THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held on Wednesday, February 1st, in the Robert Barnes Hall, 1, Wimpole Street, by kind permission of the President and Council of the Royal Society of Medicine. The President (Mr. F. W. F. Arnaud) was in the chair.

Certificates were read for the first time in favour of Alfred Thomas Symonds Babb, B.Sc., Sidney Harold Cakebread, B.Sc., A.I.C., Henry Phillips, D.Sc., F.I.C., and Walter Desmond Raymond, B.Sc., A.I.C.

The following were elected members of the Society: Clifford Kenneth Boundy, A.I.C., Raphael Heber Callow, M.Sc., A.I.C., Miles Ernest Catti-Camfield, John Dewar, B.Sc., Clifford Walter Herd, B.Sc., Ph.D., F.I.C., Henry Humphreys Jones, F.I.C., Frederick Leigh Okell, F.I.C.

JOINT MEETING OF THE SOCIETY WITH THE FOOD GROUP OF THE SOCIETY OF CHEMICAL INDUSTRY

After the Ordinary Meeting a joint meeting was held to discuss "The Changes taking place during the Storage of Fruit." The President (who was in the chair) extended a cordial welcome to the members of the Food Group of the Society of Chemical Industry, to which Dr. L. H. Lampitt (Chairman of the Food Group), when opening the discussion, replied.

Dr. Ezer Griffiths gave a detailed description of the technique of pre-cooling and transport of fruits. This was followed by a general discussion in which the following speakers took part:—Messrs. Harold Williams, T. Rendle, A. Samson, A. More, T. McLachlan, C. E. Barrs, Coyne, F. R. Stephens, and the President.

NORTH OF ENGLAND SECTION

THE Eighth Annual General Meeting of the Section was held in Manchester on February 11th, 1933. The Chairman (Mr. J. Evans) presided over an attendance of thirty-seven.

The Secretary read the report and financial statement for 1932, which were adopted.

The following officers were elected:—*Chairman :* John Evans; *Vice-Chairman :* Professor W. H. Roberts; *Committee :* W. Gordon Carey, G. D. Elsdon, J. W. Haigh Johnson, W. B. Marston, S. E. Melling, H. E. Monk, and J. G. Sherratt. *Honorary Auditors :* U. A. Coates and W. Marshall. *Honorary Secretary :* J. R. Stubbs.

The following papers were read and discussed:—"Some Observations on the Behaviour of Artificial Creams," by Muriel Roberts, B.Sc., F.I.C.; "Citric Acid and its Detection," by G. D. Elsdon, B.Sc., F.I.C., and Arnold Lees, F.I.C.; and "The British Pharmacopoeia, 1932: Some Notes on the Changes and Additions," by J. R. Walmsley, F.I.C.

Some alterations of the rules of the Section were approved for submission to the Council.

Some Properties of Ergosterol and Calciferol

By A. L. BACHARACH, B.A., F.I.C., E. LESTER SMITH, M.Sc., A.I.C., and S. G. STEVENSON, B.Sc., B.Pharm., F.I.C.

(*Read at the Meeting, December* 7, 1932)

Ergosterol and calciferol (crystalline vitamin *D*) are both now articles of commerce. Following Steenbock's discovery that inactive material could be made antirachitic by the action of ultra-violet radiation (*J. Biol. Chem.*, 1924, **61**, 405), it was fairly soon announced by various investigators that the activatable material was associated with the sterols of animal and vegetable fats. Heilbron, Kamm and Morton (*Biochem. J.*, 1927, **21**, 78) pointed out that certain characteristic bands in the ultra-violet region associated with cholesterol disappeared during irradiation, that the resultant product has marked antirachitic activity, that the antirachitic fraction can be separated from unchanged cholesterol, and that this then no longer shows the bands and is no longer capable of being further activated; this led them to state that the true precursor of vitamin *D* must be sought in a substance having intense absorption in the region of the activating radiations, according to the well-known Grotthus–Draper law. Almost simultaneously it was found by Windaus and Hess (*Nach. Ges. Wiss. Göttingen*, 1927, 175) and by Rosenheim and Webster (*Biochem. J.*, 1927, **21**, 127) that ergosterol was such a substance, and subsequent investigation proved conclusively that ergosterol can be completely converted by ultra-violet radiation into a mixture of vitamin *D* and other products, though no quantitative conversion of ergosterol to vitamin *D* can be brought about. Many investigators, but notably Windaus and his colleagues (*Nach. Ges. Wiss. Göttingen*, 1932, 150), have examined the various reactions that take place during the conversion of ergosterol into vitamin *D*; the problem is extremely complex, and all that can be said at present is that a series of consecutive reactions, as well as, possibly, some side reactions, takes place during irradiation. Since nothing is known of the relative velocities of these reactions, or of the extent to which they are influenced by external conditions, such as the solvent employed, it is clear that the mixture obtained by irradiating ergosterol will contain amounts of other substances that may vary with the conditions of irradiation. Even when these are kept as constant as practice permits, it is difficult to know in what proportion lumisterol, tachisterol, the suprasterols, and other products are present as contaminants of vitamin *D* in irradiated ergosterol. There is thus the possibility that some of these may contaminate the calciferol separated from the mixture.

It follows that a precise specification of the physical constants of calciferol is highly desirable, so that vitamin *D* may be sold by weight for commercial purposes. Since, moreover, the purity of the ergosterol used may quite conceivably affect the amount both of the vitamin *D* and of the contaminating by-products produced during irradiation, similar precision of definition for ergosterol is also desirable.

Various figures for each substance are given in the literature, but none, so far as we are aware, represents determinations carried out on products prepared on a manufacturing scale, and no complete set of values for a single sample is available. We have found it necessary, in the capacity of buyers of ergosterol and sellers of calciferol, to re-determine these constants for ourselves, and think it may be of use if we record them here, so that they may be accessible together in one place, with certain observations based upon actual experience of handling relatively large quantities of both compounds.

ERGOSTEROL ($C_{28}H_{44}O$, H_2O)

1. SOURCE.—Commercial ergosterol has so far invariably been made from yeast, by fractional crystallisation of the sterols separated from the unsaponifiable matter of the yeast fat. We have examined one sample of ergosterol stated not to be made from yeast, and its physical constants were numerically higher than those of any other sample examined by us. A further supply from the same source was, however, definitely inferior. Callow (*Biochem. J.*, 1931, **25**, 79) has shown that yeast ergosterol is contaminated with appreciable quantities of dihydro-ergosterol, and that the two cannot be separated by ordinary methods of fractional re-crystallisation. It seems that even the best specimens of commercial ergosterol, such as that for which we give below the values of various constants, will contain an appreciable amount of dihydroergosterol.

2. CONSTITUTION.—According to the most recent views, ergosterol may have the structural formula shown in Figure I. The position of the two double bonds in the ring system is, however, an open question. The literature on the subject is extensive and increasing in volume; a recent survey of the whole position has been made by Heilbron (cf. *Chemistry and Industry*, 1932, **51**, 1061). It should be noted that all our observations have been made with the crystalline monohydrate. Attempts to remove the water of crystallisation by ordinary methods involve some decomposition of the sterol.

3. APPEARANCE.—Pure ergosterol is supplied in fine white needles. The sample not derived from yeast was indistinguishable in appearance from the others.

4. STABILITY.—Ergosterol slowly turns yellow on exposure to air and light. It is probable that the presence of oxygen is essential for this change, though we have no direct proof for this fact, and are arguing by analogy with calciferol. The change is accelerated by exposure to light, and is not prevented by keeping ergosterol in the dark and at a low temperature in the presence of air. The slightest yellowish tinge in ergosterol must be regarded as presumptive evidence of deterioration.

5. MELTING-POINT.—Ergosterol decomposes at its melting-point, and, to some extent, at temperatures approaching its melting-point. For this reason the appropriate precautions must be taken, if consistent values are to be obtained,

even by one observer. It is necessary to insert the capillary tube in the heating-bath when it is 5 to 10° C. below the expected melting-point, and to raise the temperature as rapidly as is consistent with the need to maintain the capillary and the thermometer bulb at the same temperature and not to cause so rapid a rise as to make an exact reading impossible. The specimens examined by us have shown m.pts. varying from 162 to 164° C. These figures are in close agreement with those given by Callow (*loc. cit.*), who found 160 to 163° C. Minor differences in melting-point will always be found by different observers, and with different apparatus. Specimens whose optical rotation and ultra-violet absorption indicate that they are of a high degree of purity should invariably have m.pts. 162° C., or above. Bills and Honeywell (*J. Biol. Chem.*, 1928, **80**, 15) have stated that a sample of ergosterol purified in their laboratories had m.pt. 183° C., but their statement has not been confirmed by any other worker, nor has it been repeated by themselves. All our melting-points were determined in open capillaries, and all the values recorded in this paper have been, to the best of our knowledge, corrected for "exposed stem."

6. Optical Activity.—Ergosterol is strongly laevo-rotatory. The highest figures to be found are those given by Callow (*loc. cit.*) for the D line and for the mercury green line ($\lambda = 546{\cdot}1 m\mu$), but only for a chloroform solution. We have accordingly determined the rotation of a specimen of melting-point 164° C. in six different solvents, using both sources of light. For this purpose we have found it convenient to use the Zeiss sodium lamp placed alongside a K.B.B. atmospheric type quartz mercury lamp, the two being separated by a small sliding mirror, whose function is simultaneously to cut off the sodium light and to reflect the mercury light through a slit in the wall of the lamp housing. By having the two lamps in appropriate positions, simple raising of the mirror causes the sodium light to replace the mercury light. It is thereby made possible to take consecutive readings with the two sources of light on the same solution, with little fear of any change having occurred meanwhile. All the rotations were taken in a 4-dm.-tube with a Schmidt–Haensch half-shadow polarimeter, fitted with the Hilger spectroscopic eye-piece; readings were made to 0·01°. All solutions contained 0·25 per cent. of ergosterol, except that in chloroform, in which a 1 per cent. solution was used. The conversion factor of 1·27, by which the rotation for sodium light must be multiplied to give the rotation for the mercury green line, appears to be quite independent of the solvent used. It is distinctly higher than that found by one of us (Analyst, 1923, **48**, 521) for lactose and sucrose, and than the closely similar values found by Quisumbing and Thomas (*J. Amer. Chem. Soc.*, 1921, **43**, 1503) for dextrose, laevulose and maltose. Our results are shown in Table I.

7. Ultra-violet Absorption at $281 m\mu$.—By this we understand the intensity of absorption of radiations with a wave-length of $281 m\mu$ calculated for a 1-cm. layer of a 1 per cent. solution in absolute alcohol. The determinations are actually carried out with a 0·02 per cent. solution in a 2-mm. cell, the results being calculated to give the value of $E\,{}^{1\%}_{1\,cm.}$ as defined. Dr. R. A. Morton and Mr. A. E. Gillam have very kindly determined for us the absorptions of both

ergosterol and calciferol; the specimen of ergosterol for whose optical rotations the values are given in Table I showed an absorption of 333, and was described by Dr. Morton as "rather better than most samples."

8. COLOUR AND OTHER REACTIONS.—Ergosterol gives with a 1 per cent. solution of digitonin in 90 per cent. ethyl alcohol an immediate precipitate of the digitonide. Ergosterol exhibits the following colour reactions:

(*a*) Antimony trichloride: red→ blue.

(*b*) Trichloroacetic acid (Rosenheim, *Biochem. J.*, 1929, **23**, 47): red→ clear blue.

(*c*) Mercuric acetate in nitric acid (Rosenheim and Callow, *Biochem. J.*, **1931**, **25**, 74): pink→ peacock blue→ magenta→ dull red. Solutions containing more than 1 per cent. give pink→ orange→ clear red; oxidised specimens give finally a green colour.

(*d*) Tortelli-Jaffé reaction (Heilbron and Spring, *Biochem. J.*, **1930**, **24**, **133**): green.

9. PHYSIOLOGICAL ACTIVITY.—There is no evidence in the literature of any physiological activity of ergosterol itself, whether in regulating calcium-phosphorus metabolism or in any other direction.

CALCIFEROL ($C_{28}H_{44}O$)

1. SOURCE.—Calciferol is made on the commercial scale by the process worked out at the National Institute for Medical Research, details of which have been published by Askew *et al.* (*Proc. Roy. Soc.*, 1932, **109**B, 488), with such minor modifications as factory procedure necessitates. It consists essentially in irradiating ergosterol in an organic solvent, removing the solvent, separating the unchanged ergosterol from the "active resin," esterifying this with dinitrobenzoyl chloride, and hydrolysing the dinitrobenzoate that separates in crystalline form from the mixture of esters.

It has also been made, but not, so far as we know, on anything approaching a commercial scale, by a method due to Windaus *et al.* (*Annalen*, 1932, **492**, 266), involving the use of the magnesium spark as the source of ultra-violet radiation, and the removal of inactive products after their condensation with citraconic anhydride.

An American pharmaceutical house claims to have made crystalline vitamin *D* by an entirely different method, but no details of this have been published.

Under certain favourable conditions the ergosterol-free active resin will deposit crystals of a product originally named by Windaus and his colleagues "vitamin D_1." This proved to be a molecular compound of "lumisterol" and calciferol, from which the latter can be separated as the dinitrobenzoate.

2. CONSTITUTION.—It is currently held that calciferol is an isomer, and possibly a stereo-isomer, of ergosterol, and the same empirical formula is given to it, but without water of crystallisation. There is no published record of any attempts further to elucidate its constitution, apart from the preparation by Windaus or his colleagues of some derivatives.

3. APPEARANCE.—Calciferol is usually hardly distinguishable in appearance from ergosterol, and, like ergosterol, it is perfectly white when pure.

4. STABILITY.—In our opinion the stability of calciferol has been somewhat exaggerated. In *Vitamins : A Survey of Present Knowledge* (Accessory Food Factors Committee; His Majesty's Stationery Office, 1932) it is described as "remarkably stable under ordinary laboratory conditions," and a similar statement has been made by several investigators. Although the description is patently a relative one, and was doubtless influenced by preconceived views about the stability of vitamins in general, we consider it rather misleading. Even in the dark, calciferol, left in contact with atmospheric oxygen, begins to turn yellow very quickly; after nine months in a corked tube a sample was noted to have changed into a deep brown resin. The change is accelerated if calciferol is powdered or heated, but does not seem to be influenced by light. The process would appear to be of an oxidative nature, for it is entirely inhibited if calciferol is kept in a sealed container in pure nitrogen. That calciferol is more oxy-labile than ergosterol is also indicated by some recent work of N. K. Adam and J. S. Danielli (Private Communication).

The change in colour is accompanied by a change in the physical constants of calciferol; its melting-point, optical rotation, and ultra-violet absorption (see below) are all lowered, and there is also evidence of a diminished biological activity. The development of the slightest yellowish tinge is, therefore, as with ergosterol, presumptive evidence of deterioration.

5. MELTING-POINT.—Our comments on the melting-point of ergosterol apply with even greater force to calciferol. For example, a specimen, that had a melting-point of 115 to 116° C., when this was determined by the method described for ergosterol, melted almost completely when it was held for half an hour at 102° C.

The figures recorded in the literature for the m.pt. of calciferol vary somewhat; the highest value we have obtained is 116° C. Askew *et al.* (*loc. cit.*) gave the value of 114·5–117° C.

6. OPTICAL ACTIVITY.—Calciferol is strongly dextro-rotatory. Like ergosterol, its optical rotation is influenced by the solvent used in the determination, but to an even greater extent. Table II gives the values found by us for the sample melting at 116° C., both for the sodium light and for the mercury green line, using the same polarimetric procedure as for ergosterol. All the rotations were taken on a 2 per cent. solution in a 2-dm. tube.

The figures are in very good agreement with those recorded in the literature, but we have been unable to obtain a specimen of the "Normalbenzin Kahlbaum" used by Windaus. This solvent gave him a lower rotation even than chloroform; the *n*-hexane used by us gave values slightly higher than in chloroform.

It will be observed that the factor for converting the sodium-light value to the mercury green-line value is distinctly lower than that for ergosterol, and is identical with the factor for sugars referred to earlier. The conversion factors for the two lights calculated from the figures given by Windaus for alcohol, ether and "Normalbenzin" vary somewhat, whereas those for the range of solvents used by us are remarkably constant.

Though we have no suggestion to offer of its significance, we would call attention to the fact that the effect of some solvents on the rotation of calciferol

is in the reverse order from their effect on that of ergosterol; this is presumably associated with the fact that their optical rotations are in opposite directions.

7. ULTRA-VIOLET ABSORPTION AT 265$m\mu$.—The sample with m.pt. 116° C. was found by Dr. Morton to give a value of 470 for $E\,{}^{1\%}_{1\,cm.}$ 265$m\mu$. Other samples have been found by Dr. Morton to give values of 485 and 483, with a probable experimental error of ± 5 in all cases. Askew *et al.* (*loc. cit.*) also gave a value of 485.

8. COLOUR AND OTHER REACTIONS.—Calciferol gives no precipitate with an alcoholic solution of digitonin. Calciferol exhibits the following colour reactions:

(*a*) Antimony trichloride: deep yellow.

(*b*) Trichloroacetic acid (*vide supra*): deep yellow→dirty red-brown.

(*c*) Mercuric acetate (*vide supra*): orange-red→yellow (in concentrated solution).

(*d*) Tortelli-Jaffé reaction (*vide supra*): blue.

9. BIOLOGICAL ACTIVITY.—As might be expected, a considerable range of figures have been quoted in the literature for the number of international units of vitamin *D* equivalent to one grm. of calciferol.

Figures are available for some dozen specimens, including six prepared and examined by Askew *et al.* (*loc. cit.*), one prepared by Windaus and examined by Askew, three prepared by ourselves, and an unknown number prepared and examined by Windaus and his colleagues. The only values below 40 million units per grm. are three given by Askew *et al.*, who nevertheless give an average figure just above 40 million; Windaus *et al.* give a figure of 40 to 45 million, but do not state how many samples were examined. The mean value for our own three samples was 42 million, and the value found by Askew *et al.* for a sample of Windaus' vitamin D_2 was just under 45 million. It will be seen that in at least one case the biological assays were carried out on a sample of outside origin; moreover, four distinct methods of separation from irradiated ergosterol were used to prepare the specimens. The figures, in our opinion, fully justify the belief that calciferol is, in fact, pure crystalline vitamin *D*, and that it has a constant biological activity of something not less than 40 million units per grm. It will be observed that this activity is four times that of the sample of irradiated ergosterol whose 0·1 per cent. solution in oil provides the International Standard Preparation of vitamin *D*. The material used in this preparation, however, was not freed from unchanged ergosterol, and a content of 25 per cent. pure vitamin *D* is quite normal for an "active resin" made under the conditions described as having been used in its production.

TABLE I

OPTICAL ROTATION OF ERGOSTEROL

Solvent	$[\alpha]_D^{20}$	$[\alpha]_{546\cdot1}^{20}$	Ratio
Chloroform	−125·25°	−158·50°	1·27
Benzene	−124·0°	−156·0°	1·26
Ethyl acetate ..	−95·0°	−120·0°	1·26
Ether	−94·0°	−120·0°	1·27
Alcohol (absolute) ..	−93·0°	−119·0°	1·28
Acetone	−92·0°	−118·0°	1·28

Average value of ratio = 1·27

TABLE II

OPTICAL ROTATION OF CALCIFEROL

Solvent	$[\alpha]_D^{20}$	$[\alpha]_{546\cdot1}^{20}$	Ratio
Alcohol (absolute) ..	+106·25°	+125·0°	1·18
Ethyl acetate ..	+95·0°	+113·25°	1·19
Ether	+88·75°	+105·5°	1·19
Benzene	+87·5°	+102·12°	1·17
Acetone	+83·5°	+99·5°	1·19
n-Hexane	+56·25°	+66·5°	1·18
Chloroform	+52·25°	+61·75°	1·18
		Average value of ratio =	1·18

It is pertinent here to refer to the deterioration in biological activity of samples whose physical condition indicated some change. The following table illustrates the correlation:

TABLE III

DETERIORATION OF CALCIFEROL

Sample	Colour	M.pt. °C.	Approximate activity in millions of units
A	White (freshly prepared) ..	115	—
	Yellowish	103–108	43
	Yellow	97–101	34
B	White (freshly prepared) ..	116	—
	Yellowish	107–110	42
	Yellow	103–107	33

CONCLUSIONS.—On the basis of the figures given above, we think it possible to lay down provisional specifications both for pure ergosterol and for pure calciferol, as follows:

	Ergosterol	Calciferol
M.pt. ..	162–164° C.	115–117° C.
$[\alpha]_{546\cdot1}^{20}$ ..	$>-157°$ (in chloroform)	$>+122°$ (in absolute alcohol)
$[\alpha]_D^{20}$ ..	$>-123\cdot5°$	$>+103°$
Ultra-violet absorption	$E_{1\text{ cm.}}^{1\%}$ 281$m\mu$ not <320	$E_{1\text{ cm.}}^{1\%}$ 265$m\mu$ not <470
Antirachitic activity ..	—	not $<40\times10^6$ international units/grm.

In our opinion all three physical constants should be determined if a high degree of purity is required, particularly for calciferol, since at least one possible contaminant raises not only the rotation, but also the melting-point.

DISCUSSION

Dr. F. H. CARR thought that the melting-point of ergosterol, when taken *in vacuo*, would be found to be about one degree higher than that in an open capillary tube. His experience had been that calciferol would keep in white crystals for a period of ten months, turning only very faintly yellow—certainly not brown, and he asked at what temperature the specimen discussed in the paper had been kept.

Mr. BACHARACH replied that the specimen in question had been kept in the bottom of a corked bottle in a laboratory cupboard. It was clear that the rate of oxidative decomposition of calciferol must be determined by the amount of accessible oxygen in the container, the amount of surface exposed by the calciferol, and other factors besides temperature.

The Determination of Tartaric Acid in Foodstuffs

BY J. KING, F.I.C.

THE Agricultural Produce (Grading and Marking) (Cider) Regulations, 1930, introduced under the Agricultural Produce (Grading and Marking) Act, 1928, adopts certain standards for the two grades of cider, "Select" and "Select Cider, Champagne process." The "Select Champagne Process" grade may not contain any acid foreign to apples, and the "Select" grade may not contain more than 1 grm. per litre of tartaric or citric acids, other acids foreign to apple juice being prohibited. For the examination of ciders under these regulations, it became necessary to devise methods for the detection and estimation of these small quantities of acids in presence of malic acid. Of the methods for the determination and detection of tartaric acid which have been studied, the most promising appeared to be:

(*a*) The method having as its basis the comparative insolubility of potassium hydrogen tartrate in dilute acetic acid in presence of alcohol, or alcohol and ether.

(*b*) The method of Kling,[1] which depends on the insolubility of calcium racemate in dilute acetic and hydrochloric acids.

Method (*a*) forms the basis of the French official method adopted in the *Journal Officiel du 22 jan.*, 1907, an account of which (by Kling) appears in *Expertises Chimiques*, Vol. III, p. 185, and it also forms the basis of the American official method in *Methods of Analysis, A.O.A.C.*, 1925, p. 366, and in the 3rd edition, 1930, p. 141.

Method (*b*) is applicable only to *d*-tartaric acid. From an examination of commercial samples of tartaric acid derived from many sources I am able to state that all commercial tartaric acid on the market in this country appears to be exclusively the *d*-acid. Briefly, this method consists in adding to the solution containing *d*-tartaric acid, a solution of calcium acetate in dilute acetic acid, and a quantity of *l*-ammonium tartrate, which, while ensuring complete precipitation of the original *d*-acid as calcium racemate, will not cause precipitation of calcium *l*-tartrate. No precipitate, therefore, will be given in absence of *d*-tartaric acid, or acids whose calcium salts are insoluble in dilute acetic acid. Meso-tartaric acid is not precipitated as the calcium salt if the conditions given in the method are adhered to, but it has been found that in presence of *d*-tartaric acid, a lengthened

period of standing with the reagents will result in the precipitation of some meso-tartrate. Racemic acid is, of course, precipitated as the racemate without addition of *l*-ammonium tartrate, and, if present, may be precipitated by adding the calcium reagent alone. It should be noted here, that the amount of *d*-tartaric acid originally present, and converted into racemate, will be one-half the amount found as calcium racemate. Oxalic acid, if present, will be precipitated by the calcium reagent, and should be eliminated before proceeding to the determination of *d*-tartaric acid.

The methods (*a*) and (*b*) referred to above have been studied by Hartmann and Hillig,[2] and with certain modifications have been adopted as tentative methods in America (*Methods of Analysis, A.O.A.C.*, 3rd edition, 1930, p. 271). Further study of the methods seemed necessary, owing to the proportionately large amount of malic acid present in cider, and also in view of the possibility of inactive malic acid appearing on the English market in the future.[3] This acid is obtained by passing benzene vapour mixed with oxygen over a heated vanadium catalyst, when maleic acid is produced. This gives malate on boiling with dilute alkali. Inactive malic acid is reported to be very pleasant to the palate, and may figure largely in beverages, provided it can compete commercially with the biochemical method of obtaining citric acid from cane sugar.[4]

The American official method for the determination of tartaric acid was found to give misleading results when a large proportion of malic acid was present. It should be borne in mind that the amount of malic acid present in cider is often five times the maximum amount of tartaric acid allowed by the regulations in the "Select" grade, and in one case a cider examined here was found to contain 7·5 grms. of malic acid per litre.

Hartmann and Hillig (*loc. cit.*) have considerably modified this official method, but their modification, also, is not satisfactory when a large excess of malic acid is present. These authors have estimated up to 0·15 grm. of tartaric acid in the presence of 0·1 grm. of malic acid and citric acid, and have found their method to give good results with this proportion of acids. It was found in experiments here that, if a reasonable titration for tartaric acid was desired, say, 5 ml. of $N/10$ alkali, representing 0·075 grm., the amount of malic acid which might be present in a portion of cider containing this amount of tartaric acid would sometimes be as much as 0·5 grm. A solution containing 0·1 grm. of tartaric acid, and 0·5 grm. of malic acid, when tested according to the method described (*loc. cit.*, p. 104), indicated the presence of 0·142 grm. of tartaric acid. High results were also given when inactive malic acid and tartaric acid were in the same solution; *e.g.* a solution containing 0·05 grm. of tartaric acid and 0·25 grm. of inactive malic acid indicated the presence of 0·074 grm. of tartaric acid. The investigation of this method was therefore abandoned.

The modification of Kling's method, referred to above, was then investigated, and was found to be satisfactory, even when the tartaric acid present was only one-seventh of the amount of a mixture of other hydroxy acids. At present, pure *l*-ammonium tartrate is somewhat expensive, and it is imperative to use the substance free from the *d*-salt. E. G. Kellett, of this department, has recently published[5] an improved technique for the preparation of the pure *l*-salt, which

should greatly facilitate the cheapening of production, and so make more generally available the present modification of Kling's method, which has proved greatly superior to all other methods. For convenience, the technique which has been used in this laboratory for over a year, and has been found satisfactory, is given here.

PREPARATION OF SAMPLE.—In many cases the sample needs no special preparation, but in some preparations, such as jellies, jams, and other fruit products, sufficient sugar or colloidal material (such as gelatin or pectin) is present to make precipitation and filtration difficult. If a considerable quantity of alcohol is present, esters may have been formed, and saponification may be necessary before determining the acid. Solid matter, which could be oxidised by potassium permanganate, must be removed by preliminary filtration. Pectin, pectic acid and gelatin may be eliminated by adding excess of alcohol with a few drops of sulphuric acid, allowing the mixture to stand for half an hour, and filtering off an aliquot portion through a coarse fluted paper or a pad of cotton wool. In no case was sufficient pectin present in ciders examined here to require precipitation by alcohol, although in some instances filtration was slow. It is essential to obtain the free acids when pectin, etc., has to be eliminated, or when sufficient alcohol is present in the original sample to depress the solubility of calcium *l*-tartrate below its precipitating point under the conditions of the determination.

DETERMINATION.—Weigh or measure such a portion of the sample as will contain not more than 0·2 grm. of tartaric acid in the final aliquot portion, adjust to 35 ml. by dilution or concentration, add 3 ml. of *N* sulphuric acid, pour into a 250-ml. flask, rinse with 15 ml. of warm water and then with 96 per cent. alcohol, and make up to the mark with 96 per cent. alcohol. Industrial methylated spirits may be used in place of alcohol. Shake the mixture, and allow it to stand for half an hour, filtering if necessary. Transfer a convenient aliquot portion of the clear alcoholic solution so obtained into a centrifuge tube, and add a slight excess of neutral lead acetate solution. Shake vigorously for two minutes, and centrifuge for 15 minutes at about 1000 revs. per minute. Drain off the supernatant liquid thoroughly, and wash once with alcohol or methylated spirit, centrifuging and draining as before. Transfer the lead salts to a beaker with warm water, and pass in a rapid stream of hydrogen sulphide until the reaction is complete. Filter, wash thoroughly, boil the filtrate until it is free from hydrogen sulphide, and adjust the volume to 150 ml. Up to this point it has been assumed that the difficulties mentioned under "Preparation of sample" have been encountered; if they have not, take a portion of the sample, which will contain not more than 0·2 grm. tartaric acid, and dilute to 150 ml.

To the 150 ml. obtained by either process, add 15 ml. of reagent (*a*), 25 ml. of reagent (*b*) and 20 ml. of reagent (*c*), stir vigorously until calcium racemate begins to precipitate, and allow the mixture to stand overnight at room temperature. Filter by decantation on to a thin, lightly-tamped pad of asbestos, and transfer the precipitate to the crucible with a portion of the filtrate. Wash the contents of the crucible five times with water, filling the crucible about half full and sucking dry each time. Treat the precipitate and mat after removal

from the Gooch crucible with 20 ml. of reagent (*d*), and wash the crucible thoroughly. Adjust the volume of solution to 150 ml. with water. Bring 50 ml. of reagent (*e*) to the boiling point and pour it through the Gooch crucible into the 150 ml. mentioned above, then bringing the temperature of the whole to 80° C. Cool, stir vigorously, and leave for at least 4 hours, stirring occasionally. Filter and wash as described in the first operation. Transfer the pad and precipitate to a beaker with 150 ml. of water, add 50 ml. of sulphuric acid, 10 per cent. by volume, and heat to 80° C. Immediately add standardised potassium permanganate solution until an excess is indicated. Again heat to 80° C., add an additional 5 ml. of the permanganate solution, and allow the beaker to stand for about 1 minute. After re-heating to 80° C., immediately add 10 ml. of the standard oxalic acid solution, and titrate back with the permanganate solution. One ml. of the permanganate solution equals 2·5 mgrms. of *d*-tartaric acid.

REAGENTS.—(*a*) Di-ammonium citrate. Dissolve 29 grms. of citric acid in about 200 ml. of water, carefully neutralise to methyl red with ammonia, add 14·5 grms. of citric acid, and make up to 1 litre with water. This solution contains 50 grms. per litre.

(*b*) Ammonium *l*-tartrate. Dissolve 3·2 grms. of the salt, entirely free from *d*-tartrate, in water, add 1 ml. of commercial formalin as a preservative, and dilute to 200 ml.

(*c*) Calcium acetate. Dissolve 16 grms. of calcium carbonate in 120 ml. of glacial acetic acid diluted with sufficient water, make up to 1 litre, and filter.

(*d*) Hydrochloric acid. Dilute 34 ml. of pure acid (conc.) to 1 litre.

(*e*) Calcium and sodium acetate. Dissolve 5 grms. of calcium carbonate in 20 grms. of acetic acid and sufficient water, add 100 grms. of sodium acetate, make up the solution to 1 litre and filter.

(*f*) Potassium permanganate. Prepare a solution in water containing 6·9745 grms. in a litre. Standardise this against pure tartaric acid, employing the complete precipitation process as for the sample taken. One ml. of the permanganate = nearly 2·5 mgrms. of the *d*-tartaric acid originally present, or nearly 5 mgrms. of racemic acid.

(*g*) Oxalic acid. Prepare a solution containing 13·8793 grms. per litre and titrate against the permanganate solution.

EXPERIMENTAL.—Active and inactive malic acids and citric acid (0·5-grm.-portions) were dissolved separately in 150-ml. portions of water, and the process described above was followed. In no instance was any precipitate observed on standing overnight in the first part of the process on adding reagents (*a*), (*b*) and (*c*). Minute crystals were observed if the mixtures were allowed to stand for 48 hours. These crystals were filtered off, and the second part of the process was continued. In no case was any precipitate given, showing that at the concentration used, the acids could not be mistaken for *d*-tartaric acid. In view of the insolubility of calcium racemate in the reagents employed, it was somewhat surprising to find that inactive calcium malate was sufficiently soluble to give no precipitate.

The effect of the presence of inactive malic acid on the determination of *d*-tartaric acid was studied by precipitating 0·15 grm. of tartaric acid alone, and a similar quantity of this acid mixed with 0·5 grm. of inactive malic acid under the given conditions. The volumes of standard permanganate solution used were almost identical in the two cases, showing that there was no appreciable precipitate due to inactive malic acid. Ordinary *l*-malic acid, and citric acid under similar conditions, did not interfere with the determination of tartaric acid.

The process was then repeated with the following mixture of acids:—*d*-tartaric acid, 0·15 grm.; *l*-malic acid, 0·50 grm.; inactive malic acid, 0·25 grm.; citric acid, 0·25 grm.

The permanganate titration was equivalent to 0·153 grm. of tartaric acid, showing that, even with this large excess of other acids, the method was reasonably accurate.

Genuine ciders, kindly supplied by the National Fruit and Cider Institute, Long Ashton, were examined, and it was found that these gave no measurable titration which would indicate the presence of tartaric acid by the method described. Weighed quantities of *d*-tartaric acid were added to these ciders, and determined. The amounts obtained by titration were almost exactly equal to the amounts of tartaric acid added in each case.

QUALITATIVE TEST OF CIDERS.—Over 300 ciders have been tested qualitatively for tartaric acid as follows: To 50 ml. of cider were added 5 ml. of reagent (*a*), 8 ml. of reagent (*b*), and 7 ml. of reagent (*c*), and the mixture was stirred vigorously at intervals during 4 hours. Ciders which contained no extraneous tartaric acid gave no precipitate in this time. Three mgrms. of tartaric acid could readily be detected by the formation of a distinct precipitate which was identified as calcium racemate, under the microscope, by the typical rosette-like form of the clusters of crystals.[6]

I wish to thank the Government Chemist for permission to publish this paper.

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Nitrite in Cured Meats

By OSMAN JONES, F.I.C.

(*Read at the Meeting, April* 6, 1932)

It has long been known that the addition of a small quantity of nitrate* to the pickle used in curing meat imparts a red colour, which is not removed when the meat is cooked. Polenske, in 1891, found nitrite to be present in used pickle, and expressed the opinion that it resulted from the bacterial reduction of nitrate to nitrite. Nothwang confirmed Polenske's findings, but considered that the reduction was due to the action of the muscular tissue. Haldane (*J. Hygiene*, **1901, 1, 115**) found that the red colour produced is due to the formation of nitroso-haemoglobin, by the interaction of nitric oxide with haemoglobin. He further showed that, upon boiling, the haemoglobin compound is converted into nitroso-haemochromogen, to which compound the red colour of cooked meat is therefore due.

The various complex changes which meat undergoes during the process of curing are still under investigation by the Food Investigation Board and the British Food Manufacturers' Research Association. The reactions which bring about the formation of the nitroso compounds are less involved than some of the other changes. There is little doubt that Haldane's and Polenske's conclusions are correct. It has been definitely established that when nitrate is used in a curing pickle, its reduction to nitrite is largely, and probably entirely, brought about by the action of nitrate-reducing bacteria, probably derived from various sources, for they are so widely distributed in nature that it is difficult to find any environment from which they are absent. Harrison (*Canadian J. Res.*, 1929, **1**, 256) isolated various organisms which reduced nitrate to nitrite, among which were the following:—*S. pyogenes aureus, Micrococcus lactis varians, Lactobacillus acidophilus, B. coli communis, Aerobacter,* etc. Wolff and Berberich found nitrate-reducing organisms in samples of commercial saltpetre. I have confirmed this, and have also found them in samples of salt.

Methods of Curing.—There are two methods ordinarily employed in the curing of meat—by the use of salt and nitrate, and by the use of salt and nitrite.† The former is the more general, but the latter is rapidly coming into use, particularly in those countries where it is permitted. As will be shown, in both cases the cured meat contains nitrite. In the former method the amount of nitrite varies and cannot be closely controlled, but in the latter the amount can be strictly limited.

It is necessary to employ nitrate in large excess of that required to produce sufficient nitrite to combine with the haemoglobin present in the muscular tissue.

* In this paper the terms "nitrate" and "nitrite" refer to the potassium or sodium salts (or both).

† Since reading this paper before the Society I have been informed that in some curing establishments, where comminuted meats are cured, the use of a mixture of nitrate and nitrite has been found to be advantageous, because it sometimes happens that products of this class do not show satisfactory colour-fixation when only nitrite is employed.

Why this should be so is not properly understood, but unless excess is used, cure is unduly delayed or never reaches completion. The reducing action of the micro-organisms is not limited to the production of just sufficient nitrite to react with the haemoglobin; its formation continues to an indefinite extent, depending upon the amount of nitrate present, the length of time of cure, the temperature and other circumstances. It is probable, therefore, that in time the whole of the nitrate will be reduced to nitrite. The following data (*J. Agric. Res.*, 1926, **33**, 54) show the change in the nitrite content of a nitrate pickle:

Time—							
Fresh	5th day	20th day	30th day	40th day	48th day	55th day	62nd day
Sodium nitrite, parts per million—							
2·6	10·0	138·0	189·0	421·0	540·0	700·0	826·0

It is the practice in many curing establishments to use the same lot of pickle more than once, its strength being restored to the original composition by the addition of salt and nitrate. No regard is paid to the fact that it contains a quantity of nitrite derived from the reduction of the previous lot of nitrate. It can be readily understood that, if such a pickle be used four or five times, it becomes comparatively rich in nitrite, as well as being highly contaminated with bacteria. Meat cured in old pickle is therefore likely to contain much nitrite.

A determination of the amount of nitrite in various samples of nitrate-cured meats indicates to what extent this substance is present. The following figures will illustrate this point:

American hams	..	Nitrite,	72	to	960	parts	per	million
Bacon (various)	..	,,	36	,,	640	,,	,,	,,
Corned beef	..	,,	2·4	,,	45	,,	,,	,,

As it is not possible to control the production of nitrite in a nitrate brine or pickle, it is not surprising that the amount of nitrite in nitrate-cured meat shows such wide variations.

If it is conceded that the presence of excess of nitrite in a food is undesirable, it must also be admitted that a desirable method of cure is one which will limit and control the amount of this substance in the finished product. If, in a pickle, nitrite is substituted for nitrate in amounts varying from about one-fifth to about one-tenth of the latter substance, the pickle so made will permit of the strict control of the amount of nitrite in the finished meat, but will nevertheless produce a satisfactorily cured article.

In some countries the use of nitrite in the preparation of meat foods is permitted, but at the same time the amount of nitrite in the finished product is limited to 200 to 250 parts per million.

Substitution of Nitrite for Nitrate.—The fact that nitrite can be satisfactorily substituted for nitrate has been known for many years. As far back as 1905 nitrite began to find some practical use in a few American packing houses, although at that time the employment of nitrite in curing meat was not officially permitted. It was found then that nitrite gave more satisfactory and uniform results than nitrate, that the curing-time could be shortened, and that by controlling

the amount of nitrite in the pickle, the amount remaining in the cured meat could be kept within quite narrow limits. Some years later the United States Department of Agriculture was approached with a view to obtaining official sanction to the use of nitrite, and that Department carried out exhaustive experiments on the question. In the *Journal of Agricultural Research* (1926, **33**, 54; ANALYST, 1927, **52**, 37) Kerr, Marsh, Schroeder and Boyer published the details of their experiments.

Their results showed that nitrites could be successfully substituted for nitrates, and that no curing difficulties were caused by the proper use of nitrites. They also found that the residual nitrites in the nitrite-cured meats were less than were commonly present in nitrate-cured meats, and that the nitrite-cured meats were also free from the residual nitrate commonly found in nitrate-cured meat. They concluded that there could be no objection to the substitution of nitrite for nitrate from the standpoint of public health, but that, on the contrary, the more accurate control of the amount of nitrite and the elimination of the residual or unconverted nitrate were definite advantages attained by the substitution. The use of nitrite in the preparation of cured meats is now permitted in the United States.

A further advantage of the nitrite cure is that it is independent of the presence of reducing micro-organisms, and it is therefore possible to work with pickles of such a p_H value as to inhibit the growth of bacteria, without interfering with the process of cure. Spoilage may, therefore, be kept in check, and the cured meat shows a lower bacteriological count. This point is of importance where it is intended to preserve such meat by canning.

The following data are instructive as showing the change in the nitrite-content of the two kinds of pickle and in the meats cured in the pickles. It has been abstracted, with the author's approval, from Moulton's *Meat through the Microscope*.

	Days	Nitrite Per Cent.	Nitrate Per Cent.
PICKLE			
Nitrate	0	0·00010	0·66
Nitrate	5	0·00011	0·55
Nitrate	15	0·00032	0·49
Nitrate	30	0·0040	0·47
Nitrate	60	0·076	0·34
Nitrite	0	0·049	None
Nitrite	5	0·040	None
Nitrite	15	0·035	None
Nitrite	30	0·026	None
Nitrite	60	0·019	None
HAMS CURED IN ABOVE PICKLE			
Nitrate-cured	5	0·00014	0·046
Nitrate-cured	15	0·00015	0·071
Nitrate-cured	30	0·0020	0·087
Nitrate-cured	60	0·0035	0·131
Nitrite-cured	5	0·0050	None
Nitrite-cured	15	0·0025	None
Nitrite-cured	30	0·0040	None
Nitrite-cured	60	0·0050	None

Reiss, Mayer and Müller (*Z. Unters. Lebensm.*, 1928, **55**, 325) record a comprehensive series of comparative experiments on the use of nitrates and nitrites in the preparation of meats, for the purpose of ascertaining if it would be expedient for the German authorities to annul the regulation as to the use of nitrite in curing meat. The conclusions at which they arrived were similar to those found in America, namely, that, as nitrate and nitrite were invariably present in nitrate-cured meat, there would be no objection to the employment of the nitrite as such in the process of curing, instead of producing it in the course of cure by reduction of nitrate. Further, that a nitrite cure was to be recommended, since it permitted of the close control of the nitrite in the cured meat and eliminated the nitrate altogether. The use of nitrite in Germany is now permitted.

The carefully-conducted experiments carried out in America and Germany, and others under my control (it is probable that work has also been carried out quite extensively elsewhere) have failed to detect any difference in the flavour, keeping quality and appearance of nitrate-cured and nitrite-cured meats, and it seems improbable that the nitrate possesses any function other than the formation of nitrite by reduction. If the investigations now being carried out should subsequently prove that such is not the case, it will probably be necessary to modify some of the views expressed in these notes.

There does not seem to be the slightest need for apprehension that nitrite-cured meats are harmful to health.

I desire to add that many of the data given above, and the views expressed, are obtained from the works of other investigators; I have confined my efforts to confirming their findings, and have, as previously stated, brought the matter forward for discussion because it appeared one that it would be desirable to ventilate.

Investigations into the Analytical Chemistry of Tantalum, Niobium, and their Mineral Associates

XXV. THE SEPARATION OF URANIUM FROM TANTALUM, NIOBIUM, AND TITANIUM

By W. R. SCHOELLER, Ph.D., and H. W. WEBB

(Work done under the Society's Analytical Investigation Scheme)

(*Read at the Meeting, November* 2, 1932)

Uranium occurs as an essential constituent of certain earth-acid minerals, such as samarskite, euxenite, betafite, etc., and has also been reported in variable or subordinate amounts in other tantaloniobates. It will be shown in this Section that the new tannin methods described in Sections XVII and XVIII (Analyst, **1929, 54, 710**; **1930, 55**, 608) can be successfully applied to the determination of uranium and to its separation from tantalum, niobium, and titanium; uranium thus takes its place among the elements of the "basic tannin group" (XXIII, *id.*, **1932, 57**, 551).

Considering the progressive character of our knowledge gained in the course of these investigations, it is satisfactory to record that the only inaccurate statement

found in the earlier papers is one referring to uranium, which was said to be included in the precipitate produced by ammonium sulphide in ammoniacal tartrate solution (I, ANALYST, 1922, **47**, 93). This statement, which was made in ignorance of the fact that tartaric acid prevents the precipitation of uranium as sulphide, was reproduced in Section XVII (*loc. cit.*, p. **714**).

In the course of our revision of the position of uranium in our scheme for the analysis of earth-acid minerals we ascertained that this element is precipitated by tannin from tartrate solution, and a preliminary notice to that effect was appended to Section XVIII (*loc. cit.*). About the same time, Das-Gupta published a paper (ANALYST, 1930, **55**, 154) on the determination of uranium by precipitation with tannin from neutralised acetate solution and ignition of the dark-brown tannin complex to U_3O_8. Das-Gupta expresses the view, which we share, that for small quantities of uranium the tannin method is safer than the usual ammonia precipitation, in which the presence of carbonate in the precipitant may cause low results.

A. PRECIPITATION OF URANIUM BY TANNIN.—This can be effected, like the precipitation of "earth"-forming elements in general, either from tartrate or from oxalate solution.

(1) *From Tartrate Solution.*—The procedure is in no way different from that employed for the precipitation of the earth acids (XVII, *loc. cit.*, 710). In Exps. 1 to 5 the solution containing 3 to 4 grms. of tartaric, and 30 c.c. of hydrochloric, acid was neutralised with ammonia and boiled with 1 grm. of tannin and 5 grms. of ammonium acetate. The quantities of oxides taken were unknown to the operator.

	Taken		Found	
Exp.	U_3O_8 Grm.	M_2O_5 Grm.	*TP* net Grm.	Error Grm.
1	0·0576	none	0·0578	+0·0002
2	0·0676	,,	0·0678	+0·0002
3	0·0808	,,	0·0809	+0·0001
Ta 4	0·0817	0·1100	0·1913	−0·0004
Nb 5	0·0514	0·1646	0·2160	0·0000

The results show that uranium is quantitatively precipitated from tartrate solution; if the earth acids also are present, the process effects the quantitative recovery of the mixed oxides, just as it did the joint recovery of the earth acids, titania, zirconia, and thoria in our earlier work.

It should be borne in mind that ignited uranic oxide, unlike the pentoxide and dioxide earths, is acted upon by dilute acids; hence, in the lixiviation of ignited tannin precipitates for the removal of traces of alkali and sulphur trioxide (XIX, ANALYST, 1931, **56**, 309) the liquid should be made feebly ammoniacal prior to filtration.

(2) *From Oxalate Solution.*—It does not appear to be generally known that uranium is not precipitated by ammonia from oxalate solutions. We satisfied ourselves that the addition of ammonium oxalate to a solution of uranium prevents its precipitation by ammonia; quantitative recovery of the uranium was, however, achieved by tannin precipitation as follows:

The slightly acid oxalate solution containing several grms. of ammonium chloride is boiled, treated with fresh, 2 per cent. tannin solution (about 10 parts

of tannin to one of uranic oxide), and a slight excess of ammonia. The liquid is kept on a hot plate until clear, then left for some hours at room temperature. The dark-brown precipitate is collected, washed with ammonium chloride solution, ignited, and weighed. The gross weight is almost invariably excessive, due to contamination with silica derived from the glass vessels. The weighed oxide is therefore dissolved in strong nitric acid, the solution is evaporated to dryness, the residue taken up with hot water and a drop of acid, and the insoluble portion is collected, ignited, and weighed. This weight is deducted from the gross weight, the difference representing U_3O_8. The test analyses in which this procedure was applied are tabulated under *B* below.

B. Separation of Uranium from Tantalum, Niobium, and Titanium.—Tannin precipitation of tantalum, niobium, and titanium (the three members of the "acid tannin group" or "Group *A*"; XXIII, *loc. cit.*) from feebly acid oxalate solution half-saturated with ammonium chloride effects their quantitative separation from uranium, which is not precipitated under these conditions. The separation is fully described in Section XXIII as a simple fractionation procedure yielding two final fractions and one intermediate one (TP^{1a}); this is re-treated. That mode of working is distinctly useful for separations involving uranium, for this element (unlike the "Group *B*" metals, zirconium, thorium, and aluminium) furnishes a coloured tannin complex which, when about to flocculate at the neutral point, imparts a brown tint to the solution. The procedure described under XXIII is outlined below, with special reference to the colour indications, which provide a convenient and reliable guide for the progress of the separation.

Fraction TP^1.—The oxalate solution of the bisulphate melt is treated with dilute ammonia until a faint cloudiness results; this is removed with a drop or two of hydrochloric acid. At this stage, a bit of blue litmus paper dropped into the solution must turn red. The liquid is treated with an equal bulk of calcium-free saturated ammonium chloride solution, boiled, stirred, and treated gradually with the requisite amount of tannin reagent. The precipitate, TP^1, is red, orange, or yellow, and the filtrate is colourless or of a pale straw colour, not brown. The ignited precipitate TP^1 is pure white, indicating freedom from uranium; it is a final product, requiring only lixiviation.

Fraction TP^{1a}.—The filtrate from TP^1 is boiled, stirred, and treated with 0·2 to 0·3 grm. of tannin, and dilute ammonia is added, drop by drop, until the precipitate, TP^{1a}, as well as the solution, acquires a permanent brown tinge. After ignition the precipitate will be yellow to pale brown, owing to a small admixture of uranic oxide.

Fraction TP^2.—In the re-treatment of TP^{1a}, the boiling oxalate solution of the bisulphate melt (30 to 40 c.c.) is neutralised, treated with ammonium chloride, and tannin reagent is added, drop by drop, until TP^2 coagulates. This is of the same colour as TP^1, and consists of the balance of Group *A* oxides. It is added to TP^1 prior to the usual purification (*vide supra*).

Recovery of the Uranium.—The combined filtrates from TP^1 and TP^2 are boiled with more tannin and a slight excess of ammonia, etc., as described under A (2).

Results of Test Separations.—In the following tests, uranium was separated

from tantalic, niobic, tantalic *plus* niobic, and titanic, oxides (see column 1), the operator being kept in ignorance of the quantities taken until the work was accomplished. The figures given under TP^1 and TP^{1a} are *gross* weights, which serve as guides, and are reproduced here to demonstrate the course of the separation. TP^2 is the result of the re-treatment of TP^{1a}; the column headed "Final P" gives the weight of the purified ($TP^1 + TP^2$). The weight of U_3O_8 found has been corrected for silica.

		Taken		Group A oxides					U_3O_8	
Exp.		Group A Grm.	U_3O_8 Grm.	TP^1 Grm.	TP^{1a} Grm.	TP^2 Grm.	Final P Grm.	Error Grm.	Found Grm.	Error Grm.
Ta	6	0·1045	0·1016	0·1038	0·0206	0·0040	0·1054	+0·0009	0·1018	+0·0002
Nb	7	0·1383	0·1104	0·1378	0·0070	0·0039	0·1385	+0·0002	0·1099	−0·0005
EA	8	0·1230	0·1084	0·1000	0·0267	0·0256	0·1237	+0·0007	0·1087	+0·0003
Ti	9	0·1146	0·1107	0·1125	0·0055	0·0044	0·1151	+0·0005	0·1113	+0·0006

C. SEPARATION OF GROUP A FROM GROUP B.—In the last two tannin experiments of this investigation, "unknown" mixtures of the oxides of seven elements were quantitatively separated into the two groups, A and B, defined in Section XXIII, uranium figuring in both tests as a member of Group B.

A substantial amount of ferric oxide was also included in Exp. 11. Iron, when present in quantity, is a less tractable member of Group B, not because the separation from Group A is indefinite, but because the inkiness of the solution at or near the neutral point obscures the more delicate colour-indications which form an important feature of the process. In such cases the operator may prefer to precipitate ferrous sulphide from ammoniacal tartrate solution, recover the earths from the filtrate by tannin precipitation (XVII, *loc. cit.*), and then separate them into Groups A and B by the present procedure. A few mgrms. of iron, on the other hand, do not complicate the group separation.

In the experiment under discussion we avoided an initial fractional precipitation, but added tannin to the almost neutral solution until the darkening of precipitate and solution indicated incipient precipitation of iron, hence complete precipitation of Group A. The ignited TP^1, which was mottled pink, was re-treated by fractional precipitation, yielding iron-free precipitates, TP^2 and TP^{2a} (white after ignition). The figures given under "Final P" are the weights of ($TP^2 + TP^{2a}$) after the usual purification.

	Taken				Found				
Exp.	Group A Grm.		Group B Grm.		TP^1	TP^2	TP^{2a}	Final P	Group A Error
10	Ta_2O_5	0·0410	ZrO_2	0·0371					
	Nb_2O_5	0·0540	ThO_2	0·0323					
	TiO_2	0·0358	U_3O_8	0·0383					
			Al_2O_3	0·0401					
		0·1308			0·1347	0·1303	0·0014	0·1304	−0·0004
11	Ta_2O_5	0·0361	ZrO_2	0·0545					
	Nb_2O_5	0·0580	ThO_2	0·0384					
	TiO_2	0·0389	U_3O_8	0·0515					
			Fe_2O_3	0·0280					
		0·1330			0·1370	0·1311	0·0021	0·1321	−0·0009

It must be conceded that this fairly simple method for separating such complex mixtures into two groups of oxides represents a definite advance in analytical manipulation, which will ease the task of the mineralogist.

D. BEHAVIOUR OF URANIUM IN TARTARIC HYDROLYSIS.—The following notes on the hydrolytic precipitation of the earth acids by hydrochloric acid from tartrate solutions containing substantial amounts of uranium, complete our Observations on Tartaric Hydrolysis (XVI, ANALYST, 1929, **54**, 704).

The tests were carried out exactly as in the earlier investigation, the mixed oxides being fused with bisulphate, the melt dissolved in tartaric acid, and the solution boiled with hydrochloric acid. The precipitate, *HP*, was mixed with paper pulp, collected, returned to the beaker, churned up with wash-liquor, again collected, and the washing completed; hence due precautions were taken to remove the whole of the soluble uranium.

The weighed precipitate, *HP*, was tested for uranium by the pyrosulphate tannin method (XV, ANALYST, 1929, **54**, 455); the filtrate from the insoluble earth-acid complex was precipitated with ammonia, the small precipitate ignited, evaporated with strong nitric acid, and the filtered extract precipitated with ammonia; the yellow precipitate was ignited and weighed. The results are tabulated below (in grms.).

Exp.	M_2O_5 Taken Grm.	U_3O_8 Added Grm.	*HP* Grm.	U_3O_8 in *HP* Grm.	Colour of *HP* after ignition
Ta 12	0·2022	0·1541	0·1973	0·0020	buff
Ta 13	0·2042	0·1515	0·2038	0·0042	yellowish
Nb 14	0·1999	0·1599	0·1436	nil	white
Nb 15	0·2024	0·1527	0·1216	nil	white
EA 16	0·1017	0·2018	0·0950	0·002	yellow

The figures disclose a remarkable difference between the tantalum and the niobium products of hydrolysis; tantalum is precipitated normally, and the precipitate contains a little adsorbed uranium which colours the ignited precipitate. Niobium, on the other hand, is incompletely precipitated, the precipitate being uranium-free. A mixture of pentoxides containing 2 parts of tantalic to one of niobic (Exp. 16) acts like tantalic oxide. The interference of zirconia in tartaric hydrolysis, noticed in Section XVI, has a reverse effect, as it affects tantalum much more strongly than it does niobium.

These observations are important, in so far as they lead us to anticipate that the tartaric hydrolysis reaction, which we consider to be of paramount importance for the detection and identification of the earth acids (XV, *loc. cit.*), will prove of more limited usefulness in quantitative work.

SUMMARY.—Like the earth acids and the earths in general, uranium is quantitatively precipitated by tannin from neutralised tartrate solution in presence of ammonium acetate and chloride. It is quantitatively precipitated from oxalate solution by tannin and a slight excess of ammonia. Uranium, like zirconium, thorium, aluminium, and iron (Group *B*), can be quantitatively separated from tantalum, niobium, and titanium (Group *A*) by tannin precipitation of the three last-named elements from feebly acid oxalate solution half-saturated with ammonium chloride. In tartaric hydrolysis, uranium interferes with the normal course of precipitation in the case of niobium, but not of tantalum or of mixed pentoxides in which tantalic oxide preponderates.

THE SIR JOHN CASS TECHNICAL INSTITUTE,
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Volumetric Determination of Potassium Dichromate and Potassium Permanganate in a Mixture

By BISHAMBAR LAL VAISH and MATA PRASAD, D.Sc.

A method for the quantitative separation of chromium and manganese has been described by Herschkowitsch (*Z. anal. Chem.*, 1920, **59**, 11) who first oxidises the two metals to permanganate and chromate and then treats it with ammonium sulphate and excess of ammonium hydroxide. The precipitate is filtered off, washed, and ignited as Mn_3O_4.

Chatterji (*Chem. News*, 1921, **123**, 232) has shown that potassium permanganate and potassium dichromate in a mixture can be determined volumetrically by first titrating the mixture with a standard solution of ferrous sulphate. Then a known volume of the mixture is treated with a hot solution of manganese and zinc sulphates which converts potassium permanganate completely into hydrated manganese dioxide; this is filtered off and washed with hot water containing a little dilute sulphuric acid. The amount of potassium dichromate is then found by titrating the filtrate (which contains only potassium dichromate) with a standard solution of ferrous sulphate.

We have found that potassium permanganate can be easily determined in a mixture of potassium permanganate and potassium dichromate by the following process:

The mixture is made alkaline by adding excess of solid sodium carbonate. Then excess of a solution of hydrogen peroxide is added until all the manganese is precipitated as hydrated manganese dioxide and the supernatant solution is distinctly yellow. The precipitate is filtered off and washed on the filter-paper with warm water, and the filtrate is boiled to remove the excess of hydrogen peroxide, cooled and acidified with dilute sulphuric acid. The amount of potassium dichromate in the mixture is obtained by titrating the filtrate with standard solution of ferrous ammonium sulphate, the end-point being indicated by potassium ferricyanide.

The validity of this method was tested in the following manner:

A solution of potassium dichromate was titrated with a solution of ferrous ammonium sulphate and then mixed with different amounts of a solution of potassium permanganate. The above process of separation was followed, and the following values for the titre for the filtered solution were obtained:

Table I

Mixtures	Potassium dichromate (0·1*N*): c.c.	25	25	25	25	25
	Potassium permanganate (0·1*N*): c.c.	0	10	25	50	60
Ferrous ammonium sulphate solution: c.c. required		24·80	24·80	24·80	24·80	24·80

We also find (Table II) that the titration of a mixture of potassium permanganate and potassium dichromate with ferrous ammonium sulphate is unaffected by the ratio in which the two salts are mixed.

TABLE II

(25 c.c. of 0·1 *N* $K_2Cr_2O_7$ solution = 24.65 c.c. of ferrous ammonium sulphate solution)
(25 c.c. of $KMnO_4$ solution = 25·40 c.c. of ferrous ammonium sulphate solution)

Mixtures	Potassium dichromate: c.c. ..	25	25	25	10	5
	Potassium permanganate: c.c. ..	5	10	25	25	25
	Ratio	5:1	2·5:1	1:1	1:2·5	1:5
Ferrous ammonium sulphate solution required: c.c.		29·70	34·80	50·5	35·25	30·35
,, ,, ,, calculated: c.c.		29·73	34·81	50·5	35·26	30·33

In these experiments A.R. chemicals and standard burettes and pipettes were used.

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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 DIPHENYL-BENZIDINE TEST FOR NITRATES IN MILK AS A MEANS OF DETECTING ADDED WATER, AND THE EFFECT OF DRENCHING COWS WITH "NITRE"

IN the ANALYST (1931, **56**, 248) we recorded our experiment of dosing two cows with 1/3 ounce (=9·4 grms.) of "nitre" twice a day for a week, testing their milk every day with the diphenylamine reagent. No trace of nitrates was found in any of the 14 samples so tested.

Our attention has recently been called to the experiments of Richmond (ANALYST, 1893, **18**, 279; 1894, **19**, 83), in which (1) he gave three cows 1 grm. of potassium nitrate per day for 3 days and obtained a strong diphenylamine reaction with their milk 8 hours after the last dose and a distinct reaction 12 hours later still; and (2) he obtained a strong diphenylamine reaction with the milk of cows accustomed to drinking water containing 18 parts per 100,000 of N_2O_5.

These results are in conflict not only with our own results, but also with those of Krause (ANALYST, 1926, **51**, 255), who found no nitrates in the milk of cows which for 30 days had drunk water containing 50 parts per 100,000 of nitrate (N_2O_5), or in the milk of cows to the food of which 7 grms. of potassium nitrate had been added. The result of Richmond's second experiment is more convincing than that of the first, for, assuming a daily ration of 10 gallons of water per cow, the daily intake of nitric nitrogen works out at 2·1 grms., as compared with 2·6 grms. in our experiment, whilst 1 grm. of potassium nitrate (as in his first experiment) is only 0·139 grm. of nitric nitrogen, and represents only 0·306 part per 100,000 in the assumed 10 gallons of water—quite a normal quantity if not rather unusually small; yet we know by experience that nitrates are not normally present in genuine cow's milk.

We considered it desirable, in view of our disagreement with Richmond, to confirm our experiment, and accordingly again sought the kind co-operation of the Principal of the Somerset Farm Institute, who readily agreed to arrange for the dosing with "nitre" and the collection of the samples. This was carried out under the direct supervision of Mr. G. G. Gregory, Vice-Principal. Instead of using diphenylamine, as in our first experiment, we used diphenyl-benzidine, which we find to be ten times as sensitive, and by means of which one can just detect 0·1 part per million of nitric nitrogen in milk, and very distinctly 0·2 part.

(*Cf.* Monier Williams, ANALYST, 1931, **56**, 397.) The greatest precautions were taken to avoid contamination of the samples. Wide-mouthed stoppered bottles were prepared in the laboratory, the final rinsings with nitrate-free distilled water being tested with the diphenyl-benzidine reagent; the cows were milked directly into the bottles after the hands of the milker and the udders of the cows had been washed and dried on a clean towel, and only the middle milk was collected. It was considered unsafe to give the nitre with the fodder in powder form for fear of it getting into the dust of the air, and it was therefore given in the form of a drench. Before making the test a blank was carried out in the actual test tubes to be used.

The following table shows the hours of milking and dosing, the next milking, and the result of the test. It will be noted that in no sample was nitrate present.

The evening milk and that of the next morning were sent to the laboratory together and tested the same morning, except on one day when there was delay in dispatching and the samples were tested in the afternoon. The acidities of all samples were determined, and in no case exceeded 0·18 per cent. of lactic acid.

Day	Cow*	Sample taken	Dosed†	Interval in hours between dosing and milking hours	Diphenyl-benzidine test
Sunday, Jan. 15, Evening	A & B mixed§	3.45 p.m.			negative
			4.0 p.m.		
Monday, Jan. 16,	A & B mixed§	6.45 a.m.		14·5	negative
			7.30 a.m.		
	A	4.0 p.m.		8·5	negative
	B	4.0 p.m.			negative
	A & B		4.0 p.m.		
Tuesday, Jan. 17	A	7.0 a.m.		15	negative
	B	7.0 a.m.			negative
	A & B		10.0 a.m.		
	A	4.0 p.m.		6	negative
	B	4.0 p.m.			negative
	A & B		4.30 p.m.		
Wednesday, Jan. 18	A	6.45 a.m.		14	negative
	B	6.45 a.m.			negative
	A & B		9.0 a.m.		
	A	4.0 p.m.		7	negative
	B	4.0 p.m.			negative
	A & B		4.0 p.m.		
Thursday, Jan. 19	A	7.0 a.m.		15	negative
	B	7.0 a.m.			negative
	A & B		9.30 a.m.		
	A	4.0 p.m.		6·5	negative
	B	4.0 p.m.			negative
	A & B		4.30 p.m.		
Friday, Jan. 20	A	7.0 a.m.		14·5	negative
	B	7.0 a.m.			negative
	A & B		7.30 a.m.		
	A	4.0 p.m.		8·5	negative
	B	4.0 p.m.			negative
	A & B		4.30 p.m.		
Saturday, Jan. 21	A	7.0 a.m.		14·5	negative
	B	7.0 a.m.			negative

* Cow A—"Lovely." Cow B—"Violet."
† Dosing with 1/3rd ounce of potassium nitrate twice daily.
§ The first two samples were mixed by mistake, not intentionally.

The diphenyl-benzidine test is extremely sensitive when applied as a spot test. The suspected solution is evaporated to dryness, and two drops of the reagent are added. This procedure is not applicable to milk, but we found it of value in testing our distilled water and rinsings of bottles. One can detect 0·0001 mgrm. of nitric nitrogen. The method of applying the test to milk is exactly the same as that described by Lerrigo (ANALYST, 1930, **55**, 433), except that diphenyl-benzidine is used in the place of diphenylamine.

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THE USE OF AMYL ALCOHOL FOR MILK-TESTING

THE composition of fermentation amyl alcohol, which is used in the Gerber acido-butyrometric method for determining the percentage of butter-fat in milk and milk products, depends largely on the original materials employed in the manufacture of ethyl alcohol. Some difficulty has been experienced in this laboratory in ensuring that supplies of amyl alcohol were suitable for milk-testing. Of 10 samples examined, 5 gave errors which exceeded 0·10 per cent. of butter-fat. The errors found were always in a positive direction, indicating that the disturbing factor was, in all probability, some impurity in the alcohol which was thrown out of solution, and, after centrifuging, remained dissolved in the fat column. Since the Gerber method is widely used in creameries and in other places where an examination of chemicals is not possible, it is desirable that a more rigid standard should be adopted for amyl alcohol used in milk-testing.

The tests for the suitability of amyl alcohol for milk-testing, described in most text-books of dairy chemistry, are inadequate, for 3 of the 5 samples giving erroneous results in this investigation had boiling ranges within the prescribed limits, while all the samples were free from moisture, furfural and petroleum. The alcohols were examined by means of a polarimeter, a refractometer and a stalagmometer, but the results obtained were valueless. The polarimeter served to show that there was considerable variation in the proportion of active alcohol present in the different samples, and both suitable and unsuitable samples gave high and low readings. The refractive indices and surface tensions were more or less constant, and did not give any indication of quality. The actual results of these tests are set out in the accompanying Table.

PHYSICAL CHARACTERISTICS OF 10 SAMPLES OF AMYL ALCOHOL

Sample No.	Boiling range* °C.	Polarimeter scale readings in 4-dm. tube	Refractive index (Abbé)	Surface tension (No. of drops)	Error in percentage of butter-fat†
1.	130·3–132·0 (131·3)	19·5	1·4101 (15° C.)	77	0
2.	128·5–132·4 (131·2)	12·0	1·4092 (15° C.)	78	−0·03
3.	131·2–132·2 (131·8)	9·6	1·4099 (15° C.)	78	−0·03
4.	122·0–135·2 (131·7)	9·0	1·4085 (15° C.)	77	+0·02
5.	129·8–132·5 (131·4)	9·6	1·4098 (15° C.)	77	−0·04
6.	127·4–132·0 (131·1)	20·6	1·4100 (15° C.)	77	+0·14
7.	Insufficiency of sample	15·0	1·4092 (15° C.)	78	+0·20
8.	131·2–132·6 (131·8)	8·5	1·4076 (20° C.)	77	+0·12
9.	130·2–132·2 (131·7)	11·3	1·4091 (15° C.)	78	+0·31
10.	123·8–135·0 (126–129)	0	1·4109 (15° C.)	75	+0·53

* Temperature in brackets is that at which the bulk of the sample distilled over.

† These are the average errors obtained with several samples of milk, the results being compared with those obtained by the Röse-Gottlieb method.

Data were obtained to show that the error in the butter-fat test was constant over a wide range of milks containing different percentages of butter-fat. It would appear, therefore, that the impurity is insoluble in the acid-milk solution and of such a nature that it might be possible to eliminate it by means of careful fractional distillation.

Fractional distillations were carried out by means of a Young–Thomas still-head with 5 sections, and measured fractions were collected without regard to the rise in temperature which took place during the process. With No. 2, an alcohol which has given satisfactory results over a number of years, it was found that there was no significant difference between the butter-fat tests when different fractions were used in the Gerber method. With No. 10, on the other hand, it was found that the impurity was mostly concentrated in the first fractions coming over. As this sample was of very inferior quality, not likely to be met with often in practice, its further examination was abandoned. With samples Nos. 6 and 9, the impurity was easily concentrated in the last portion of the distillate, butter-fat tests nearly twice as great as the original butter-fat tests being obtained with small end fractions. Finally, the impurity was eliminated from samples Nos. 6 and 9 by continued fractional distillation. With one portion, the last 10 per cent. of the volume was rejected each time for three successive distillations. With another portion, only 5 per cent. was rejected. In each case the greater bulk of the alcohol was obtained in a form suitable for milk testing, *i.e.* the errors in the butter-fat test were reduced to less than 0·05 per cent. of butter-fat.

There appears to be no one satisfactory chemical test which will distinguish an alcohol suitable for milk-testing. Amyl alcohol for milk-testing should be guaranteed to give, when used in the Gerber method, a butter-fat test with an error of not more than ± 0·05 per cent. of butter-fat, as compared with the result obtained by the Röse-Gottlieb method. It would be very desirable if a chemically pure substitute for amyl alcohol could be found, even if this involved an alteration of the graduations of the Gerber butyrometer. Five supposedly pure isomeric amyl alcohols were tried with this end in view, but, as their boiling ranges varied as widely as that of the fermentation product, there would be no advantage in using them instead of amyl alcohol.

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THE DETERMINATION OF LEAD IN ACID CALCIUM PHOSPHATE

THE difficulty of obtaining a satisfactory determination of the amount of lead in acid calcium phosphate was brought to our notice recently, when we were shown the results obtained by a number of analysts on a particular sample. In this connection we applied the method suggested by Allport and Skrimshire (ANALYST, 1932, **57**, 440), which seemed to offer a reasonable prospect of success without being an unduly long or difficult procedure.

Two grms. of the sample are dissolved by heating in 10 c.c. of concentrated hydrochloric acid and water. To the cooled solution it is necessary to add 40 c.c. of ammonium citrate solution (J. R. Nicholls, ANALYST, 1931, **56**, 594) and 15 c.c. of ammonia (sp.gr. 0·880). After the addition of 1 c.c. of 10 per cent. potassium cyanide solution and thorough cooling, the lead is extracted with the diphenyl-thiocarbazone reagent, three extractions being used in all cases (10 c.c., 5 c.c., and 5 c.c. of reagent). The red colour of the lead compound is not seen in the presence of this large amount of ammonium citrate. The Allport and Skrimshire

procedure is then followed, except that we add 1 c.c. of sulphuric acid to the residue, instead of 0·5 c.c. in the digestion.

Using this procedure, we made satisfactory determinations with different amounts of lead added to calcium phosphate, provided the amount of lead did not exceed 0·0004 grm. This, on a 2-grm. sample, is equivalent to 200 parts per million. If larger quantities are present, smaller quantities of the acid calcium phosphate must be taken.

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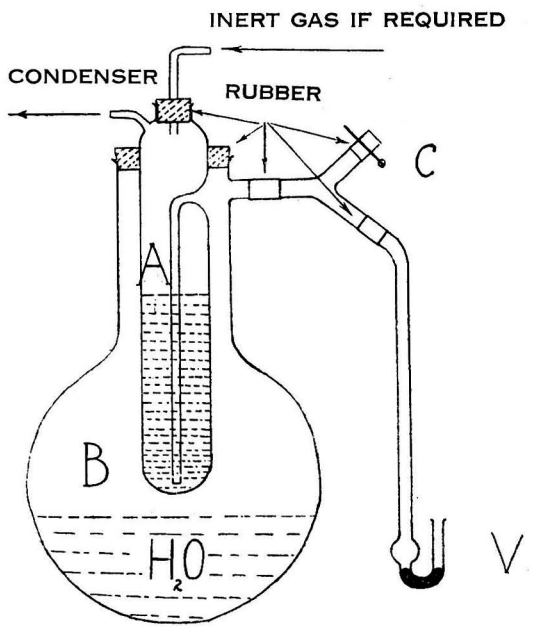
A STEAM-DISTILLATION APPARATUS

THE apparatus shown in the diagram has been found very useful for the rapid distillation of small quantities of organic preparations (300 c.c.). A two-litre flask with a wide neck, having a side tube, is fitted with a bung carrying the other portion of the apparatus. The substance to be distilled with steam is placed in the inner vessel A, and in B is placed water slightly in excess of the quantity required in the distillate. The stop-cock at C is opened, and the water in B is heated until the steam issues freely at C, when the stop-cock is closed. The valve, V, assists in keeping the distillation steady, and when the distillation is finished the heating may be discontinued and the apparatus left without fear of anything being sucked back. Directly C is closed the steam passes through the tube leading into A. The diagram is self-explanatory. The advantages are: (1) a saving of time, bench space and heating; (2) no risk of the vessel containing the preparation cracking, as it is heated at the same rate and as uniformly as the water; (3) minimum condensing surface to this vessel.



The apparatus has also been used for the determination of sulphites in foods, and gives results comparable in accuracy with those of other methods, but much more rapidly obtained. The following results were obtained with one sample (in duplicate) by three methods:

(1) Ordinary steam-distillation apparatus, followed by determination as barium sulphate: Found, 96 parts per 1,000,000;

(2) Monier-Williams' Method: Found, 102 parts per 1,000,000;

(3) With this apparatus, the distillate being passed into hydrogen peroxide, and titrated as in method 2: Found, 100 parts per million (calculated amount present 102 parts per 1,000,000).

The apparatus was made by Baird and Tatlock (London), Ltd.

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THE OIL FROM THE GLANDS OF THE MUSK-RAT

THE musk-rat, which is a rodent related to the beaver (*Castor fiber*, L.), is found largely in North America, and millions of rats are there trapped annually in order to obtain their pelts, which constitutes the well-known musquash fur.

The rats have also been imported into Europe, and a good deal has been heard in this country lately of danger caused to embankments, etc., by their burrowings. An interesting description of these rats was recently given by C. A. Oldfield (*Soap Trade & Perfumery Review*, 1932 [April], p. 277).

As its name implies, the musk rat has a distinct musk-like odour, derived from glands contained in the abdomen. From time to time the use of these glands in perfumery has been suggested, but hitherto no serious attempt has been made to collect them. Recently, however, the Hudson Bay Company have collected a considerable quantity, and we have examined a sample of these.

The glands had been slightly smoked to prevent putrefaction, and had an average weight of about 3 grms. per pair of glands. They are very oily on the surface, any paper in which they are wrapped quickly becoming very greasy. On extraction with petroleum spirit, they yield 50 to 54 per cent. of an oil, one specimen of which had the following values:

Acid value	80·6
Saponification value	145·9
Iodine value	136·9
Unsaponifiable matter	22·8 per cent.
Insoluble fatty acids	70·0 ,, ,,

The fatty acids give the following results:

Mean molecular equivalent	307
"Titre"	28·3° C.
Refractive index (n_D^{40})	1·4558
Iodine value	87·3
Solid acids*	34 per cent.
M.pt. of solid acids	43·0° C.
Iodine value of solid acids	57·5
Mean molecular equivalent of solid acids	338
Iodine value of liquid acids	112

* Determined by the modified lead salt and alcohol method (ANALYST, 1931, **56**, 376).

The unsaponifiable matter has, for perfumery purposes, been separated into two fractions—44·5 per cent. soluble, and 55·5 per cent. insoluble, in 90 per cent. alcohol—and the iodine values of these were: alcohol-soluble, 122; alcohol-insoluble, 333.

The nitrogen-content of the extracted glands was 12·2 per cent.

The most interesting of the above figures are (1) the high iodine value and mean molecular equivalent of the solid acids, indicating the presence of unsaturated acids of high molecular weight; and (2) the very high iodine value of the unsaponifiable matter insoluble in 90 per cent. alcohol. It is hoped, later, to investigate both these points more fully.

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SYMPOSIUM ON THE FREEZING-POINT OF MILK

ALL members of the Society are invited to attend the next meeting of the North of England Section on this subject, at the Engineers' Club, Albert Square, Manchester, on Saturday, April 8th, at 1.15 p.m.

Official Appointments

THE Minister of Health has approved the following appointments:

HARRI HEAP as Public Analyst for the Borough of Glossop, in place of John White (who is retiring). (February 18th.)

C. V. REYNOLDS as a Public Analyst for the County Borough of Plymouth, in addition to Thomas Tickle (February 18th).

WILLIAM PARTRIDGE as Public Analyst for the Metropolitan Borough of Fulham, in place of C. H. Cribb (decd.) (March 4th).

Department of Scientific and Industrial Research

REPORT OF THE FOREST PRODUCTS RESEARCH BOARD*

DURING the year under survey 689 specimens of structural timber were tested, and 3470 test pieces, cut from material free from knots, etc., were submitted to almost every conceivable mechanical test. Tests on physical properties, etc., numbered over 9500. A total of 4800 specimens, treated in various ways with eight wood preservatives (including low temperature tar and its distillates), have been exposed for tests, and are kept under constant observation. The new experimental building at Princes Risborough has made possible large-scale investigations of dry rot and the action of the death-watch beetle. Most of the pulp boards tested have been found susceptible to dry rot when damp enough. Incense had no observable effects on the larvae of *Xestobium*, and the study of the effects of various insecticides on the beetle has brought out the difficulties of making satisfactory tests; for not only is standard material needed, but also much information on the penetrating power of liquids and gases into the timber.

A vigorous policy of development and exploration of the forest resources of the Colonies and Dependencies would apparently render the whole Empire almost, if not quite, independent of foreign supplies of hardwood, whilst a considerably greater consumption of Canadian soft woods is possible.

The photo-electric cell is now being used in the study of wood structure to measure the percentage of empty space in the interstices of timber.

TOXICITY TESTS OF PRESERVATIVES.—The testing of preservatives has been continued, and particular attention has been paid to the toxicity of creosotes obtained from low-temperature tar, and the derivative phenolic fractions, and these creosotes appear to be at least as toxic as ordinary creosote. In the course of the work being carried out to devise methods of test which shall be internationally comparable, a comparison of five strains of *Coniophora cerebella* has led to the most vigorous being adopted for all toxicity tests, and different strains of various other test-fungi are now being tested. Salicylanilide ("Shirlan") has been proved to be very toxic to wood-destroying fungi.

D. G. H.

* Obtainable at H.M. Stationery Office, Adastral House, Kingsway, W.C.2. Price 3s. 6d. net.

International Commission for Uniform Methods of Sugar Analysis

REPORT OF PROCEEDINGS OF THE EIGHTH SESSION*

THE chief recommendations made by the Commission at the Eighth Session, held at Amsterdam from September 5th to 8th, 1932, are as follows:

100° *S. Point of the Saccharimeter.*—A standard scale for the saccharimeter, to be known as the "International Sugar Scale," should be adopted, rotations expressed in this scale being designated as degrees sugar (°S.). The polarisation of the normal solution (26·000 grms. of pure sucrose dissolved to 100 ml. and polarised at 20° C. in a 200 mm. tube with white light, and the dichromate filter as defined by the Commission†) should be accepted as the basis of calibration of the 100° point on the International Sugar Scale. It is suggested that new saccharimeters should be graduated in accordance with this scale, and inscribed by the makers with the phrase "International Sugar Scale." The reading of the normal sugar solution on the Herzfeld-Schönrock scale should be accepted as 99·90° S. The normal quartz plate of the International Sugar Scale has the following rotation.

Normal Quartz Plate $=100°$ S. $=40{\cdot}690° \pm 0{\cdot}002$ ($\lambda = 5461$Å) at 20° C.
,, ,, ,, $=100°$ S. $=34{\cdot}620° \pm 0{\cdot}002$ ($\lambda = 5892{\cdot}5$Å) at 20° C.

The question of the French Saccharimeter Scale is held in abeyance.

Ash (Mineral Matter) in Raw Sugars.—The incineration and electrical conductivity methods are both recommended, but the latter is preferred.

Determination of Reducing Sugars.—The following ten methods are proposed for further study: (1) Baerts', (2) Brown, Morris and Millar's, (3) Lane and Eynon's, (4) Main's "Pot" Method, (5) Müller's, (6) Ofner's, (7) Pellet and Babinski's, (8) Pick's, (9) Saillard's, and (10) Schoorl's Method. If defecation is employed prior to determination of reducing sugars, neutral lead acetate should be used, followed by sodium phosphate or potassium oxalate as de-leading agent.

Sulphur Dioxide in Consumption Sugar.—Direct titration with iodine solution is sufficient for the running control of sulphur dioxide in sugar factory products. For exact work the sulphur dioxide should be distilled from the acidified solution after expulsion of air with carbon dioxide, oxidised to sulphuric acid, and the latter determined gravimetrically.

L. E.

* *Int. Sugar J.*, 1933, **35**, 17–19, 62–64.

† "A solution of potassium dichromate of such a concentration that the percentage-content of the solution multiplied by the length of the column of the solution in cm. is equal to nine."

Ministry of Health

SALE OF FOOD AND DRUGS ACT

EXTRACTS FROM THE ANNUAL REPORT FOR 1931–1932, AND ABSTRACT OF REPORTS OF PUBLIC ANALYSTS FOR THE YEAR 1931*

THE number of samples submitted to Public Analysts during the year 1931 was 136,169, a decrease of 346 on the record year, 1930. Of these, 6324, a percentage of 4·6, the lowest recorded, were reported as adulterated or not up to standard.

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

PRESERVATIVES.—Of the 561 contraventions of the Public Health Regulations, the use of preservative would have been permissible in 207 cases if its presence had been declared on the label; boron preservative was present in 85 samples, chiefly of sausages, cream, butter, meat, potted fish and cake. Prohibited preservatives were present in meat, sweets, ginger, and desiccated soup, and excessive preservative was found in jam, marmalade, dried fruits, a few samples of non-alcoholic wine, candied peel and other foods. Three samples of "preserving powders" and one of "bacon-dusting powder" contained boron preservative.

MILK.—The highest recorded number of samples (70,201) were analysed by Public Analysts, and, of these, 4507 (6·4 per cent.) were found to be adulterated. Of the "appeal-to-cow" samples, 39·1 per cent. were not up to standard, the high percentage being accounted for by their having been taken from farms whose supplies had been found below standard when previously sampled in transit to the consumer. In 27 samples of milk, visible dirt was reported; 17 contained colouring matter, 5 formalin, and 1 boron preservative. In one case a deficiency in fat of 8 per cent. was reported, and the sample contained colouring matter and formalin. In 6 cases, graded milk was deficient in non-fatty-solids.

DRIED AND CONDENSED MILK.—Of the 245 samples of dried milk examined, 6 were reported deficient in fat, as were 4 of the 1309 samples of condensed milk, and 4 samples of the latter were deficient in non-fatty-solids, one in fat and non-fatty-solids, and 6 were improperly labelled.

CREAM.—Fourteen of 2163 samples contained boron preservative; 13 consisted of reconstituted artificial cream; 4 tinned samples were deficient in milk-fat; 1 contained 65 parts per million of tin; 3 contained foreign fat; and 2, sold as "ideal table cream," were mixtures of starch and sugar.

BUTTER AND MARGARINE.—Ninety-six samples of 10,502 of butter were reported against, 73 for excess of water, 5 for containing foreign fat, 6 for consisting of margarine, 10 for containing preservative, and 2 for excess of free fatty acids. Of 3472 margarines, 29 had excess of water, 1 contained boron preservative, and 5 were improperly labelled. A "butter-substitute" vendor was convicted of "exposing margarine not properly labelled for sale"; "selling margarine otherwise than as margarine"; and "selling margarine not duly labelled."

LARD AND OTHER FATS.—Twelve of 3008 samples of lard, 29 of 346 of suet, and 17 of 673 of dripping, were reported against; the majority of lards, for consisting partly or wholly of fat other than that of the hog; of the suets, for excess of rice flour; and of the drippings, for excessive acidity.

CHEESE.—Of 1573 samples of cheeses, 20 wrapped cheeses contained tin in proportions up to 7 grains per lb., but the degree of contamination appears to be lessening; whilst 16 samples were deficient in fat, and one was infected with mould and mites. Three "cheesettes" should have been labelled "margarine cheese."

BREAD AND FLOUR.—All the 312 samples of bread were reported as genuine; of 1395 samples of flour, 4 self-raising flours were condemned, 3 for containing sulphate of lime, and one for containing no bicarbonate of soda.

JAM AND MARMALADE.—Of 1621 samples, 58 contained excess of sulphur dioxide; others were deficient in soluble solids, or contained juices of fruits other than those specified; one contained string and packing material; one strawberry jam only had one-quarter of its fruit strawberries; a raspberry jam had 21 per cent. of apples and one per cent. of gingelly seeds.

VINEGAR.—Of 1571 samples, 102 were deficient in acetic acid, and 44 were wholly or partly artificial vinegar.

SPIRITS AND BEER.—In 160 cases, brandy, gin, rum or whisky were found to be diluted so as to reduce the spirit below the limit of 35 degrees under proof.

Two beers contained excess of preservative, and one traces of arsenic, which was also found in 2 black beers.

MISCELLANEOUS FOOD ARTICLES.—Of 1297 samples of confectionery, 35 contained excess of sulphur dioxide; arsenic was present in 6, talc in 4, and chalk in 2; Cornish cream toffee contained neither cream nor butter. Copper was reported in mushrooms-in-butter, tinned tomatoes, dried peas, and cider, and the paper tips of some chocolate cigarettes contained 800 parts of copper per million. Tin was reported in 57 samples (not including wrapped cheese), such as fish, fruit salad, vegetables, apricots, cherries, peaches, etc., brawn and tongue; lead in a sample of aerated water. Rice was found with talc or other mineral matter in 48 samples.

DRUGS.—The 236 samples reported against, of a total of 5257 examined, included sweet spirit of nitre; camphorated oil; mercury, zinc and other ointments; ground ginger; seidlitz powders; bismuth and aspirin tablets. Several samples sold as turpentine consisted wholly of mineral oil or paraffin; Glauber salts were found to consist of dehydrated sodium sulphate, and ammoniated quinine tablets were almost devoid of ammonia; tartaric acid was found to be cream of tartar or Rochelle salts; borax was sold as carbonate of soda, and paregoric was reported to be devoid of opium, and liniment of turpentine to be wholly soft soap. Tablets sold as "digestive" had no digestive properties, and 5 prescriptions were inaccurately dispensed.

D. G. H.

Palestine

ANNUAL REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1931

IN his Annual Report to the Palestine Department of Health the Government Analyst (Mr. G. W. Baker) states that 8550 samples were examined, as compared with 6359 in 1930, nearly half of this increase being due to Customs and Excise samples.

OILS AND FATS.—Of the 1153 samples examined 1043 were samples of semneh and butter from the Customs (5 per cent. adulterated), and 106 were from local factories (18 per cent. adulterated). Much Australian butter is imported in bulk and then packed in wrappers printed locally. Much of the so-called local butter is made from cream imported from Egypt.

Cotton-seed oil is now little used as an adulterant of olive oil and sesame oil.

Sunflower seed and areca nuts imported for oil production are now free of duty, and the extent to which these compete with the indigenous oils as adulterants or substitutes is being investigated.

Though acid oils and offals for soap-making are free of duty, unrefined olive oil (except virgin oil) is now a prohibited import. For Customs purposes virgin unrefined oil must contain not more than 2 per cent. acidity (as oleic). The fluorescence test has been of some value in distinguishing between refined oil and virgin oil.

The extent to which olive oil is being replaced by other materials in soap manufacture is indicated by the fact that 872 samples of acid oils and offals have been examined during the year.

FIREARMS AND PROJECTILES.—The exhibits submitted included 3 rifles, 11 cartridge cases, 5 revolver bullets, and 2 hand-grenades. In one case it was demonstrated that the suspected rifle did not fire the cartridge cases found on the scene of the crime, and in another case it was proved that a cartridge case had been

fired from the suspected rifle. It was also possible to show that a cartridge case found on the scene of a recent crime had the same bolt impressions as a cartridge case connected with a previous crime.

Considerable time has been spent upon improving the technique in the forensic examination of cartridge cases and firearms. A preliminary examination with a comparator microscope is followed by close examination of an image of about 10 diameters magnification on the focussing screen of a long extension camera with short focus lens. During this examination the cartridge case is rotated in oblique illumination from an arc lamp, which shows up any marks impressed upon the case by the bolt-head or striker. The photographs obtained in this way require no subsequent enlargement. The whole examination is carried out in the dark room.

ULTRA-VIOLET LIGHT AND ALTERED DOCUMENTS.—In two cases of alterations on cheques ultra-violet light revealed traces of substances with a yellow fluorescence beneath the words and figures of the amounts to be paid.

DANGEROUS DRUGS.—Of 20 samples of confiscated material, 2 were identified as novocaine adulterated with boric acid, 4 as raw opium, and 10 as hashish. Novocaine and cocaine are readily distinguished by the fluorescence test (Smith and Glaister, *Recent Advances in Forensic Medicine*).

INTERNAL CORROSION OF ALUMINIUM RADIATOR HEADS.—It has been proved that aluminium radiator heads are unsuitable for motor vehicles working in Palestine.

Speaking generally, the corrosion is associated (as was to be expected) with high salinity, much of which is present as chlorides of sodium and magnesium, and it appears that for this reason serious corrosion has, so far, been confined to vehicles working in the coastal plain. Some of the other factors influencing corrosion will be concentration and temperature in the radiator and the running hours under those conditions. Electrolytic action set up between the aluminium and other metals in the aluminium and the copper or brass parts is also a factor to be considered.

The Royal Air Force find the average life of a radiator using water from bores at Ramle and Sarafand to be less than one year. The Ramle water has chlorine 75 and total solids (anhydrous) 162 parts per 100,000, while that from Sarafand (which is said to be better) has chlorine 28, total solids 115, p_H 7·7, nitric nitrogen 6·0, and ammonia 0·02. The Sarafand water, after 26 days in a radiator, contained chlorine 48, total solids 125, nitric nitrogen 2·3, and ammonia 3·4 parts per 100,000. This reduction of the nitrates to ammonia, which was confirmed by laboratory experiments, may play a part in the corrosion. With a Jaffa water containing chlorine 11, total solids 45, nitric nitrogen 0·9, ammonia 0·002, seven days in a radiator changed these to chlorine 15, total solids 32 (the temporary hardness had been deposited), nitric nitrogen 0·1, ammonia 0·45 parts per 100,000.

On an ambulance using this Jaffa water the radiator had a life of 4 years, in which time 48,500 kilometres were run. Another ambulance, on which the radiator lasted 3 years, ran 28,000 kilometres, using Haifa water containing chlorine 159, total solids 320, nitric nitrogen 0·3 parts per 100,000. These two ambulances are the only vehicles concerning which a full history could be obtained, and the radiators from these have been examined and photographed in the laboratory.

The corrosion takes the form of pitting associated with a whitish deposit, samples of which contained 68 per cent. Al_2O_3. The corrosion is most intense on the edge of the flange which bolts on to the tube assembly, and it is near this flange that leaks and, finally, complete perforations first show themselves. Before actual perforation takes place pieces of corroded metal fall into the tubes and cause serious over-heating troubles. It has been ascertained that, while working, these two radiators were, as a rule, emptied weekly, and after any long runs. In brass

or copper radiators frequent emptying and re-filling tend to produce scale trouble, but where aluminium is concerned corrosion may be more serious than scale, and therefore high concentration of salinity by frequent "topping up" is to be avoided.

At the time when the report on this investigation was submitted orders for new ambulances had already been placed, and it is now learnt that they are, in consequence, to be supplied with brass radiator tanks.

Federated Malay States

ANNUAL REPORT OF THE CHIEF CHEMIST FOR THE YEAR 1931

As in previous years, most of the chemical work carried out by the Institute for Medical Research (Chief Chemist, Mr. R. W. Blair, F.I.C.) is concerned with the examination of water supplies and of milk, and with criminological investigations.

WATER.—In addition to the usual processes (storage, sedimentation and filtration), chlorination has been adopted as a final method of purification in an increasing number of supplies. At the intake works of the Kuala Lumpur supply the dosage of chlorine has been steadily increased from 0·2 to 0·6 part per million, with satisfactory results as regards taste and purity.

MILK.—According to The Sale of Food and Drugs Enactment, 1913, milk must contain not less than 3·25 per cent. of fat, and not less than 8·5 per cent. of solids-not-fat. Of the 889 samples submitted by the Health Authorities, 12 were deficient in fat, 55 in solids-not-fat, and 1 in both fat and solids-not-fat.

CONDENSED MILK.—The standards prescribed under the Food and Drugs Enactment, 1913, are as follows:—Sweetened condensed milk: 9 per cent. of milk fat, 31 per cent. of milk solids (including milk-fat); unsweetened condensed milk: 8 per cent. of milk-fat, 28 per cent. of milk solids (including milk-fat). Of 21 samples examined, 16 were deficient in milk-fat.

Machine-skimmed Condensed Milk.—This can be imported only under special licence; one sample was examined. No standards have as yet been laid down for milk of this nature.

TOXICOLOGICAL EXAMINATIONS.—The total number of exhibits examined was 212. The poisons identified were the alkaloids of datura, morphine, strychnine, cyanide, acetic acid, formic acid, alcohol, lysol, and mercury.

Gelsemium Poisoning.—A male Chinese died shortly after drinking a decoction made from a root supplied to him by a medicine man. The viscera and a specimen of the root were submitted for examination. The root, which closely resembled *Gelsemium*, contained an alkaloid, and a small quantity of an alkaloid was also isolated from the viscera. This was not identified, but a small quantity injected into a rat caused death with signs similar to those occurring in a second rat which had been injected with the alkaloid obtained from the root.

Bamboo Hairs as Poison.—A specimen of faeces was forwarded in connection with a case of diarrhoea and abdominal pain, in which poisoning was suspected. On shaking the faeces with saturated salt solution and centrifuging, a scum was obtained which, on microscopical examination, proved to contain numerous fine bamboo hairs. This material is sometimes employed as a poison (Gimlette, *Malay Poisons and Charms*).

LEAD IN COSMETICS.—In 1930 Professor R. B. Hawes observed symptoms suggestive of lead poisoning in a number of Chinese girls in Singapore, and it was subsequently discovered that certain brands of face powder contained lead compounds. Samples of local face powder were accordingly submitted for examination by the Health Authorities of the Federated Malay States, and of 54 such samples, 14 were found to contain lead, usually in the form of the carbonate,

one specimen containing as much as 48 per cent. (as $PbCO_3$). As the result of this investigation the following rule was gazetted under section 27 of "The Sale of Food and Drugs Enactment, 1913": "No lead or any compound thereof shall be added to, or be an ingredient or component part of any cosmetic preparation." Subsequently, a further rule was made prohibiting the sale, advertisement for sale, or importation of cosmetics containing lead or any compound thereof. (*Cf.* ANALYST, 1931, **56**, 812; 1932, **57**, 655.)

FIREARMS AND PROJECTILES.—Exhibits in connection with shooting cases included 23 rounds of ammunition, eight empty cartridge cases, six bullets, five pistols, two revolvers, and one gun.

An automatic pistol from an arrested person, together with empty cartridge cases, found at scenes of various gang robberies, were submitted. Characteristic marks on these cartridge cases, caused by striker-pin and ejector, were similar to those seen on cases fired by the automatic pistol in the laboratory. It was thus demonstrated that all the cartridge cases had been fired from this pistol, and that several robberies were the work of one gang.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs Analysis

Eggs (Detection of Preservation and Ageing). K. Elbe, H. Pfeiffer and R. Bretschneider. (*Z. Unters. Lebensm.*, 1933, **65**, 100–104.)—If 1 c.c. of the egg-white and 2 c.c. of amyl alcohol are shaken with 0·5 c.c. of a solution of alizarin, the alcohol layer develops a blue-violet colour with fresh eggs, but becomes wine-red or brown-yellow with eggs preserved in lime or water-glass. The test will not distinguish new from old eggs, and it is preferable to dip the tip of the egg in a dilute solution of thymol blue in alcohol, when a well-defined yellow stain, changing to a greenish tinge, is obtained with new or old unpreserved eggs, the final colour from eggs preserved with lime or water-glass being a blue tint. Bell and Doisy's phosphate reaction (ANALYST, 1921, **46**, 13) has been adapted as a test for new-laid eggs as follows:—Two c.c. of the egg-white and 8 c.c. of water are mixed with 5 c.c. of a 2 per cent. solution of hydroquinone in 0·1 per cent. sulphuric acid, and 5 c.c. of a 5 per cent. solution of ammonium molybdate in *N* sulphuric acid. After 5 minutes 25 c.c. of a mixture prepared from 1000 c.c. of 20 per cent. sodium carbonate (anhydrous) solution, 250 c.c. of water and 37·5 grms. of sodium sulphite are added; eggs up to 2 weeks old give no colour, or a slight green tint, whilst older eggs (preserved or otherwise) give a blue-green colour; the reagents should be tested to ensure the absence of phosphates. Ultra-violet light may be used to detect erasure of the mark of origin, whether by acid or abrasion, as a deep blue stain on the violet background of the shell becomes visible (*cf.* ANALYST, 1929, **54**, 664). The determination of the p_H value of the methyl alcohol serum does not afford a trustworthy means of distinguishing between fresh and preserved eggs.

J. G.

Mercurimetric Determination of Chlorides in Milk. E. Geyer and A. Rotsch. (*Z. Unters. Lebensm.*, **1933**, **65**, 66–71.)—Votoček's method (ANALYST, **1923**, **48**, 192) has been adapted to milk serum, and gives results in close agreement with those obtained by Mohr's method in which aluminium sulphate is used. Of the other methods tested, Volhard's procedure gives low values, and the following is recommended as giving the most exact results:—Fats and proteins are removed either (*a*) by shaking 20 c.c. of milk in a 200-c.c. flask with 10 c.c. of a 20 per cent. solution of aluminium sulphate and 8 c.c. of 2 *N* sodium hydroxide solution, and then diluting to 200 c.c.; or (*b*) by precipitation of 10 c.c. of milk (diluted with 30 c.c. of water) with 1 c.c. of 10 per cent. acetic acid, the mixture being diluted to 100 c.c. The mixture is then filtered in either case, 50 c.c. of the filtrate are diluted to 200 c.c. with water and 10 drops of concentrated nitric acid, and 6 drops of a solution of 6 grms. of sodium nitroprusside in 30 c.c. of water, and 10 drops of concentrated nitric acid are added. Titration is carried out with a 1/35·5 *N* solution of mercuric nitrate, until a blue white opalescence results. The reading is corrected by deducting 0·1 c.c., and, when multiplied by 20, gives the number of mgrms. of Cl in 100 c.c. of milk. The reagent is prepared by diluting a solution of 3·1 grms. of mercuric oxide in dilute nitric acid to 1 litre, and standardising this against a 1/35·5 *N* solution of sodium chloride. J. G.

Soya Bean Flour and Pea Flour. D. Kaltschewa. (*Z. Unters. Lebensm.*, **1932**, **64**, 540–545.)—(1) *Soya Bean Flour.*—Yellow Bulgarian soya beans are extracted for 30 hours at 20° C. with a solution containing 5 per cent. each of sodium carbonate and sodium chloride. This is continually renewed until bitter and colouring substances have been removed; the beans are then shelled and heated in steam under a slight pressure to remove the characteristic odour and taste. Finally the residue is pressed, dried at 100° C. in thin layers, and ground. Percentage analyses are given in the following tables:—

State	Water	Nitrogenous compounds	Oil	N-free extractives	Ash	Crude fibre
Unshelled	7·75	33·82	18·87	30·75	4·18	4·63
Shelled (dry substance)	—	40·31	23·18	28·43	2·97	5·14
Flour	4·45	39·36	24·46	24·43	2·71	4·59

The percentage nitrogen-content was made up as follows (results calculated on the dry substance):

State	Protein	Peptone	Amide	Indigestible	
				Nitrogen	Nuclein
Raw	—	—	—	0·256	1·626
Shelled	5·92	0·37	0·16	—	—
Flour	6·31	0·13	0·15	0·284	1·822

The oil from the flour had the constants:—Saponification value, 183·5; iodine value, 118·2. The ash contained 32 per cent. of phosphoric anhydride (mainly soluble), and the lecithin phosphate amounted to 0·1839 and 0·2014 per cent. of the raw and prepared material, respectively.

Pea Flour.—This is obtained from *Cicer arietinum*, L. by a similar process,

the resulting flour being heated for 1 hour at 120° C., when a yellow colour and almond flavour develop. The following percentage results were obtained:

State	Water	Nitrogenous compounds	Fat	N-free extractives	Crude fibre	Ash
Raw	9·97	20·90	5·65	58·33	2·74	2·42
Prepared	1·98	24·22	7·64	62·12	1·74	2·30

The constants for the extracted oils were:—Saponification value, 239·2 and 232·8; refractometer reading at 25° C., 74 and 71; iodine value, 128·6 and 114·0.

	Indigestible		Ash	
State	Nitrogen	Nuclein	Total P_2O_5	Lecithin phosphate
Raw	0·143	0·908	0·983	0·127
Prepared	—	—	0·972	0·143

The digestible nitrogen was determined by the Sjolema-Wedemayer method, and the results given are calculated on the dry substance. These flours are mixed with other vegetable flours or with dried milk, and should be suitable for use in certain specified nutritional diseases. J. G.

Detection of Dried Plums in Plum Pulp Mixtures. G. Kappeller and W. Reidemeister. (*Z. Unters. Lebensm.*, 1932, **64**, 558–560.)—The three reactions proposed by Rudolph and Barsch (ANALYST, 1932, **57**, 106) have been tested. The fuchsin test gave a yellow and a red colour, respectively, with 50 and 38 per cent. of the 16 fresh plum pulps tested; with 52 and 15 per cent. of the 27 dried plums; and with 31 and 21 per cent. of the 29 mixtures. As a red colour is also produced in the presence of iron (*e.g.* 0·12 per cent.), with an intensity which increases roughly with the iron-content, the test is considered untrustworthy. The reactions with sodium hydroxide solution, with iodine and with ammonia have also been found unsatisfactory, and the authors therefore prefer their own method (*loc. cit.*).

J. G.

Detection of Sorbitol in Wine. B. Bleyer, W. Diemair and G. Lix. (*Z. Unters. Lebensm.*, 1933, **65**, 37–41.)—A number of aldehyde derivatives of sorbitol, which may assist in its identification (*cf. id.*, 1930, **60**, 305), are described. The general method of preparation was to shake 2 grms. of sorbitol with 1 c.c. of water, 1 c.c. of 50 per cent. sulphuric acid or concentrated hydrochloric acid, and 1 grm. of the aldehyde (dissolved in xylene or butyl acetate, if a solid). After 1 day in the ice-chest the precipitate was removed by filtration, and was washed in succession with 50 and 80 per cent. alcohol and dry ether, and then re-crystallised from a mixture of ethyl acetate and alcohol (3:1). The following are the derivatives:—*o*-Chloro-monobenzal sorbitol (fine crystals), m.pt. 170° C.; *o*-chloro-tribenzal sorbitol (fine radiating needles), m.pt. 217° C.; 2-nitro-5-chloro-monobenzal sorbitol (tufts of crystals), m.pt. 250·5° C.; *m*-nitro-monobenzal sorbitol (long needles), m.pt. 180° C.; *m*-nitro-dibenzal sorbitol (prisms), m.pt. 228·5° C.; *m*-nitro-tribenzal sorbitol (spear-shaped crystals), m.pt. 168° C.; *o*-nitro-tribenzal sorbitol (fine needles), m.pt. 181° C.; 2.6-dichloro-benzal sorbitol (prisms), m.pt. 204·5° C. J. G.

Determination of Citric Acid in Wine. W. Bartels. (*Z. Unters. Lebensm.*, 1933, **65**, 1–37.)—The following methods have been compared, and are criticised in the light of the results obtained:—(1) The pentabromo-acetone reaction; (2) Denigès' reaction; (3) Conversion into acetone which is determined as (*a*) the complex mercury compound, or (*b*) iodoform; (4) precipitation as calcium or barium citrate. The last method (*cf.* Kogan, *Z. anal. Chem.*, 1930, **80**, 112) gives the most satisfactory results, even for red wines and in the presence of glycerin, if carried out exactly as follows:—A mixture of 50 c.c. of wine, 1·5 c.c. of 25 per cent. ammonia, 10 c.c. of 10 per cent. barium chloride solution, and sufficient 96 per cent. alcohol to bring the alcohol-content to 20 per cent. by volume, is filtered after 24 hours, the residual barium citrate being washed twice with 30 per cent. (by volume) alcohol. It is then transferred to a beaker with sufficient water to give a total volume of 45 c.c., and the mixture is heated (pumice being added) until all the alcohol is removed and the volume is halved. One c.c. of 80 per cent. acetic acid is then added, and the mixture is transferred to a 100-c.c. distillation-flask, fitted with a tap-funnel and leading into a cooled 200-c.c. flask, so that the total volume is 60 c.c. Pumice is added and heat is applied gently, and when the liquid just boils 10 drops of a 1·5 per cent. solution of potassium permanganate are added over a period of 5 minutes, without interrupting the boiling. The addition is continued until a permanent precipitate of manganese dioxide is obtained (2·5 and 3 hours for pale and red wines, respectively, if the content of citric acid is less than 1 grm. per litre), and, when the volume is halved, 25 c.c. of water are added without interrupting the boiling. The distillate is shaken with 5 c.c. of 25 per cent. sulphuric acid and 3 c.c. of a 5 per cent. solution of potassium permanganate, and if after 25 minutes no permanent violet colour results, more permanganate is added. The solution is then decolorised with the minimum quantity of hydrogen peroxide, and a 10 per cent. solution of sodium hydroxide is added until a precipitate is produced, followed by 20 c.c. in excess; the excess of hydrogen peroxide may then be removed by a slight excess of manganese sulphate. The mixture is diluted to 200 c.c., and filtered through a dry filter, and (for amounts of citric acid below 1 grm. per litre) 10 c.c. of a 0·1 *N* solution of iodine are added in a fine stream to 100 c.c. of the filtrate. The solution is shaken well for 15 minutes, 10 c.c. of 25 per cent. sulphuric acid are added, and the iodine is titrated with 0·1 *N* sodium thiosulphate solution with starch as indicator. The apparatus and solution are best standardised under the above conditions by a test experiment with a known quantity of citric acid. Results are given for 19 wines of various types, which were found to contain 0 to 29 mgrms. of citric acid in 50 c.c.; on addition to these of 8 to 19 mgrms. of citric acid per 50 c.c., 92 to 102 per cent. was recovered. The occurrence of citric acid as a natural constituent of the wine is discussed, and it is concluded that it may be derived from the grapes or produced during fermentation. J. G.

Tests for Coffee Substitutes [Malt Coffees]. A. Heiduschka and H. Thomas. (*Z. Unters. Lebensm.*, 1933, **65**, 95–97.)—The authors confirm the usefulness of Merl's method (ANALYST, 1930, **55**, 760), but record mean maltol-contents of 5·2 to 5·7 mgrms. per 10 grms. for 4 normal malt coffees, *i.e.* less than

the minimum value (6 mgrms. per 10 grms.) for such coffees specified by Merl. The results may be influenced by adsorption of maltol by the active charcoal. Other maltol values were:—Normally roasted barley, 5·5; wheat, 5·6; rye, 6·1; dried, roasted and glazed brewery malt, 1·3; steeped, roasted and glazed brewery malt, 5·7; rye, treated similarly, 3·9 and 5·2; wheat (similarly), 3·5. It therefore appears possible to distinguish between malted and unmalted products, but the value of the method is limited. J. G.

Investigations on Tobacco Smoke. II. E. Waser and M. Stähli. (*Z. Unters. Lebensm.*, 1932, **64**, 569–573.)—Further investigations (*cf.* ANALYST, 1933, **58**, 46) on substances intended to be placed in the tips of cigarettes to absorb nicotine have failed to confirm the claims of the manufacturers. The following results were obtained by smoking 5 cigarettes (about 5 grms. in all) in about 6½ minutes (4 seconds' "pull," yielding 40 c.c. of smoke, followed by 11 seconds pause); the tobacco contained 1·31 per cent. of nicotine and 1 per cent. of water, and all but 15 mm. of the length (68 mm.) of the cigarette was smoked. The effective removals of nicotine from the smoke were:—No absorbing agent, 15 to 30 per cent.; 1·3 grm. of silica gel *A*, increasing from 30 per cent. for a smoking-period of 6·5 minutes to 100 per cent. for over 8 minutes; 1·8 grm. of silica gel *B*, 29 to 42 per cent.; 0·45 grm. of active carbon, 40 to 54 per cent.; 3·4 grms. of glass beads similar in size to the granules of silica gel, 14 to 30 per cent.; 1·9 grm. of porous clay granules (similar size), 22 to 32 per cent. As a rule, silica gel is more effective as a denicotinising agent for solutions (*loc. cit.*) than for smoke. J. G.

Strophanthins of *Strophanthus Eminii*. W. A. Jacobs and N. M. Bigelow. (*J. Biol. Chem.*, 1933, **99**, 521–529.)—In a study of the digitaloid glucosides and aglucones which occur in the different species of *Strophanthus* plants the seeds of *Strophanthus eminii* have been examined, and the glucoside mixture has been found to be of complex character. It consists of easily hydrolysable glucosides and of more stable glucosides. The former, which were not isolated in crystalline form, are glucosides of an α-desoxy sugar. At first the aglucone obtained on hydrolysis of the labile glucosides gave analytical figures which suggested a formula $C_{23}H_{34}O_6$, but it was found to be a mixture of strophanthidin, the aglucone of *Strophanthus kombe* and *Strophanthus hispidus*, and of periplogenin, which has heretofore been found only as the aglucone of the glucosides of *Periploca graeca*. On repeated re-crystallisation of the mixed aglucones, strophanthidin itself was finally obtained from the mixture. The presence of periplogenin was shown by the isolation of derivatives such as dihydroperiplogenin and *dihydroperiplogenin benzoate*. The more stable glucosides were isolated as a chloroform-soluble *monoside*, $C_{30}H_{46}O_9$, and a *bioside*, $C_{36}H_{56}O_{14}$, which was sparingly soluble in chloroform; both of these proved to be derivatives of the same aglucone, $C_{23}H_{34}O_5$, which, however, could not be isolated as such. By a special procedure, in which methyl alcoholic hydrochloric acid was used, a *trianhydro derivative*, $C_{23}H_{28}O_2$, was obtained from both the *monoside* and the *bioside*; it proved to be isomeric with *trianhydroperiplogenin* produced by similar treatment of periplogenin itself. Although this would indicate that the previous

aglucone is not periplogenin but a closely related isomer, it is still possible that periplogenin in stable glucosidic union could give such an isomeric trianhydro derivative owing to an altered course of dehydration. The new glucosides were obtained from seeds nearly three years old. This fact, together with the low toxicity of these glucosides, gives rise to the suspicion that a certain amount of allomerisation may have occurred during this lapse of time, and that the monoside and bioside may therefore be glucosides of an alloperiplogenin. Fresh seeds will be used to investigate this point. In the monoside, the aglucone appears to be conjugated with a *methyl ether* sugar, $C_7H_{14}O_5$, which is either identical or isomeric with digitalose, the sugar of *Digitalinum verum*. In the bioside there is an additional hexose, possibly glucose, attached to this sugar. Full experimental details are given. P. H. P.

Water-soluble Iodine Content of Desiccated Thyroid Gland. C. R. Harington and S. S. Randall. (*Quart. J. Pharm.*, 1932, **5**, 629–632.)—The fraction of the total iodine of thyroid gland which dissolves in distilled water is very small, and in only one of eight samples examined did it greatly exceed 10 per cent. Following the inclusion in the B.P. (1932) of a limit for this water-soluble iodine-content, attention has been drawn to the fact that thyroid preparations dried at temperatures below 40° C. may yield a considerable proportion of their iodine to water. With two of four commercial samples of desiccated thyroid, dried at low temperatures, extraction of the iodine by water was almost complete, whilst the other two gave up 70 and 76 per cent., respectively, of their total iodine. Moreover, most of the iodine thus extracted passes into the precipitate, obtained by acidifying the extract with acetic acid and boiling, and is hence present in combination with protein. This solubility of the iodine in water is due to the fact that, under the drying conditions employed, the thyroglobulin does not undergo denaturation, and compliance with the requirements of the new B.P. evidently involves such denaturation. There is no evidence to show that denaturation of thyroglobulin results in impairment of its physiological activity, and it is possible that the increased digestibility thus produced may actually be advantageous. If the dried thyroid preparations are left in contact with alcohol for 17 hours and are subsequently dried at 55° to 60° C., the proportion of their total iodine extractable by water is greatly reduced, and this treatment is suggested as a means of satisfying B.P. requirements. T. H. P.

Occurrence of Dehydrodeguelin and Dehydrotoxicarol in Derris Root. E. P. Clark and G. L. Keenan. (*J. Amer. Chem. Soc.*, 1933, **55**, 422–423.)—Rotenone has been absent from several samples of derris root examined during the past two years, but, instead, mixtures of yellow crystals were isolated with m.pts. ranging from 200° to 225° C. On purification by re-crystallisation from various solvents the materials yielded mixtures of dehydrodeguelin and dehydrotoxicarol, identified by the optical immersion method. In two instances dehydrotoxicarol predominated sufficiently to allow of its isolation in a chemically pure state. Details of the examination of a particular mixture are given, and an application of the optical-immersion method to the original dehydro compound

showed it to have consisted of such a mixture of dehydrodeguelin and dehydrotoxicarol as could not be resolved by repeated re-crystallisation. The presence of such conditions may account for the fact that earlier workers on derris root recorded the presence of various yellow compounds melting between 200° and 230° C. Dehydrotoxicarol has not hitherto been reported as a natural plant product. D. G. H.

Testing Aluminium Foil for its Suitability as a Wrapping Material for Cut Bread. K. Seidel. (*Z. Unters. Lebensm.*, 1933, **65**, 104–106.)—The solubilities of a number of such foils have been determined in 0·25 *N* and 0·5 *N* acetic acid, 0·25 *N* and 0·5 *N* lactic acid, 0·25 *N* phosphoric acid, and an extract of 20 grms. of sterilised pumpernickel (containing originally 38 per cent. of water, and the equivalent of 3 per cent. of lactic acid). The test pieces were 5 × 8 square cm. in area, and 2·2 to 3·0 mgrms. per square cm. in weight, and they were immersed for 3 days at 20° C. in 100 c.c. of solvent. The respective losses in weight in the best and worst of the samples tested were (for the solvents in the above order):—Acetic acid, 0, 6·7; 1·0, 14·8; lactic acid, 2·8, 3·6; 4·5, 30·9; phosphoric acid, 135·3, 200·8; and pumpernickel, 0, 5·8 mgrms. per 100 square cm. J. G.

Biochemical

Biological Distinction of Egg- and Plant Lecithin by means of the Complement-Combination Method. O. Mezger, H. Jesser and M. Volkmann. (*Z. Unters. Lebensm.*, 1933, **65**, 49–54.)—Neither Uhlenhuth's precipitin method nor an adaptation of the Wassermann blood haemolysis reaction (*cf.* Uhlenhuth and Weidanz, *Praktische Arbeiten zur Ausführung des biologischen Eiweissdifferenzierungsverfahrens*) can be used satisfactorily to distinguish plant lecithin (*e.g.* from soya beans or wheat) from one another or from egg-lecithin. **J. G.**

Cobalt in Animal Nutrition. F. J. Stare and C. A. Elvehjem. (*J. Biol. Chem.*, 1933, **99**, 473–483.)—Until recently the biological significance of cobalt attracted the attention of relatively few investigators. Waltner and Waltner (*Klin. Woch.*, 1929, **8**, 313), in a study of the toxicity of various metals, observed that cobalt, when added to the diet of rats, produced a marked increase in the number of red blood cells, and, in studies on haemoglobin regeneration, other workers have since reported a polycythaemia produced by the addition of cobalt to a diet of milk, iron and copper. Owing to these results the authors have studied the cobalt-content of normal rats and those in which a polycythaemia had been produced by cobalt-feeding. A rapid, convenient and accurate colorimetric method for the determination of cobalt in biological materials has been based upon the observation recorded by Van Klooster (*J. Amer. Chem. Soc.*, 1921, **43**, 746), that the nitroso derivatives of R salt (sodium 2,3,6-β-naphthol disulphonate, when treated with cobalt salts, form a stable red dye, $(C_{10}H_5NO_8S_2Na_2)_3Co$, the colour of which is not destroyed by acids. Iron, nickel, copper and zinc also form

coloured compounds with the R salt, but these colours are destroyed by boiling with nitric acid. The colour is best developed at 70° C. in a solution made slightly alkaline with sodium acetate; on then adding nitric acid in slight excess and boiling, the red colour formed by cobalt is produced. The method is applicable to samples containing 0·01 to 0·5 mgrm. of cobalt. The results obtained when the method was applied to a few biological substances are given. Data are given (1) showing that if cobalt does occur in the normal animal organism it does so in extremely minute amounts, and (2) revealing the distribution of cobalt in the bodies of rats and pigs given a diet containing cobalt. The entire body of rats fed on a diet of milk, iron, copper and manganese was found to contain less than 0·01 mgrm. of cobalt. Definite amounts of cobalt were found in all rats given a similar diet plus cobalt, and the quantity present was proportional to the amount given. The presence of 0·04 to 0·05 mgrm. of cobalt in the entire body of a rat was sufficient to produce a decided polycythaemia. No cobalt was detected in tissues taken from pigs on a diet of milk, iron, copper and manganese, but definite quantities were detected in practically all the tissues taken from pigs fed on a similar diet plus cobalt. P. H. P.

Milk Diastase. H. Kluge. (*Z. Unters. Lebensm.*, 1933, **65**, 71–84.)—Rothenfusser's method (ANALYST, 1930, **55**, 758) for the estimation of diastase has been improved as follows:—Six c.c. of German Pharmacopoeia lead acetate solution are added, drop by drop, to 100 c.c. of milk; the mixture is then shaken and filtered through a dry paper, and 100 c.c. of the filtrate are again shaken with 2 c.c. of a saturated solution of sodium chloride and again filtered. Ten tubes containing the quantities of reagents shown in the table are then prepared.

Tube No.	Sodium chloride (0·85 per cent.) c.c.	Milk Serum c.c.	Starch c.c.	Sodium hydroxide (0·25 *N*)	Diastase unit
1	10	9	0·12	3 drops	0·073
2	10	9	0·25	3	0·146
3	9·5	9	0·50	3	0·292
4	1	18	1	11	0·583
5	7	12	1	6	0·876
6	11	8	1	4	1·312
7	13·7	5·3	1	2	1·980
8	15·5	3·5	1	2	3·003
9	16·7	2·3	1	2	4·566
10	17·5	1·5	1	1	6·993

The starch solution is made by boiling 1 grm. of soluble starch for 15 minutes with a mixture of 50 c.c. each of solutions containing 11·944 grms. of disodium hydrogen phosphate and 4·539 grms. of dipotassium hydrogen phosphate in 500 c.c.; the solution is diluted to 100 c.c. for tubes 1 to 3, and this is again diluted with an equal volume of water for the other tubes. It is particularly important that the p_H value of the liquid in the tubes should be 6·9, and this should be checked by brom-thymol blue, and adjusted, if necessary, by means of 0·1 *N* acid or alkali. After 3 hours at 38° to 40° C., 2 c.c. are removed from each tube without disturbing the sediment, and mixed with 2 c.c. of a 0·002 *N* iodine solution (1 drop for tubes

1 to 3). The tube which marks the transition from red-brown to pale blue gives the diastatic value in the units shown. The value is 1·980 to 0·583 (mean 1·312) for raw milk, less than 0·292 for permanently-pasteurised milk, and less than 0·146 for milk heated momentarily at 75° C. ("stassanisation" or "biorisation"). In raw milk it is unchanged on keeping, but it is destroyed by boiling, and the fall produced after heating for 30 minutes at 55° C. is almost as great as at 60° to 63° C.; preservatives (especially mercuric chloride) lower the diastatic action, and there is a recovery in its value on storage of lightly pasteurised milk. The Orla–Jensen method (*id.*, 1932, **57**, 383) has been found satisfactory for permanently-pasteurised or "stassanised" milk, and it is noted that there is a weakening in the creaming-capacity of milk (diluted to 50 per cent. strength) after 30 minutes at 60° to 63° C., but none after 30 minutes at 55° C. (*cf. supra*). J. G.

Lipases of Wheat. I. B. Sullivan and M. A. Howe. (*J. Amer. Chem. Soc.*, 1933, **55**, 320–324.)—The arbitrary method used for measuring the lipase activity of wheat consisted in incubating 0·5 grm. of finely-ground wheat for 24 hours at 37·9° C. in a 250-c.c. flask with 10 c.c. of water, 2 c.c. of toluene, the substrate (1 c.c. of liquid or 1 grm. of solid fat) and 10 c.c. of buffer solution. Subsequently 100 c.c. of a mixture of acetone and ether (3 : 1) were added to the sample, and to the blank (boiled before incubating); 0·1 *N* sodium hydroxide solution was used for the titration, with 1 per cent. phenolphthalein as indicator. The substrata used were triacetin, tripropionin, tributyrin, tri-*n*-valerin, tricaproin, tricaprylin, trimyristin, tripalmitin, tristearin, ethyl propionate, ethyl butyrate, ethyl stearate, ethyl oleate, and olive oil, in alkaline and acid range. The glycerides of the lower fatty acids, particularly triacetin and tripropionin, were acted on to the greatest extent, and triacetin gave considerably higher results than any other substrate tried with the time and temperature employed. With triacetin as substrate the enzyme was most active at p_H 7·3 to 8·2, with a wide optimum range. Low-grade wheat flour, rather than germ or bran, gave the most pronounced action of all wheat products in hydrolysing triacetin, and germination of wheat increased its lipase activity with the higher triglycerides, but made little difference with triacetin and the glycerides of the lower fatty acids as substrata. D. G. H.

Technical Refinements for the Micro Colorimetric Method of Iodine in Blood. R. G. Turner and M. Z. Weeks. (*J. Amer. Chem. Soc.*, 1933, **55**, 254–258.)—Small amounts of iodine in blood are determined by comparing the blue colour produced with starch with a standard in a microcolorimeter (ANALYST, 1930, **55**, 707). Two difficulties may be encountered; at times a complete or partial failure of colour development, or the development of an intense black. The first occurs if interfering substances, such as aldehydes, are present in the alcohol, and purification of the alcohol should be carried out by allowing 0·5 grm. of iodine crystals to stand in 300 c.c. of absolute alcohol for 24 hours. When the alcohol is distilled off, the first 25 c.c. of the distillate are discarded, and distillation is stopped when 50 c.c. of the residue are retained. The distillate is shaken with 100 grms. of granulated zinc until the yellow colour disappears, the alcohol is distilled as before,

and, to the distillate, another 100 grms. of zinc are added, and the mixture is shaken continuously for 15 minutes and re-distilled as before, when the distillate is fit for use. Development of blackish colour in the final reaction is due to oxidation of the excess potassium iodide, and is attributable, either to iodine in the potassium carbonate, or to iron in the sulphuric acid. To purify the potassium carbonate, 500 c.c. of 95 per cent. alcohol are added to 100 grms. of the salt, the mixture is stirred electrically for 10 hours, the alcohol is decanted, and the residue is dried between filter-papers and dissolved in 200 c.c. of boiling water. The filtrate is evaporated to a third of its volume, and crystallisation induced in an ice-box, and, when equilibrium is reached, the liquid is filtered and the residue is dried at 110° C. The powder is dissolved in 100 c.c. of hot water, and, after cooling, the liquid is shaken with an equal volume of absolute alcohol in a separating funnel for 30 minutes, after which the aqueous layer is evaporated to a third of its volume, crystallised in an ice-box and filtered, and the residue is dried at 110°. Chemically pure potassium hydroxide may be used for the 4 *N* solution instead of potassium carbonate solution, and no purification is then necessary. To test the sulphuric acid used for the 2 *N* solution 3 drops of purified (1 per cent.) potassium iodide solution are placed in a test tube graduated to 1 c.c., water added to the mark, then 5 drops of starch solution, which are followed by two drops of the acid to be used. No colour should be developed in 15 minutes, *i.e.* not more than 0·00008 per cent. of iron should be present. With these precautions, the method of analysis can be carried out with the accuracy given in the original paper. D. G. H.

Method for the Quantitative Determination of Indoxyl Compounds in Urine. H. Sharlit. (*J. Biol. Chem.*, 1933, **99**, 537–545.)—Jolles (*Z. physiol. Chem.*, 1913, **84**, 310) introduced alcoholic thymol as a reagent for the detection of indican in urine, and, later, developed a colorimetric method based thereon (*Z. physiol. Chem.*, 1915, **94**, 79; *Arch. Pharm.*, 1928, **266**, 40), for the determination of indican in both urine and blood. Repetition and manipulation of this procedure with both urine and indican solution showed that the method of Jolles fails to give satisfactory quantitative results, that the use of many times the amount of acid advised produces pigments of greater colour intensity per unit of indican, and that the addition of trichloroacetic acid to the acid reagent induces in the purple hue a red value which increases in direct proportion to the increase of indican in the reaction mixture. It was possible to adjust the amount of trichloroacetic acid used to form readily within the reaction mixture ethyl trichloroacetate, which served as an organic solvent for the indoxyl-thymol condensate produced. A method is therefore presented for the quantitative determination of urinary indoxyl compounds which makes use of the findings mentioned, and is more than twice as sensitive as Jolles' reaction. For quantitative measurements, the technique has been adapted to the comparison colorimeter by the use of a colour filter and an appropriate coloured solution (cobalt sulphate) as a standard; a table shows the indican equivalent of the standard. Determinations by the new procedure of indican excretion in 24 hours gave results several times higher than heretofore reported (40 to 150 mgrms.). The method has also been adapted to the quantitative determination of indoxyl compounds in blood. P. H. P.

The *o*-Quinone Test for Cysteine. O. Baudisch and E. Dyer. (*J. Biol. Chem.*, 1933, **99**, 485–492.)—Dyer and Baudisch (*J. Biol. Chem.*, 1932, **95**, 483) described a simple qualitative test for cysteine, which involved the use of *o*-benzoquinone; the reaction resulted in the formation of a red-coloured complex soluble in chloroform. Owing to the simplicity of the technique, the investigation of this reaction has been continued. An additional group of substances has been studied in order to ascertain what molecular groups are necessary to give this coloration. The method of preparing the quinone has been improved chiefly by the use of a cooling mixture composed of solid carbon dioxide and alcohol, described by Goldschmidt and Graef (*Ber.*, 1928, **61**, 1868). A mixture of 0·5 grm. of powdered catechol, 1·2 grm. of dried silver oxide, 2 grms. of anhydrous sodium sulphate and 15 c.c. of anhydrous ether is placed in a test-tube previously chilled in the cooling mixture. The tube is removed from the cooling bath and shaken vigorously for 1 to 2 minutes, and the mixture is filtered quickly. The ethereal filtrate, when gradually cooled to −70° C., deposits red crystals of the quinone. If the cooling is too rapid, the colourless, more colloidal form is obtained. The crystals are freed from liquid by decantation, washed twice with 1 c.c. of cold ether, and dissolved in 20 c.c. of dry cold chloroform. When 4 c.c. of the greenish tan-coloured solution which results are shaken for 2 to 3 minutes with 2 c.c. of a 0·1 per cent. aqueous solution of cysteine hydrochloride, a cherry-red colour is developed in the chloroform layer. For the test, about 2 c.c. of a dilute aqueous solution of the substance to be tested are shaken for 2 to 3 minutes with 3 or 4 c.c. of a cold chloroform solution of the quinone, prepared as described. If the substance is soluble in chloroform, but not in water, an aqueous suspension of the material is treated with a chloroform solution of the quinone, as usual. The test should be carried out in dilute hydrochloric acid solution. From a consideration of tables which show (i) compounds producing no red coloration with *o*-quinone, (ii) compounds producing a coloration in the aqueous layer, and (iii) compounds producing a red coloration in the chloroform layer, it appears that cysteine, in aqueous hydrochloric acid solution, may be distinguished from all the other compounds tested, except aniline and benzidine, by means of the *o*-quinone reaction. This reaction, therefore, provides a means of distinguishing cysteine from a large number of related nitrogenous and sulphur-containing substances. Two products have been isolated from the reaction of cysteine with *o*-quinone—catechol (from the aqueous layer) and a red-brown substance containing sulphur and nitrogen (from the chloroform layer), which is under investigation. The formation of the characteristic red compound is not affected by a change of solvent. It appears that the initial stage of the reaction between cysteine and *o*-quinone involves the reduction of the quinone to form catechol. Nevertheless, neither a mixture of cysteine and catechol, nor a mixture of cystine, catechol and *o*-quinone produces a coloration. P. H. P.

Fatty Acids of Liver Lecithin. R. H. Snider and W. R. Bloor. (*J. Biol. Chem.*, 1933, **99**, 555–573.)—It is shown that in the lecithin of ox liver the proportion of liquid (unsaturated) to solid (saturated) fatty acids is about 55 : 40. The saturated acid of lecithin consisted of about 71 parts of stearic to 29 parts of

palmitic acid; the neutral fat of liver contains more palmitic acid than stearic acid. The distribution of the unsaturated acids was found to be: linolic, 45 per cent.; arachidonic, 31 per cent.; and oleic, 21 per cent. Acids with three double bonds were not found. Acids of larger molecule than C_{20} were present in small amount in one series of samples. P. H. P.

Bacteriological

Identification and Biological Oxidation of α-Glucoheptulitol. Y. Khouvine and G. Nitzberg. (*Compt. rend.*, 1933, **196**, 218–220.)—The chemical individuality of α-glucoheptulitol, obtained (instead of the β-glucoheptitol expected), together with α-glucoheptitol, by reduction of α-glucoheptulose, is confirmed by *X*-ray analysis. In a yeast-water medium, the sorbose bacterium oxidises α-glucoheptulitol to α-glucoheptulose. This result, although inadequate as the basis of a structural formula for α-glucoheptulitol, shows that the formula generally accepted for β-glucoheptitol is inapplicable to α-glucoheptitol. T. H. P.

Biological Reagent for the Determination of Galactose. V. J. Harding, T. F. Nicholson and G. A. Grant. (*J. Biol. Chem.*, 1933, **99**, 625–628.)—Harding and Grant (*Analyst*, 1932, **57**, 183) described a method for the determination of galactose, applicable to human blood and urine; bakers' yeast, adapted or acclimatised to the fermentation of galactose, could be used for its quantitative determination. It has since been found that this "galac" yeast might remove small amounts of maltose, and that ordinary bakers' yeast could not be depended upon to remove maltose completely beforehand. However, these criticisms do not affect the use of galac yeast for the original purpose for which it was intended, *viz.* the determination of galactose in blood, urine and tissues, in an effort to follow the course of metabolism of that sugar after its oral administration. It is now shown that *Saccharomyces marxianus* can be used to determine galactose in mixtures of certain sugars in aqueous solutions, in urines and in Folin-Wu blood filtrates. It is devoid of action on maltose. When cultured and used under the experimental conditions described, it removes glucose, fructose, mannose, sucrose, and galactose, but has no action on maltose, arabinose or xylose. If glucose, fructose and mannose are first removed from the solution, any further loss in reducing power following the use of *Saccharomyces marxianus* becomes specific for galactose; 0·5 grm. of washed *Saccharomyces marxianus* completely removes the galactose from 10 c.c. of a 20 mgrms. per cent. solution. Under conditions where the presence of maltose may be suspected, *Saccharomyces marxianus* is a reagent for galactose superior to galac yeast, but, under more limited conditions, galac yeast is perhaps to be preferred. The keeping properties of *Saccharomyces marxianus* have not been investigated; it was always used within 24 hours of its preparation. P. H. P.

Toxicological and Forensic

Solanocapsine, a new Alkaloid with a Cardiac Action. J. M. Watt, H. L. Heimann, and E. Epstein. (*Quart. J. Pharm.*, 1932, **5**, 649–656.)—The minimum lethal doses and the general effects of the hydrochloride of this alkaloid, isolated from the leaves of *Solanum pseudocapsicum*, L., have been determined. The alkaloid is irritant, producing vomiting when given by the stomach, and marked local irritation when injected subcutaneously. Its systematic action is wholly intracardiac, the effects being exerted mainly on the sinus. In toxic concentrations, it causes wide disorganisation of the heart's action, resulting in sinus arrhytmia, auricular extra-systoles, sinus block, A-V block, and weakening of the muscle. Judging from the minimum doses which are lethal when injected intravenously into the rabbit, the toxicity of solanocapsine hydrochloride is between those of nicotine and atropine sulphate, and is about the same as that of cocaine hydrochloride. The alkaloid may prove of therapeutic value as a corrective for the abnormal rhythms of the heart known as paroxysmal tachycardias.

T. H. P.

Toxicity of Mixtures of Poisons. B. A. Southgate. (*Quart. J. Pharm.*, 1932, **5**, 639–648.)—Investigation into the causes of the toxicity of the waters of the polluted Tees Estuary showed that the toxicity was due mainly to the presence of cyanides, but that other poisons, mainly phenolic, were present in sub-lethal concentrations. Experiments have, therefore, been made (with yearling rainbow trout) to determine the effects on the toxicity of a polluted water produced by the introduction of a fresh toxic substance in proportions lower than the lethal ones. Such effects may be classified under two headings: (1) If the new poison has a physiological action different from those of the poisons already present, the addition may cause either no change in toxicity (as with addition of *p*-cresol to either potassium cyanide or 1:2:6-xylenol), or a slight increase in toxicity (addition of either potassium cyanide or 1:2:6-xylenol to *p*-cresol). (2) If the new poison is interchangeable with one already present, large increases in toxicity may occur, as when *p*-cresol is added to phenol or *vice versa*. The numerical results indicate that potassium cyanide and *p*-cresol exert independent actions on the trout, and that 1:2:6-xylenol is partially interchangeable with *p*-cresol and with 1:3:5-xylenol.

When placed in a solution containing a toxic substance in lethal concentration, the trout first display symptoms characteristic of the poison, and, finally, overturn and remain helpless in this state. The reciprocal of the time elapsing between the initial exposure and the overturning is taken as a measure of the toxicity of the substance.

T. H. P.

Organic Analysis

Reaction of Sulphydryl-containing Substances. A. Giroud and H. Bulliard. (*Bull. Soc. Chim. Biol.*, 1932, **14**, 278–279.)—To stabilise the nitroprusside reaction a salt of zinc (chloride, sulphate or acetate) is added to the reduced nitroprusside. This results in a red, instead of a violet, coloration.

When testing a tissue for the –SH group the frozen material is cut with a microtome, and the sections are immersed for a few seconds in a 5 per cent. solution of zinc acetate, quickly washed and treated with nitroprusside. The sections may then be treated with alcohol, followed by toluene and Canada balsam, when the colour is kept for a long time. The procedure may be adapted for characterising soluble –SH groups. Proteins containing the reducing group –SH show the colour, as also do those of the glutathione type, also cysteine hydrochloride and alkaline monosulphides, which, in contact with water, give hydrosulphides; but the reaction is not given by substances, such as cystine, in which the sulphur is present as disulphide S–S. It is suggested that the reduced nitroprusside group forms a relatively stable compound with the zinc salt. D. G. H.

Chlor-iodo and Bromo-iodo Compounds Precipitated from Fish Oils. S. Ueno and M. Iwai. (*J. Soc. Chem. Ind. Japan*, 1932, **35**, 592B–594B.)—A white precipitate is formed on addition of concentrated Wijs or Hanus solution to ethereal solutions of the fatty acids of fish oils. In both cases the precipitate blackens on heating, the Wijs precipitate decomposing at 170° C., and the Hanus precipitate at 150° C. Yields of the insoluble precipitate were constant under similar conditions for the same kind of fish oils. Four to seven c.c. of Wijs solution (of 1 mol.-litre concentration), according to the degree of unsaturation, when added to 0·5 grm. of the fatty acids, and allowed to stand in a stoppered flask at 15° to 20° C. for several hours, produced a precipitate, which was filtered off, washed with ether and weighed. The results were as follows (as percentage yields of chloro-iodo-compounds): Japanese sardine oil, 43·0; large herring oil, 7·1; small herring oil, 10·8; shark-liver oil (Hiragashira), 22·5; a small shark oil, 19·0; Californian gray whale oil, (blubber) 21·7, (bone oil) 21·9, (oil from internal organs) 24·1; sperm oil, (blubber) 1·6, (bone) 0·8, (internal organs) 2·7; humpback whale oil, (blubber) 6·9, (bone) 13·1, (internal organ) 15·5; finback whale oil (internal organs), 5·1. Such figures are useful for identifying fish oils in other oils, and ascertaining the nature of the fish oil. Hanus solution is not so satisfactory as Wijs solution, since it gives a similar precipitate with linolenic acid. Analysis of the precipitates shows that Wijs solution precipitates the tetra-chloro-tetra-iodo or penta-chloro-penta-iodo derivatives from the ethereal solution of the fatty acids, and Hanus solution the tetra-bromo-tetra-iodo, or penta-bromo-penta-iodo derivatives, whilst with linseed oil fatty acids the tri-bromo-tri-iodo derivatives of linolenic acid are formed. D. G. H.

Determination of Acids in Dyed Wool. S. R. Trotman and G. N. Gee. (*J. Soc. Dyers and Colourists*, 1932, **48**, 321).—Existing methods for the determination of mineral and organic acids in dyed wool have been investigated and certain modifications and new methods are suggested. Some of the methods involve continuous washing. It was found that this was not satisfactorily accomplished by a continuous process. Efficient washing is best achieved by placing the wool in an open cylindrical tap-funnel attached to a suction-flask. Water is poured on to the wool in sufficient quantity to immerse it, and is then sucked away by means of a pump, and the wool is pressed "dry," the process being frequently

repeated. (i) *The "ammonia method."*—Wool is allowed to stand in contact with a known volume of 0·1 *N* ammonia for 12 hours, and a portion of the solution is then back-titrated. The results are vitiated by the fact that wool absorbs ammonia. Better results are obtained if 0·1 *N* triethanolamine is used instead. This is not absorbed by wool. (ii) *The "sodium bicarbonate method"* of Meunier and Ray is greatly improved if the washing of the wool is carried out as described above. (iii) *The "magnesium carbonate method,"* in which the wool is heated with a suspension of magnesium carbonate, is useful with dyed wools, since the colour is not stripped. Sulphuric acid is determined gravimetrically, but, if the wool contains sodium sulphate, this is also included. The stripping effect produced in methods (i) and (ii) can be minimised by the use of a saturated solution of sodium chloride instead of distilled water. (iv) *Woodmansey's method* presents difficulties, and acid-free cloth is not always available. (v) *The "terephthalic acid method"* of Hirst and King is very trustworthy. Calcium soaps must be determined and allowed for. (vi) *The "sodium acetate method"* consists in adding 1 grm. of sodium acetate to 2 grms. of the wool in a 500-c.c. distilling flask and steam-distilling until 650 c.c. have been collected. The distillate is titrated with sodium hydroxide in the presence of phenol phthalein, the acetic acid thus found being equivalent to the mineral acid in the sample. The water in the steam generator must be made faintly alkaline with lime water and boiled for a short time to prevent carbon dioxide from interfering with the final titration. If the wool contains formic or acetic acid alone this can be determined by direct steam-distillation. If sulphurous acid is present, the wool should be steam-distilled into hydrogen peroxide or bromine water. (vii) *The "calcium carbonate method."*—Two grms. of the wool are boiled for 15 minutes with carbon dioxide-free water and precipitated calcium carbonate, the carbon dioxide liberated being absorbed in barium hydroxide and determined by titration. If boiling is continued for longer than 15 minutes, the carboxyl groups of the wool become gradually hydrolysed and liberate carbon dioxide.

R. F. I.

Analysis of Weighted Silk. R. T. Mease. (*U.S. Dept. Comm. Bur. of Stds. J. of Research*, 1932, **9**, 669; *Research Paper* No. 498.)—In the analysis of silk the most important constituent to evaluate is the silk fibroin. A strip of the full width of the fabric (3 to 5 grms.) is dried by spreading it loosely on a watch-glass in an electric oven at 105° to 110° C. for 1½ hours, and weighed in a tared weighing bottle. The dry weight is called weight A. Soluble finishing materials are removed from the dry material by two immersions of two minutes each in 30-c.c. portions of ether, at laboratory temperature, with squeezing after each immersion. The fabric is then similarly treated with ethyl alcohol at 50° to 60° C., followed by 20 minutes' immersion in water at 65° to 70° C., and three half-minute immersions, after which it is well squeezed by hand and dried as before. The weight at this stage is weight B; $\frac{(A-B)\times 100}{A}$ = the percentage of soluble finishing materials.

The more firmly held weighting and finishing materials are now removed by immersing the treated fabric for 20 minutes in 90 to 100 times its weight of a solution

containing 2 per cent. hydrofluoric and 2 per cent. hydrochloric acid (equivalent to N in HF and $N/2$ in HCl) at 55° C. ± 1° C. (This solution should be contained in a Pyrex vessel coated with ozokerite.) The acid solution is poured off, and the material is rinsed with two portions of water at 55° to 60° C., followed by hand-squeezing after each rinsing. This acid treatment is repeated. The squeezed fabric is then given a similar treatment in which the acid is replaced by 2 per cent. sodium carbonate solution, followed by two more acid treatments and several rinsings with water, and is then dried as before (weight C). The percentage of silk fibroin equals $\frac{C \times 100.}{A}$ The various immersions are carried out in a circular oil-bath, capable of holding several beakers, so that several analyses can be carried out simultaneously.

Qualitative tests are briefly described for silica, lead, aluminium, tin, zinc and phosphate, and references are given in case further details should be required. Inorganic impurities can be detected on burning a small piece of the fabric. Any ash will retain the form of the original threads. R. F. I.

Inorganic Analysis

Qualitative Separations in the Alkaline-Earth and Alkali Groups. J. Kunz. (*Helv. Chim. Acta*, 1933, **16**, 3–6.)—The chloride solution is treated successively with ammonium chromate, sulphate, and oxalate, drop by drop, for the identification of barium, strontium, and calcium, respectively; the precipitate, if any, being filtered off prior to the application of the next reagent. The oxalate filtrate is evaporated to small bulk and treated with excess of ammonia; any precipitate is filtered off, and the filtrate tested for magnesium with a minimum of ammonium arsenate. The filtrate is evaporated to dryness and the residue heated as usual. The residue is extracted with water and the extract tested for potassium and sodium. Potassium may be identified by means of magnesium cobaltinitrite or bitartrate. In either case the filtrate is evaporated to dryness, and the residue gently calcined and extracted with water. The extract contains sodium chloride, which is obtained in characteristic crystals by evaporation.

W. R. S.

Conditions for Determining Antimony by the Permanganate Method. W. Pugh. (*J. Chem. Soc.*, 1933, 1–4.)—A study of the titration of tervalent antimony in dilute sulphuric-hydrochloric acid solution with potassium permanganate has shown that theoretical results may be obtained, provided that the degree of acidity and the relative proportions of sulphuric and hydrochloric acids present are adjusted between proper limits; when the most recent value for the atomic weight of antimony (121·76) is used in the calculation, the amount of antimony may be calculated from the normality of the permanganate solution, as determined by titration with sodium oxalate, to an accuracy within 1 part in 3000. In a series of titration experiments, the end-point was observed both visually and potentiometrically, a platinum wire immersed in the solution in conjunction with

a saturated calomel electrode being used; the change of potential in the neighbourhood of the equivalence-point was large, being about 300 millivolts. The antimony solutions, which contained approximately 0·1 grm. of antimony in 180 c.c., were titrated at the ordinary temperature with 0·1 *N* potassium permanganate solution. Accurate results were obtained when the following proportions of sulphuric and hydrochloric acids were present in a total volume of 200 c.c.:

Conc. H_2SO_4, c.c.	0	10	20	30
Conc. HCl, c.c.	30–50	30–35	15–20	10–15

Too little hydrochloric acid leads to low results because of the precipitation of a basic antimony salt, and too much leads to the reduction of permanganate by chloride ions, with liberation of chlorine, the end-point becoming barely perceptible.

Determination of antimony in antimony-rich lead-tin alloys.—These alloys may be readily dissolved in boiling concentrated sulphuric acid, but considerable error may be caused by occlusion of antimony salts in the lead sulphate precipitated on subsequent dilution. Three methods of avoiding this difficulty were tested, and the following gave the most satisfactory results: A sample of the alloy (containing about 0·1 grm. of antimony) was dissolved completely in 20 to 50 c.c. of concentrated sulphuric acid by heating; the solution was cooled, diluted to 100 c.c., and the precipitate of lead sulphate was allowed to settle. The clear liquid was decanted and boiled for 5 minutes to expel any sulphur dioxide. The precipitate was dissolved, by heating, in the appropriate quantity of hydrochloric acid (see table above), diluted with an equal volume of water, and the solution was added to the main sulphuric acid solution. The liquid was slowly titrated with 0·1 *N* permanganate solution at 40 to 60° C.; near the end-point a few drops of methyl orange solution were added, and the titration was continued until the indicator colour was discharged. Good results were obtained in tests with alloys and synthetic mixtures containing lead, tin, and from 11 per cent. upwards of antimony; in all the tests approximately 0·1 grm. of antimony was determined; no results are quoted for determinations of amounts of antimony varying over a wide range, as might be met with in practice. S. G. C.

Microchemical

Influence of Electrical Charge in Micro-gravimetric Analysis. W. M. Sperry. (*Mikrochem.*, 1932, **12**, 151–152.)—Pyrex glass or other borosilicate glass of similar composition is found to be unsuitable for micro-gravimetric work, owing to the tenacity with which it holds an electric charge. A number of centrifuge tubes, weighing approximately 6 grms., were washed and dried by various methods, and finally wiped first with moist and then dry chamois leather, before being weighed, as in the usual Pregl technique. In most cases the tubes showed an apparent loss in weight after being placed on the balance pan. In some cases the loss was 0·5 mgrm. or more, and equilibrium was not always obtained even in half an hour. If the finger is placed on the tube a few minutes before weighing the charge is discharged, showing the phenomenon to be electrical. A

definite spark may be drawn from a 200-c.c. flask after being wiped vigorously. Ordinary soft glass and Jena glass tubes, as used in the Pregl methods, show no such loss of weight on the balance. J. W. B.

Micro-Determination of Methoxyl. V. Bruckner. (*Mikrochem.*, 1932, **12**, 153–160.)—Micro-methoxyl determinations usually give low results, even when a correction factor (+0·12 mgrm. to the weight of silver iodide) is used. Good results without correction are obtained as follows: The sample is weighed out in a small glass holder (stoppered for volatile substances), which will stand on the balance pan, and will slip down the side arm of the distillation apparatus. When the substance is soluble with difficulty it is dissolved in the small glass holder in 0·1–0·2 grm. of phenol and 0·1 c.c. of acetic anhydride before being placed in the apparatus, as low results are frequently due to incomplete solution. As the substance is not weighed in tin-foil holders, about 10 to 15 mgrms. of tin-foil rolled into a ball are added separately, to ensure even boiling. Hydriodic acid (sp.gr. 1·70) is used, and the trap of the apparatus contains a wash-liquid consisting of freshly-prepared red phosphorus suspended in 10 per cent. cadmium sulphate solution. Jena glass filter tubes with fritted glass filters (Type 13f. G.4) are used in preference to a filter surface of asbestos, which does not remain constant in weight. Very good results were obtained with a large number of substances. Good results were also given by the titration method of Vieböck and Brecher (*Ber.*, 1930, **63**, 3207). J. W. B.

Microchemical Identification of Novocaine (Procaine). M. Wagenaar. (*Mikrochem.*, 1932, **12**, 143–150.)—Novocaine (procaine) base forms a white crystalline mass, melting at 62° C. The crystals are insoluble in water, but easily soluble in alcohol, ether or benzene. The base crystallises from dilute alcohol with two molecules of water, in white needles melting at 51° C., soluble in fatty oils and fatty acids. The hydrochloride crystallises from alcohol in colourless prismatic polarising crystals with a typical angle of 120° at the top. The crystals do not decompose on heating to their melting-point, and are soluble in cold water in the ratio 1:1 and in cold alcohol 1:30. *Sublimation* gives only oily drops, which neither by scratching nor by treatment with acetone become crystalline. *Precipitation*, by means of ammonium chloride, is a good test; 0·1 mgrm. of novocaine in 1:50 dilution may be detected. The crystals are in the form of thin plates, showing good interference colours in polarised light. The top angle of the crystals is 60°. (i) *Crystal formation with gold salts.*—Former workers obtained only cloudiness (Grutterink, *Z. anal. Chem.*, 1912, **51**, 214). Crystals are obtained when dilute hydrochloric acid is added to the solution, and then the gold salt, followed by a few crystals of ammonium acetate. The addition of sodium bromide causes the crystals to become dark brown, and to show a flower-like formation. (ii) *Crystal formation with platinum salts.*—A very small amount of the reagent should be used; 0·01 mgrm. of novocaine in 1 : 1000 dilution can be detected. (iii) *Crystal formation with mercury salts.*—The best method is to add a solution of mercuric chloride to the novocaine solution, to dissolve the precipitate in dilute hydrochloric acid, and to add a crystal of sodium acetate

to the almost clear solution. Good crystals come down at once. The smallest amount recognisable is 0·025 mgrm., and the limit of dilution is 1 : 1000. (iv) *Crystal formation with picrolonic acid.*—Ammonium picrolonate may be used instead of the acid. Star-like crystals are formed, anisotropic and obliquely extinguished. The smallest amount recognisable is 0·025 mgrm., and the limit of dilution is 1:1000. (v) *Crystal formation with picric acid.*—Ammonium picrate gives even better results, and the first amorphous precipitate rapidly changes to the crystalline form. The smallest amount recognisable is 0·02 mgrm. (vi) *Crystal formation with alkali bichromate solution.*—Good crystals are formed at once, having a top angle of 78°, polarised light is extinguished in the diagonal direction and additional colours are given in the long direction. The smallest amount detectable is 0·025 mgrm., and the limit of dilution is 1 : 100. Up to a dilution of 1 : 30 the reaction is instantaneous. (vii) *Crystal formation with bromine water.*—An amorphous precipitate, formed at once, rapidly becomes crystalline; 0·001 mgrm. can be detected in 1:1000 dilution. In dilute solutions the test should be treated with a solution of potassium bromide and potassium bromate, covered with a cover slip and a drop of dilute hydrochloric acid allowed to run in carefully. The crystals are formed at once. (viii) *Dye formation with furfural.*—Crystals of novocaine are turned to a deep red colour when treated with a drop of a 15 per cent. solution of furfural in oleic acid, which dissolves furfural but not novocaine. The purity of a novocaine product can be tested in this way, as all crystals of pure novocaine are coloured with deep red flecks of the dye, and any foreign substance (such as cocaine) remains uncoloured. The test can be carried out on the dry substance or in solution. (ix) *Crystal formation with alkali perchlorate.*—Characteristic crystals of novocaine perchlorate are formed by treating novocaine with a 5 per cent. solution of sodium perchlorate. The crystals are well-formed: large prisms with an angle of 74°. The smallest amount recognisable is 0·05 mgrm. in 1:50 dilution. Several photo-micrographs of the crystals are given.

J. W. B.

Physical Methods, Apparatus, etc.

New Universal Viscometer. F. Höppler. (*Chem.-Ztg.*, 1933, **57**, 62–63.)—The viscometer (shown in Fig. 1) works on the principle of the time taken for a ball to travel under gravity through a specified distance in an inclined tube filled with the fluid to be tested. The falling-ball principle has previously been employed in other viscometers, *e.g.* Stange's and Valenta's, in which the ball falls vertically; its successful use in these instruments depends on the ball falling slowly and co-axially in the tube, and is, therefore, restricted to comparatively highly viscous liquids; with liquids of lower viscosity the ball tends to fall with a rotatory and periodic lateral motion, which gives rise to serious inaccuracies. By employing an inclined tube, this difficulty is stated to be overcome; an inclination of 80° to the horizontal was chosen, as small deviations from this were found to have less effect on the results than at other inclinations. The cross-section of the liquid in the path of the ball is crescent-shaped, as shown in Fig. 2, and remains

mathematically constant throughout the length of the path. The inner glass tube of the instrument, which contains the fluid to be tested, is made to limits of ±0·002 mm. on diameter, and is engraved with distance marks. This tube is surrounded by a jacket of liquid which can be maintained at any desired temperature by electrical heating, and is kept stirred by drawing a stream of air through

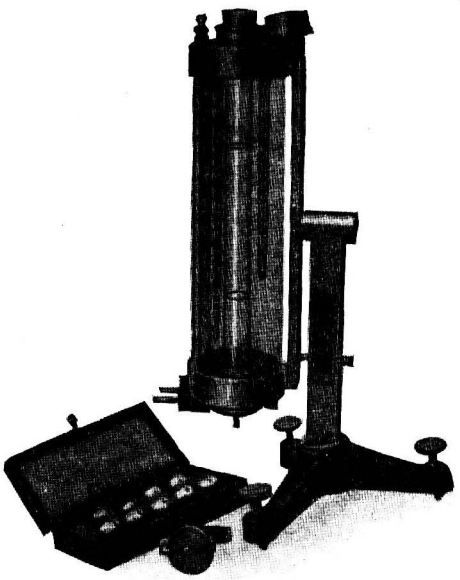

Fig. 1

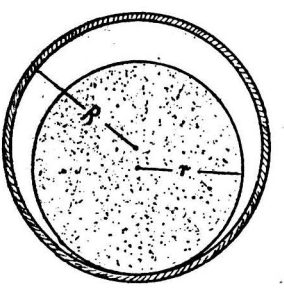

Fig. 2

it; two thermometers, one of narrow and the other of wide range, are immersed in the jacket. The inner tube is fitted with a special stopper-mechanism which is stated to permit filling and the removal of air-bubbles without any loss of sample. Before use, the inner tube is adjusted to the 80° inclination by means of the "levelling" device provided. A set of 9 different balls, gauged to ±0·001 mm. diameter, is provided; the largest is a gold-plated steel ball for determining the viscosity of gases; the others are specially hardened balls of corrosion-resisting steel, varying in size and covering a range of viscosities from 0·5 to 100,000 centipoises. When the appropriate ball is used the time of fall is, normally, 50–300 seconds. Tables are supplied with the instrument giving the viscosity in absolute units from the time of fall of the ball and the sp.gr. of the liquid. It is claimed that an accuracy to 0·1 per cent. is obtainable with both gases and liquids. Small differences in viscosity, *e.g.* between distilled water and conductivity water, can be detected. Numerous applications to scientific and industrial purposes are suggested. The instrument goes under the name of the Höppler Viscometer, and is manufactured by Gebrüder Haake, Medingen, Dresden. S. G. C.

Application of Ultra-violet Rays and Fluorescent Indicators. W. Holthoff. (*Z. anal. Chem.*, 1933, **91**, 263–266.)—Acidimetric or alkalimetric methods can be applied to coloured or cloudy solutions if fluorescent indicators

are employed. The determination of the end-point (fluorescent to non-fluorescent or *vice versa*) is greatly facilitated by the use of a suitably screened 200-watt Osram-Nitraphot lamp (the light of which is rich in ultra-violet rays) and observation of the solution through a "Callophane"—a small portable light-filter in the shape of a collapsible camera, which transmits only the ultra-violet rays close to the visible part of the spectrum. W. R. S.

Reviews

QUANTITATIVE CLINICAL CHEMISTRY. Volume I, INTERPRETATIONS; Volume II, METHODS. By JOHN P. PETERS and DONALD D. VAN SLYKE. First Edition. Pp. xvi+1264 and xix+956, with 124 and 95 figures in the text. London: Baillière, Tindall & Cox. 1931 and 1932. Price £3 and £2 12s. 6d.

These volumes are the joint efforts of two well-known American workers. The first volume is devoted to the interpretation of results obtained from the chemical and physical examination of body fluids and excreta in normal conditions and pathological states. The second volume describes and critically examines various methods available to the analyst who is called upon to carry out the determinations. The magnitude of this undertaking is shown by the fact that the text of the two volumes occupies 2220 pages.

It was originally intended to review Volume II only of this valuable contribution, but, through the courtesy of the publishers, both volumes have been made available.

Those who are not concerned with physiology or pathological chemistry will naturally have a limited interest in Volume I, but Volume II, containing, as it does, full details of suitable processes for the determination of a large number of substances of biological importance, will be of definite interest to those whose analytical practice takes them in these directions. It will be of interest to them to know that the volumes may be purchased separately.

VOLUME I.—The impression gained by the reader is one of the amazing thoroughness with which modern literature has been searched in order to put before him every aspect of the subject under discussion. In this volume, each chapter is devoted to some chemical substance or group of substances, *e.g.* urea, uric acid, fats, lipoids. Each chapter opens with an account of the chemical nature of the substance or substances under discussion. This is followed by the normal metabolic story, which occupies a considerable space, and the chapter concludes with a description of such changes or deviations from the normal as may be observed in pathological states. To the biochemist, who perhaps deals more with substances than with states, such an arrangement is ideal, but to the clinician for whom the book is, in part at least, intended, such an arrangement is less satisfactory. For example, if he desires to study the chemistry of uraemia, a search must be made in as many as six different chapters, with the results that he cannot get a vivid picture of the condition.

As one cons the pages the impression remains that a more informative title to this book would have been "Quantitative Physiological and Clinical Chemistry." Physiology and medicine are in practice much too widely separated, partly because the physiologist is too much occupied with animal experimentation, and the physician has so often but a nodding acquaintance with the normal human. Any attempt at their marriage is to be welcomed, and surely such an attempt has been made here.

An ambitious work of this type, if it is to arrest the attention of the physician, must be prepared to give him information on the chemistry of all those conditions which may occur in his regular practice. It must be recorded that the chemistry of certain morbid states, such as jaundice (including the work of van den Bergh), and that of the cerebro-spinal fluid, receive but cursory mention. A chapter is devoted to sulphur, but iodine (although it must be admitted that most of the conclusions drawn concerning the metabolism of this element are based on faulty methods of determination) is dismissed with a few references in the text. Certain of the rarer states, which are worthy of description in a book of this size, receive little or no mention, *e.g.* alkaptanuria, sulphaemoglobinaemia and haematoporphynuria.

The book, as has been suggested, will probably be of limited value to the clinician, but to the biochemist and the chemical pathologist, who perhaps study their subjects from a more academic point of view, who are often appealed to by the clinician for assistance, and who have the greatest difficulty in keeping themselves abreast of the immense amount of literature being produced on these subjects, a wealth of information is unfolded. The data and conclusions given by the authors have been fully weighed by them before they have permitted them to appear, and those who seek further information will find at the end of each chapter a full bibliography, the accuracy of which is vouched for by the authors. Space does not permit mention of each chapter, but that on Haemoglobin and Oxygen is probably the best in the book, and it includes an admirable description of the chemical phenomena observed in the clinical states of anaemia and polycythemia; anoxaemia and carbon monoxide poisoning are particularly worthy of note. Of the other chapters, those dealing with the carbohydrates, lipoids and acid-base balance are notable contributions.

The plentiful use of graphs, tables and diagrams is of assistance to the reader, although, in some cases, their complicated nature tends to detract from their value. Misprints are few, and nearly all are corrected by the only proper method, namely, the placing of an erratum slip in the actual page concerned, and not in the front of the book. Of the uncorrected misprints, "special" for "specific" (p. 73), "absorbed" for "adsorbed" (p. 224), and "has been" for "will be" (p. 268), should be noted. Mention of "ovariotomised dogs" is made, and may be contrasted with the sentence following it, which discusses castration in "male rabbits."

VOLUME II.—The authors realise at the outset that it would have been impossible to describe, or even summarise, all the available methods of clinical importance for the determination of substances present in the body and the excreta. They have, therefore, described selected methods which they consider

suitable, and which, from personal trial, in the majority of cases, they believe to be satisfactory.

They have endeavoured to give a gravimetric, volumetric, colorimetric and a gasometric technique for the determination of each substance dealt with in the text, when satisfactory processes are available, and, in certain cases, to give macro- and micro-methods as well.

The chapters open with a discussion of the principles on which the methods are based, and are critical. In the course of the discussion other methods, not described in detail, are mentioned, and, should the reader desire further information on these methods, references are given at the end of each chapter to the original papers, as well as to those on the methods receiving full description.

Chapter I discusses apparatus, its construction, standardisation, use and maintenance, and possible error. Many ingenious and valuable pieces are mentioned for micro-determinations. The preparation of standard solutions, colorimetry and nephelometry are described, but a timely word of warning is given concerning the avoidance, if possible, of the last-named process. It is a chapter which should be well digested by all those who aspire to the practice of clinical chemistry.

Chapter II deals with the special problems arising from the nature of specimens sent for examination, and includes the collection and preservation of samples.

Chapters III to VII are devoted to gas analysis, including those gases which may be obtained from body fluids. The well-known manometric methods, which have been so brilliantly developed by one of the authors (Van Slyke), are given in detail, including many modifications which have been suggested by his co-workers. This section is especially valuable, for, so far as the reviewer is aware, this is the first time that these have been collected together in complete form.

Some of the applications of these methods are of interest, for example, practical methods for the estimation of 0·1 mgrm. of calcium, by the measurement of the volume of carbon dioxide which is given off from the oxalate when it is treated with acid permanganate, and that of 0·3 mgrm. of nitrogen, by the determination or the measurement of the volume of gas obtained by a micro-Kjeldahl process and its subsequent treatment with hypobromite. The volumes of carbon dioxide and nitrogen measured are of the order of 0·05 and 0·2 c.c., respectively, at N.T.P. Actually, the gases are measured under reduced pressure, so that greater volumes are read in the burette. It is claimed that the accuracy is at least as good as that obtained by any of the other recognised methods, and, in some cases, better.

These new methods have many possibilities in spheres other than biological chemistry, and are well worth study.

The remaining chapters (VIII to XXXII) deal with individual substances, or groups of substances, for which a choice of methods is given. From an examination of the pages it is realised at once what a great debt is owed to American workers for methods of analysis of biological products. It is only natural, perhaps, that the authors should have studied more closely these methods, and paid less attention to the efforts of workers in other countries. In the chapter on the determination of sugar, the polarimeter is dismissed as being too expensive for sugar in urine;

nevertheless, there is an excellent instrument for this purpose sold in this country at a moderate price, which will give satisfactory results for clinical purposes very quickly.

Although Bang was the first to publish a method of determining sugar in small quantities of blood, it was Maclean who first (in 1915) described a simple method based on re-oxidising the reduced copper with iodate. It was from this method that Shaffer and Hartmann later developed their process (which is given in the text). Maclean is not mentioned, and it is pertinent to enquire the reason, as his method is still widely used. For urine, too, Bertrand's method might also have been described.

It is surprising to find no mention, in Chapter XX, of the Hartridge reversion spectroscope for the determination of carbon monoxide in blood.

An Appendix gives details of many processes which, although perhaps not falling within the scope of the preceding chapters, nevertheless are of importance to the clinical chemist, *e.g.* the examination of gastric contents, the van den Bergh reaction, etc.

Space does not permit of the mention of the remaining chapters, save to say that descriptions are ample, so that any method can safely be embarked on without reference to the original publication. The methods may be relied on to give accurate results, except in those cases where accurate determinations are not possible. For these the best methods available are given.

The volume, like its companion, is very free from misprints; "mgrms." for "grms." was noted on p. 493, and one or two minor errors were found in the index.

The printing is clear, and the technical descriptions are printed in heavier type. One fault may be found in this connection: the heading or title of each method of analysis which is described in full might have been spaced out a little more and printed in larger type. At present they do not catch the eye as one turns over the pages.

In conclusion, it is right to say that these volumes are notable contributions to science; both volumes are indispensable to the biological chemist, and the analyst may read Volume II and gain many new ideas for attacking problems.

The authors are to be heartily congratulated.

G. ROCHE LYNCH

QUALITATIVE ORGANIC ANALYSIS: AN ELEMENTARY COURSE IN THE IDENTIFICATION OF ORGANIC COMPOUNDS. By OLIVER KAMM. Pp. 311+ix. New York: John Wiley & Sons, Inc.; London: Chapman & Hall. Second Edition, 1932. Price 16s. 6d. net.

Ten years have elapsed since Mr. Kamm's book was first published. In the interim, several reprints have appeared, and now we have also a new and much enlarged edition. Although his system of analysis, based as it is primarily on solubility data (see ANALYST, 1925, **50**, 44), may not, without modification, commend itself to every teacher of organic analysis, yet the undeniable popularity of the book is proof enough of its general merit.

In the 2nd edition, although no fundamental changes have been made, many modifications appear, and the additions are responsible for an increase of

50 pages. The new matter brings the book up to date; thus we find a description of Rast's camphor method, and reference is made to *Recent Advances in Analytical Chemistry*, Vol. I, *Organic*, 1932. The admirable chapter on the choice and preparation of derivatives, which the reviewer has found so useful in his own teaching work, is, in the new edition, much enlarged and modernised. The substance 2:4-dinitro-phenylhydrazine is, for example, recommended as a reagent for aldehydes and ketones (ANALYST, 1926, **51**, 77).

Another excellent feature of the new edition is the consecutive numbering of the 2158 compounds listed in the "Classified Tables," and the addition of a new index to fit this numbering makes the book more useful than ever before.

Mr. Kamm has done a great service to future generations of students by summarising his knowledge of organic compounds and placing it before them. All wishing to gain skill in organic analysis will do well to consult his book.

HAROLD TOMS

HANDBUCH DER PFLANZENANALYSE. By G. KLEIN. Vienna and Heidelberg. Dritter Band. Spezielle Analyse. Zweiter Teil. Organische Stoffe II. With 67 illustrations. Erste Hälfte (pp. xiii+806). Zweite Hälfte (pp. 807). Berlin: Julius Springer. 1932. Price, unbound, RM. 162.

The two sections under review form the first and second halves of the third volume of this comprehensive Handbuch; owing to the very considerable proportions which the completed work will ultimately assume a large amount of subdivision has been necessary, with the result that it is not easy at first sight to disentangle the numerous sub-headings.

The second volume, which was reviewed last year (ANALYST, 1932, **57**, 347), dealt with "Spezielle Analyse—Erster Teil," and this comprised the analysis of inorganic materials and the first portion of the organic compounds occurring in plants. The present volume, continuing with "Spezielle Analyse," deals with the second half of this subject, and, therefore, bears the sub-heading "Organische Stoffe II." Owing to the extensive nature of the text it has been found necessary to divide it into two sections of equal size—as indicated in the heading. The first part (pp. 1–806) opens with a section on cell-wall constituents, entitled "Membranstoffe." Prof. Hans Pringsheim here contributes monographs on Cellulose, Hemicelluloses, Gums, Mucilages and Chitin; then follows one on Pectin by Prof. Felix Ehrlich; these are followed by similar monographs on Lignin, Cork, Cutin and Suberin, the cell-wall constituents of bacteria, fungi, mosses, ferns, algae and fossil plants by a variety of authors. As may be seen from this list, no section of the plant-world has been ignored. Next follows a section on natural tannins by Prof. Karl Freudenberg, and then sections on Lichen Acids, Essential Oils, Rubber and Guttapercha, and, finally, Resins. To review each of these sections separately would be impracticable; suffice it to say that they are written by experts whose reputation is sufficient to guarantee the value of their contributions, and the reader who did not find the volume up to his expectations would indeed be hard to please.

The second part (pp. 807–1613) contains monographs on Glucosides by Prof. Max Bergmann, on Flavones, etc. by Prof. Hans Rupe, on Anthocyans by Prof.

Karrer, on Anthracene-, Cyanogenic- and Indoxyl-glucosides by Prof. Rosenthaler, as well as others on Mustard Oil Glucosides, Saponins, Digitalis Glucosides, Carotinoids, Chlorophyll, and Pigments of Algae, Fungi and Bacteria by various experts.

Throughout the two parts the authors have not confined themselves to the purely analytical aspects of the subject, but have dealt with the chemistry of the substances concerned sufficiently to provide a *résumé* of the established and essential facts. As in the previous volume, each section has attached to it an account of the occurrence and distribution in the plant world of the substances dealt with. The handbook has, so far, set itself a high standard which, if maintained, will render it indispensable as a book of reference to all workers in this field.

P. HAAS

BIBLIOGRAPHICAL SURVEY OF VITAMINS, 1650–1930. With a section on PATENTS by MARK H. WODLINGER. Compiled by ELLA M. SALMONSEN, Medical Reference Librarian, the John Crerar Library, Chicago. Pp. 334. Chicago: Mark H. Wodlinger, 86, East Randolph Street. 1932.

This book, which should be of value to workers in nutrition, consists of a collection of references to most, if not all, of the printed matter connected in any way with vitamins or vitamin research. The fact that it contains 11,098 separate items is a guarantee that the sins of omission cannot be many. This imposing total includes popular articles, chapters from books (popular and technical), and even informative advertising matter on the subject of proprietary preparations, together with accounts of original scientific work.

The most valuable section is that dated 1650–1910, which contains many interesting and little-known books and articles. After 1910 the arrangement is yearly, and after 1915 the annual sections are sub-divided into vitamins *A*, *B*, *C*, *D* and *E*, and General. This method, though useful in some ways, is inconvenient in many. It renders laborious the task of collecting the work of one author or school of workers or of tracing one particular paper of which one knows the author but not the date. A simple author-index would be a great improvement.

It seems ungrateful to criticise too severely so monumental a work, but a more careful proof-reading would have increased one's confidence in it. When nine obvious spelling errors are found during a cursory glance down 10 pages, taken at random, one wonders, uneasily, how many of the volume and page references are given accurately.

The section on patents is interesting, and it will come as a surprise to many people that there are 240 patents dealing with vitamins and vitamin preparations.

The general make-up is simple and pleasing, and the authors have earned the gratitude of many for their lengthy and tedious labours.

M. A. B. FIXSEN