as internal indicator, which yields a red barium salt at the point of complete precipitation of the sulphate. Tetrahydroxyquinone may be prepared by treating glyoxal sodium bisulphite with sodium carbonate (Homolka, *Ber.*, 1921, **54**, 1393); it is unstable in solution, necessitating its being kept in solid form; it dissolves rapidly in water when finely powdered in intimate mixture with 400 parts of potassium chloride, thus providing a satisfactory method of introduction into the test solution.

Method.—The cold sulphate solution (25 ml.), containing between 2 and 20 mgrms. of sulphate, is rendered faintly acid to phenolphthalein with hydrochloric acid, and 25 ml. of alcohol are added; 0·2 grm. of the tetrahydroxyquinone-potassium chloride mixture is dissolved in the solution, giving it a deep yellow colour. To the solution is added barium chloride solution (0·025 *N*) at a steady rate, with constant shaking, until a brown colour begins to form. From this point, barium chloride solution is added 2 or 3 drops at a time, with shaking, until a red colour appears throughout the bulk of the solution, marking the end-point. A "blank" of 0·1 ml. of barium chloride solution, which is required to colour the indicator, is deducted from the total volume used; 1 ml. of 0·025 *N* barium chloride solution is equivalent to 1·2 mgrm. of SO_4. In test experiments, results within ±0·3 mgrm. were obtained with up to 20 mgrms. of sulphate, but above this amount the results were low. The maximum amounts of other ions which, it is stated, may be tolerated without causing error are: 7·5 mgrms. of carbonate, 5 mgrms. of aluminium, 25 mgrms. of silicate, and 15 mgrms. or more of

magnesium. Calcium, up to the amount at which calcium sulphate is precipitated, is without effect, and chloride is without effect, but iron in amounts above 0·1 mgrm. interferes and must be removed. The whole subject of volumetric determination of sulphate is reviewed, and 120 references are cited. S. G. C.

Colorimetric Determination of Iodine by the Chloroform Method. K. L. Maljaroff and W. B. Matskiewitsch. (*Mikrochem.*, 1933, **13**, 85–91.)—The effect of interfering salts on the colorimetric determination of iodine by the chloroform method has been investigated. Both the volume of the solution under examination and that of the chloroform must be the same in every test, and the volume of chloroform must not be too small, as the partition coefficient for iodine in water and chloroform is 135, so that when the volume-ratio of chloroform to water is 1:4, an error of about 3 per cent. is introduced. This ratio was used in all the experiments described. Test-tubes with glass stoppers were used, the test solution was first introduced, then chloroform, then 4 drops of dilute (1 : 1) hydrochloric acid, and finally a small drop of a concentrated solution of sodium nitrite to oxidise the iodine. The tubes were shaken for three minutes, allowed to stand for an hour in the dark, and then compared colorimetrically. Standard solutions were made up to contain 10, 20 and 40 mgrms. of iodine (as potassium iodide) per litre. The method is not suitable for concentrations higher than 40 mgrms. per litre, but concentrations as low as 0·125 mgrms. per litre can be used. The following salts in concentrations up to 200 grms. per litre had no effect on the results:—Sodium sulphate, magnesium sulphate and calcium sulphate. Sodium chloride (up to 15 grms.), calcium chloride (up to 12·5 grms.), and magnesium chloride (up to 10 grms. per litre) did not interfere. Larger amounts of these salts affected the results proportionally to the concentration, owing to their effect on the partition coefficient of the iodine. The following table shows the results obtained:

Concentration of salt Grms. per litre	Percentage loss of iodine in the presence of		
	Sodium chloride	Calcium chloride	Magnesium chloride
0·10	0·0	0·0	0·0
12·5	0·0	0·0	5
25	3·2	5	10·8
50	20	11·7	18·3
100	24·6	32·4	51·2
150	34·7	—	71·3
200	50	70·3	100
350	100	—	—

The results may be plotted graphically and a correction applied. Empirical formulae, calculated from the figures, give the corrected values as follows:—

$$\text{Sodium chloride } \frac{100a}{104{\cdot}05-0{\cdot}270b} = x$$

$$\text{Calcium chloride } \frac{100a}{104{\cdot}65-0{\cdot}373b} = x$$

$$\text{Magnesium chloride } \frac{100a}{105{\cdot}26-0{\cdot}526b} = x,$$

where x represents the corrected value, a the iodine-content as found in mgrm. per litre, and b the concentration of interfering salt in grms. per litre. Bromides, up to a concentration of 1·5 grms. per litre, have no effect on the results.

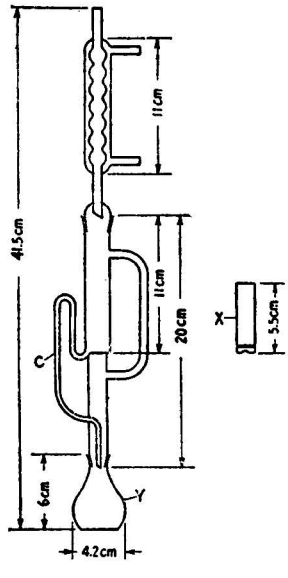
J. W. B.

Microchemical

Torsion Micro-balance. J. Donau. (*Mikrochem.*, 1933, **13**, 155–164.)—The modification of the Nernst air-damped micro-balance previously described (ANALYST, 1931, **56**, 342) has been further improved. The beam is made of duralumin instead of glass, and at the centre of gravity it is attached to a rider or carrier which is fixed to the quartz thread in two places, instead of being supported at only a single point. The beam is 7 cm. long, and at the ends there are two fixed riders which support the pans from quartz threads. These two riders or carriers, can be raised or lowered by a screw to adjust the sensitivity of the balance. The pointer, which is fixed in the middle of the beam, consists of a fine glass rod pulled out to a hair-fine tip where it reaches the scale; swings are read with a telescope. Apart from the disadvantage of small load-capacity (about 0·5 grm.), the advantages of the balance are the following:—Simplicity of construction, so that no cleaning or adjustment is necessary when it is set up correctly; the balance is much less sensitive to temperature effects than the Kuhlmann type, as the beam weighs only 1·5 grm. temperature equilibrium is reached almost instantaneously, weighing is much more rapid, and is complete in about 15 seconds. Ordinary riders are not used, but only small tares of known weight, so that the error of placing the rider at the wrong angle which can be quite appreciable in weighing with the Kuhlmann balance, does not occur. The weights should always be corrected for null point variation, which, however, is small.

J. W. B.

Micro-extractor. L. Titus and V. W. Meloche. (*Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 286–288.)—The micro-extractor, which is of Pyrex glass, consists of the following parts: The lower part, S, was made from a 40-mm. tube with an ether-tight ground-glass joint, the inner member, R, of the joint being sealed to the condenser, and the outer member, A, sealed up to form a cup. The extraction thimble, T, was made from a 16-mm. tube, constricted as shown, with a shorter length of 15-mm. tube fitting by means of a ground joint into the end of the longer 16-mm. tube, and hooks were sealed at the top of the thimble to support it in the aluminium ring, E. Before use, the thimble is fitted with a filter-paper bottom; for this purpose, it is taken apart, an 18-mm. disc of No. 50 Whatman filter-paper, wetted with water, is placed over the lower end of the outer tube of the thimble, and forced into place by firmly pressing the shorter tube into the ground end of the larger tube. The weighing bottle, D, blown from soft glass, weighed 4 grms., and when in position in the apparatus, an annular clearance



of at least 1 mm. was found desirable between the mouth of the bottle and the thimble. During the functioning of the apparatus, it is necessary to keep the main joint slightly warm to prevent ether from condensing there; this was accomplished by passing one strand of No. 26 nichrome wire around the joint at P, and passing sufficient current to maintain it at 50° C.; a covering of adhesive tape (presumably "insulating tape" is meant) over the wire was found necessary to maintain satisfactory heating of the joint.

Method of use.—The bottle D and the sample (about 15 mgrms.) are weighed on a micro-balance with the usual precautions, and the sample is introduced into the thimble. Five ml. of pure ether are added to the bottle D, which is placed in the bottom part of the apparatus. The aluminium ring E is placed on the top edge of A, and the thimble is lowered into position. The cup A is then joined to the upper part of the extractor. The apparatus is evacuated to a pressure of about 20 cm. of mercury and the stopcock is closed. Current is passed through the heating wire, and such heat is applied to the cup A, by means of an electric heater-box, that the rate of condensation of ether is not high enough to cause the condensed ether to rise above the constriction in the thimble. After the extraction, the ether is removed from the apparatus under reduced pressure. After the gradual admission of air, the lower part of the extractor is detached, and the bottle D is removed and weighed, giving the weight of extract. Close agreement between the results of this and of the Soxhlet method was obtained in tests on samples of dry skim milk, casein and residues from the evaporation of lake water. A micro-extractor of the Soxhlet type is also described, but it gave less satisfactory results than the above. S. G. C.

Microchemical Tests IX. L. Rosenthaler. (*Mikrochem.*, 1933, **13**, 317–320.)—1. *Potassium perrhenate as a precipitant for alkaloids.*—The reagent precipitates benzene as well as veratrine (*C. Agte. Z. anong. Chem.*, 1931, **196**, 129) and a large number of other alkaloids. The alkaloids given below give a crystalline precipitate when a drop of a 1 per cent. solution is added to a drop of the aqueous solution of the alkaloid, or when a few crystals of the solid alkaloid are placed on the edge of the drop of reagent. *Aconite* gives small rods and needle-shaped crystals, some forming stars. *Alypine* forms branched needles and plates. *Berberine* forms stars and groups of small needles. *Brucine* forms small thick groups of needles. *Quinidine* at first gives an amorphous precipitate which slowly becomes crystalline, finally forming groups of small needles. *Diocaine* gives at first an amorphous precipitate, and then gives rosettes of leaf-shaped crystals. *Heroin* forms rosettes of many-cornered leaf-shaped crystals. *Hydrastinine* gives large irregular-shaped crystals. *Pantocaine* gives plates and groups of needles. *Strychnine* slowly forms prisms, some crossed, and forming stars. With the solid hydrochloride, prisms are also formed.

2. *Crystal precipitation with alkaline hydroxyquinoline.*—The reagent solution is a 10 per cent. solution of hydroxyquinoline in 30 per cent. acetic acid, treated with potassium hydroxide solution until the precipitate first formed dissolves. If the solution is not clear, it should be filtered through kieselguhr. A large number of metals are precipitated by the reagent. *Thallium* gives an amorphous

precipitate changing to the crystalline form. *Nickel* and *cadmium* give only an amorphous precipitate. *Chrome alum* (1 : 100) gives first an amorphous precipitate, and then groups of long fine needles which sometimes disappear again. *Copper sulphate* and *lead acetate* both react with the reagent to give crystals in dilutions between 1 : 10,000 and 1 : 20,000. *Calcium acetate* gives crystals in dilutions between 1 : 10,000 and 1 : 20,000, but barium and strontium are less sensitive; for barium acetate the limit of dilution lies between 1 : 500 and 1 : 750, and for strontium nitrate between 1 : 1000 and 1 : 2000.

3. *A sensitive test for thiocyanates.*—The test combines two known reactions—oxidation to hydrocyanic acid and the detection of this as silver cyanide (Melitzky and Koslowsky, *Mikrochem.*, 1929, **7**, 94). A drop of the solution under examination is mixed with a few drops of dilute sulphuric or acetic acid (acetic acid in the presence of chlorides), and a little potassium permanganate is added. The small glass container is covered with a cover glass holding a drop of the silver nitrate methylene blue solution of Melitzky on its under side. After a period of time, varying with the concentration, blue crystals of silver cyanide are formed. The limit of dilution is 1 : 100,000.

4. *Volatile amines and reagents for ammonia.*—All the tests for ammonia described by Feigl (ANALYST, 1933, **58**, 641), with the exception of the formation of a precipitate with Nessler's reagent, are also applicable to monomethylamine, dimethyl- and trimethyl-amine. Seven photomicrographs are given.

J. W. B.

Micro Gas Analysis and its Applications, especially in Biological Work (Collected References). H. Schwarz and F. Rappaport. (*Mikrochem.*, 1933, **13**, 235–274.)—The micro methods of gas analysis are described, with experimental details, and references are given to 14 text-books and pamphlets and to 27 original papers.

(i) *Methods involving measurement of gas volume.*—These include the Haldane method, its use for different gases and mixtures of gases, and its various modifications. The Krogh method for microscopic volumetric measurements, which is suitable for the measurement of the respiration of insects, is described. The useful but less accurate spirometric methods are mentioned. References are given for different types of nitrometers.

(ii) *Methods involving the measurement of gas pressure.*—These include the van Slyke, the Barcroft, and the Warburg methods, all of which are described in detail.

(iii) *Optical methods*, in which interference and refractive phenomena are measured, are briefly described.

(iv) *Thermo-electric methods.* (v) *Weighing and vapour tension* methods are described. (vi) Brief mention is made of other methods, and references are given to facilitate choice of apparatus. J. W. B.

Detection and Determination of Small Amounts of Mercury (Collected References). F. Cucuel. (*Mikrochem.*, 1933, **13**, 321–364.)—The literature of the micro-chemistry of mercury is critically surveyed, and experimental details of many methods are given. There are five diagrams and 180 references.

I. The *qualitative* tests described are included under the following headings: (i) The amalgam test; (ii) aluminium test; (iii) the wire methods, in which the mercury is deposited on iron or copper wire, either by placing the wire in the test solution or electrolytically. The mercury may be finally identified by the iodine test. (iv) Crystal formation. Five tests are described for divalent, and 3 for monovalent mercury. (v) Spot tests, of which 7 are given. (vi) Tests on impregnated threads. (vii) Spectral analytical tests.

II. The *quantitative* methods include methods under the following headings:— (i) Volumetric methods; (ii) Colorimetric and nephelometric methods; (iii) Gravimetric methods, which include weighing the mercury as (*a*) sulphide, as (*b*) the complex $[HgI_4]$ $(Cuen_2)$ which is formed by precipitation with potassium iodide and copper ethylenediamine nitrate, and (*c*) deposition in metallic form on copper, platinum or gold; (iv) Spectrographic methods. (v) Micro-metric methods, in which the mercury is separated and distilled and its volume measured under the microscope; (vi) The methods of Bodnár, Szép and Stock (*J. Amer. Chem. Soc.*, **1926, 48, 1815**) are described in detail.

III. Details of methods are given for the detection and determination of mercury in urine and other organic and biological material.

IV. A section deals with the detection and determination of mercury in the atmosphere. J. W. B.

Physical Methods, Apparatus, etc.

Gas-Holder for Constant Pressure. J. Lindner. (*Mikrochem.*, **1933, 13**, 313–316.)—A gas-holder of simple construction for maintaining constant pressure is described. It consists of two 10-litre aspirators; the upper aspirator is fitted up as a Marriotte flask, the glass water-outlet tube (which for convenience may be constructed in parts with rubber connections) passes down through a rubber stopper in the lower outlet hole of the lower aspirator, and is bent up, by means of a rubber connection, to reach the gas in the neck of the aspirator immediately under the gas-outlet tube. When the apparatus is filled, the gas escapes at a pressure depending on the difference in height of the bottom of the air-inlet tube of the upper aspirator (which is usually made to reach to the bottom of the upper aspirator) and the height of the end of the water-outlet tube from the higher to the lower aspirator, which is usually just below the gas outlet of the lower aspirator. The pressure may be adjusted by raising or lowering the upper aspirator. This aspirator is fitted with a short air-inlet, provided with a screw clip, and the water-outlet has an arm, also fitted with a screw clip, to reach only to the bottom of the lower aspirator, so that the gas-holder can be filled in the ordinary way by opening both these clips. The apparatus, set up in a suitable holder, is obtainable from P. Haack, Vienna. J. W. B.

Precision Gas-Holder for Constant Pressure. H. O. Hohl. (*Mikrochem.*, 1933, **13**, 189–200.)—A patented gas-holder for constant pressure is described. It is constructed on the same principle as the apparatus described in the preceding abstract, but is made entirely of glass, so that any liquid may be used for the expulsion. It holds about 6 litres of gas. The apparatus is obtainable from P. Haack, Vienna. J. W. B.

Reviews

RECENT ADVANCES IN PHYSICAL CHEMISTRY. By S. GLASSTONE, Ph.D., D.Sc. 2nd Edition. Pp. 498. London: J. & A. Churchill. 1933. Price 15s.

It is but two years since an appreciation of the merits of the first edition of this volume of Churchill's "Recent Advances" series was published in THE ANALYST (1932, **57**, 68), a fact which clearly demonstrates two things: one, the intrinsic merits of the work; and the other, the rapidity of the advances which are taking place in the realm of physical chemistry. Indeed, now that heavy hydrogen is being studied so much, a third edition will be needed almost before the ink is dry on the second. It is unnecessary to review the work again at length, and it suffices to point out the much new matter which has been included and some which has been excised to make room for it. The chapter on solubility has gone; perhaps one may linger in regret because it was so interesting and useful. In its place is a discussion of wave mechanics, calculation of energy of activation, nuclear disintegration, surface potentials, surface mobility, the kinetics of photochemical reactions, adsorption both activated and continuous, the rotation of dipole moments and the study of molecular beams.

We think it was Kant who said that the amount of true science in any matter was strictly proportional to the amount of mathematics in it. It this be so, there is much truth in *Recent Advances in Physical Chemistry*, and much more truth in the second edition than in the first. We must congratulate Dr. Glasstone on the readableness of his work, despite the complexities of its subject-matter. He greatly helps those of us who do not specialise in this field to follow the almost bewilderingly rapid strides of those who do: would that he helped us more easily to remember the mathematical details! H. E. Cox

INTRODUCTION TO PHYSICAL CHEMISTRY. By ALEXANDER FINDLAY. Pp. vii+492. London: Longmans, Green & Co. Price 7s. 6d.

On the cover of this book it is stated that its aim "is to provide a text-book which shall not only serve as an introduction to the study of physical chemistry, but shall also carry the student on to such a point that he can read with profit the numerous special monographs now available. The work is designed more especially to meet the needs of the student of chemistry who desires to build his later specialised study on a broad foundation." This it is likely to do, which is more than can be said of many books which are obviously designed to enable students to pass certain examinations and then to pay the minimum of attention to a boring subject.

Professor Findlay has the gift of making a difficult subject interesting. By adopting a somewhat historical treatment and giving the full names of the many workers who have helped to unravel the mysteries of the relations of electrons, protons, neutrons, atoms, and molecules to one another in time and space, he has succeeded in humanising physical chemistry.

Many of us must either remember hearing the Faraday Lecture of Wilhelm Ostwald, in which he sought to explain all that we then knew of chemistry without invoking the aid of atoms, or have read his Chemical Conversations, with their

Berkeleyan repudiation of substance. The researches of many workers in different tracts of the borderland between physics and chemistry have not only fully established the atoms, but have demonstrated their complexity. How this was done, and the light which such work as that of Jean Perrin on the curious movement of small particles in a liquid, discovered by Robert Brown in 1827, threw on facts hidden in the lumber room of science, is well described in the earlier chapters of this book. It may here be noted that Waterson's earliest development of the kinetic theory of gases in 1845, which was dug out of the archives of the Royal Society and made public by Lord Rayleigh in 1892, is mentioned.

Towards the end of last century inorganic chemistry seemed a dead subject, and physical chemistry merely a barren field yielding only a few weeds uninteresting to right-minded chemists or physicists. The brilliant light thrown on the constitution and spacial configuration of organic compounds, by researches in which chemistry was aided by the polarimeter and goniometer, had diverted most chemists from the basic facts of the subject, except as applied to a large and tractable group of compounds of a very few elements. The periodic law of Newlands, Mendeléef and Lothar Meyer was found to present almost as many anomalies as coincidences, and the similarities and differences of the known elements were inexplicable. The discovery of the rare gases and of radioactive elements, with J. J. Thomson's work on the electrical conductivity of gases, opened up a new field of speculation for those blessed with imagination. The application of the gas laws to solutions and the development of Faraday's conception of ions broke down for ever the barriers between physics and chemistry, greatly to the advantage of both sciences. All these things, and the energetic conditions and time relationships governing chemical reactions, with the effect of chemical constitution on physical behaviour of chemical entities, are ably set forth by Professor Findlay.

The present grave danger is that students whose temperament should lead them to take mathematics as their principal subject may be tempted to build on chemical observations, made uncritically, a vast and unstable superstructure of formulae, forgetting that the chemist's place is the laboratory, not the armchair, and that pens and paper are not his principal pieces of apparatus. The great physical chemists have all been good experimenters.

On reading on p. 65 the statement "it has been found that when water vapour has been very carefully freed from dust particles its temperature may be lowered considerably below the point of condensation before liquefaction takes place," one supposes that the author's reading on hygroscopic nuclei has been confined to the work of his countryman, Aitken, and has not included that of Wigand, Nolan and other later workers, who have shown that dust particles do not act as condensation nuclei.

An omission which is all the more remarkable, in view of the wide range of subjects treated and of the very up-to-date character of the book, is the absence of all reference to the highly interesting and important metallic state, concerning which sufficient is known for the author, had he been so minded, to have given us a few illuminating paragraphs.

It is hardly correct to describe chloroform, as the author does on p. 148, as being insoluble in water, when *aqua chloroformi* is a well-known pharmaceutical

preparation; the experiment there described does not seem to show the desired result until after some days.

The book appears to be remarkably free from errors and misprints; it can be confidently recommended to the student who really wants to know, and to those of us whose work lies mostly in other branches of chemistry, but who wish to have a readable manual from which to obtain information of a reliable kind on points of physical chemistry which may bear on our own problems, with references enabling us to read what the original authors themselves had to say about their work.

The publishers are to be congratulated on the production of this important work in an attractive form and at a very low price. J. H. COSTE

THE PHYSIOLOGICAL EFFECTS OF RADIANT ENERGY. H. LAURENS, Ph.D. American Chemical Society Monograph. Pp. 610. New York: The Chemical Catalog Company, Inc. 1933. $6.00.

This is a monumental review of published work on the effects on the human body, and on some other organisms, of ultra-violet, visible, and infra-red radiation. The principal criticism of this book is anticipated by the author himself in his preface, where he says: "No doubt many readers will be annoyed at the inconclusiveness of some of the statements and arguments." Some sections do indeed present long bewildering accounts of numerous experiments carried out by different workers under different experimental conditions, often imperfectly controlled and only qualitatively recorded, leading to inconsistent, if not quite contradictory, conclusions. But this, of course, is hardly the author's fault, except that his passion for completeness has led him to include work which, on internal evidence, might have been rejected as comparatively worthless. Some attempt might also have been made to summarise the evidence at the end of each chapter. At least, the method followed has the merit of demonstrating most convincingly the need for much more accurate and carefully controlled work in the field, and it is to be hoped that in later editions it will be possible to present a less confusing picture of the state of our knowledge.

In so far as the author presents his own views at all, they are extremely sane. He points out that the undoubtedly specific effect of ultra-violet radiation in the treatment of rickets, and other established cases of the benefits accruing from light therapy, have led to indiscriminate and inadequately controlled use of solar and artificial radiation for all manner of complaints. The sun is "an heroic remedy," whilst artificial sources, and particularly quartz mercury-vapour lamps, are by no means perfect substitutes for sunlight. It would appear that in many cases the value of irradiation lies chiefly in the penetrative power of the short infra-red and visible rays, producing a rise of temperature several mm. below the skin. Carbon arcs may replace the sun for this purpose, but the light from mercury-vapour lamps is relatively deficient in infra-red, and contains ultra-violet radiations, extending far beyond the limits of the solar spectrum, and not by any means necessarily beneficial.

After a brief introduction, the book begins with a long chapter on the physics of radiant energy, dealing with the measurement of radiation and the characteristics

of sources, natural and artificial. There follow chapters on the effects on the skin, on wounds and certain skin diseases, on the eye, on the circulatory system and the blood, on metabolism (five chapters), and on body temperature and respiration; the next chapter deals with photodynamic sensitisation, others with the effects on tuberculosis, and on bacteria, toxins, and so on; to conclude there is a short and halting chapter on the mode of action of radiant energy on physiological and pathological processes. This arrangement seems susceptible of improvement, since it leads to some scattering of information on a particular subject: for example, tuberculosis is the subject of Chapter XI, but skin tuberculosis is relegated to Chapter IV; skin and subcutaneous temperatures are mentioned in six different chapters.

It is a curious fact that the word "colour" does not even appear in the index; there is no mention of observations on the effect of colours, for example, of wall-coverings, on well-being; but, perhaps, this is relegated to a later volume on the psychological effect of radiant energy?

On the whole, however, the book is well conceived and accurate, though a few sections bear evidence of careless proof-reading. The historical survey of the chemistry of vitamin *D*, for example—a subject outside the main field of the work—is excellently presented, but within ten pages occur such errors as "ergot" for "ergosterol" (p. 411), omission of the word "destroyed" (p. 416), "solute" for "solution" (p. 421). It is a little confusing, moreover, when hypotheses later abandoned by their authors, or disproved, are presented as though they were still accepted.

There is a bibliography of nearly a thousand references, including many of recent date, a good author index, and fairly complete subject index.

E. LESTER SMITH

PRACTICAL PHYSIOLOGICAL CHEMISTRY. Ninth Edition. S. W. COLE, M.A. Pp. xii+419. Cambridge: W. Heffer & Sons, Ltd. 1933. Price 12s. 6d.

Reviewing a previous (seventh) edition of this book some years back (ANALYST, 1926, **51**, 273), I commented that a new edition of "Cole" must always mark a minor epoch for chemists whose work brings them at any point in touch with physiological problems. That remark is even truer of the ninth than it was of the seventh edition, for the eighth edition of 1928 has here not only been fully revised, but almost wholly re-written. This has had several very valuable results, not least of which is a reduction in the number of pages from nearly 500 to a little over 400, a result that has been achieved by technical devices of the publisher and printer, as well as by the author's revision.

It may be noted with satisfaction that the "literals" that disfigured the seventh edition to a rather unnecessary extent appear to be absent from the new one, as far as a cursory examination reveals; also that some of the minor errors found in the seventh edition, and cited in the previous review, have been either corrected or avoided altogether in the course of the revision.

The author, however, still persists in attributing to cholesterol a melting point three degrees lower than that of pure specimens, and evidently differs from

the reviewer as to the advisability of introducing students to the Donnan membrane equilibrium theory. Nevertheless, in my opinion, it is of such importance in biochemistry that its introduction to students at the earliest possible moment ought to be attempted. The same remarks apply to the concept of oxidation-reduction potentials, which has become of increasing prominence during the last few years; this might well have been discussed, however briefly, in Chapter XI which is concerned with "Oxidising and Reducing Mechanisms." The appropriateness of such discussion must have been in Dr. Cole's mind, for this chapter contains the sole reference to the concept in question. He writes that the energy conditions of oxidation and reduction re-actions are related to the relative affinities for hydrogen of the oxidised and unoxidised re-actants, and that "this is related to what is known as 'oxidation reduction potential' which cannot be discussed here."

This text-book has for many years been one of the most useful for students of biochemistry, and its revision has certainly in no way diminished its utility. Moreover, like all good text-books, it contains information and suggestions of value to the qualified practitioner as well.

A. L. BACHARACH

Publications Received

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THE CONTROL OF WATER SOFTENING AND BOILER WATER CONDITIONING. Issued by Imperial Chemical Industries Ltd., London, S.W.1. Pp. 39.

CONTENTS.—Introduction—Summary of Tests—Control Tests for Water Softening—Alternative Estimation of Hardness of Water—Boiler Feed Water Conditioning—Analyses for Blowdown Control—Table of Daily and Weekly Tests—Bibliography.

THE COLORIMETRIC DETERMINATION OF OXIDATION-REDUCTION BALANCE. The British Drug Houses, Ltd., Graham Street, London, N.1. (A copy will be supplied by the British Drug Houses, Ltd., to any chemist who cares to write to them for one.)