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Death

WE deeply regret to announce the death, on June 14th, of Mr. E. R. Dovey, Government Analyst for Hong Kong.

The Chemical Assay of Thyroid Gland

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

IODINE was first discovered in the thyroid gland by Baumann in 1895, and it soon became known that the physiological activity of the gland was due to an iodine compound. Thereafter the percentage of iodine was accepted as the basis of evaluation of thyroid, although it had not been proved that all of the iodine was present in the form of physiologically active compounds.

Kendall, in 1914 (*Thyroxine*, New York, 1929, p. 22) was the first to isolate from the thyroid a pure substance, thyroxine, possessing the characteristic physiological activity of the gland; the yield, however, was very small. Later, Harington (*Biochem. J.*, 1926, **20**, 293), using a more efficient method of extraction, obtained a much higher yield of thyroxine, which he showed to be β–[3:5-di-iodo-4-(3′:5′-di-iodo-4′-hydroxyphenoxy) phenyl] α-amino-propionic acid. Harington and Barger (*Biochem. J.*, 1927, **21**, 169) next synthesised it, and later Harington and Randall (*Biochem. J.*, 1929, **23**, 373) showed that the only organic iodine compounds present in the gland were thyroxine and physiologically-inactive di-iodotyrosine. As the result of these discoveries it became evident that the assay of thyroid gland should be based on thyroxine rather than on total iodine.

Thyroxine occurs in the gland in combination with protein, and is set free on prolonged boiling with alkalis. For this purpose barium hydroxide appears to be the most suitable alkali, the thyroxine being recovered both from the fraction insoluble in the alkali and from the precipitate obtained on acidification of the alkaline solution. This method, used by Harington (*loc. cit.*) for the preparation of thyroxine, is too complicated to form the basis of assay.

By the hydrolysis of thyroid with sodium hydroxide, followed by acidification, there are obtained two fractions, respectively insoluble and soluble in acid. Harington and Randall (*loc. cit.*) have produced evidence that the whole of the acid-soluble iodine is present as di-iodotyrosine, whilst the whole of the acid-insoluble iodine belongs, in all probability, to thyroxine; and they state that, "the only reasonable chemical assay of the therapeutic value of a thyroid preparation must be based on the acid-insoluble iodine and not on the total iodine-content of the material." The examination by this process of a number of commercial samples of thyroid tablets (Harington and Randall, *Quart. J. Pharm.*, 1929, **2**, 501) showed that the proportion of acid-insoluble iodine to total iodine varied between 28 and 60 per cent.

Although the evidence on which Harington and Randall base their proposed assay does not constitute strict proof that this process effects a complete separation of the thyroxine from the di-iodotyrosine, there is no doubt that a standardisation based on the acid-insoluble iodine is a definite advance on one based on the total iodine.

The assay for acid-insoluble iodine must comprise two stages: a separation of the material into acid-insoluble and acid-soluble fractions, and a determination of the iodine in the former fraction.

The known methods for the determination of the total iodine in thyroid gland are suitable for the determination of the iodine in the fractions, but the method employed for the separation of these two fractions must be adapted to the process used for the determination of the iodine.

DETERMINATION OF THE TOTAL IODINE IN THYROID GLAND.—The destruction of the organic matter may be carried out by ignition with an alkali, with or without the addition of an oxidising agent, or by igniting in a current of oxygen and collecting the iodine in alkali. The method now generally adopted for the determination of the iodine in the solution obtained is the oxidation of the iodide to iodate, addition of potassium iodide, and titration with thiosulphate—a method which has the great advantage that six equivalents of iodine are titrated for each one present in the original substance. Although the combination of alkali fusion and oxidation to iodate is employed by most workers who determine iodine in thyroid gland, the methods generally employed are subject to error. It is necessary, therefore, to consider the sources of error at each stage of the analysis.

Destruction of the Organic Matter.—For the destruction of the organic matter an oxidising agent is commonly added. Thus Baumann (*Z. physiol. Chem.*, 1898, **21**, 1) heated the material with dry caustic soda, subsequently adding sodium nitrate. Krauss (*J. Biol. Chem.*, 1915, **22**, 515) and Hunter (*J. Biol. Chem.*, 1910, **7**, 321) used a mixture of potassium and sodium carbonates with potassium nitrate. Kendall (*J. Biol. Chem.*, 1920, **43**, 161) used caustic soda with a little water, potassium nitrate being added after the water had been boiled off; and a similar method was used by Kelly and Husband (*Biochem. J.*, 1924, **18**, 251). Grützner (*Chem.-Ztg.*, 1914, **38**, 769) fused with caustic soda and sodium or barium peroxide.

As the use of an oxidising agent is very liable to cause trouble at a later stage (see below), Schulek and Stasiak (*Pharm. Zentr.*, 1928, **69**, 113) used powdered caustic potash, Smith (*Quart. J. Pharm.*, 1928, **1**, 372) powdered caustic soda,

and Pickworth (*Biochem. J.*, 1925, **19**, 769) a strong solution of caustic soda, which was evaporated down and ignited. In these cases no oxidising agent was added to the melt.

Methods in which a substance is evaporated with caustic alkali solution require much attention to prevent creeping, and it is impossible to avoid some loss as spray. If the ignition is carried out with dry powdered caustic alkali, creeping still occurs, while there is some doubt whether the whole of the iodine is recovered. This is particularly the case with dried thyroid, as it is very difficult to grind the undiluted material to a fine powder, and, in consequence, it is often found that the apparent iodine-content of the material increases after it has been diluted and ground with lactose.

In order to avoid the errors due to the addition of oxidising substances to the melt, and to loss of iodine by volatilisation, I proposed the ignition of the thyroid with sodium carbonate in a double crucible (*Quart. J. Pharm.*, 1929, **2**, 536). In this method the substance is mixed with powdered sodium carbonate in a small crucible which is then filled with the carbonate and inverted on a layer of carbonate in a larger crucible, the space between the two being filled up with the carbonate. The two crucibles are heated in a large and fairly hot flame, so that the carbonate at the bottom and sides of the large crucible is hot before the gases produced by the decomposition of the organic matter pass through it. No attention is required during the ignition, and there is no danger of loss of iodine by creeping or volatilisation. Thorough extraction with hot water, however, is essential, so that after the carbonate has been dissolved in water and the solution filtered, the residue is again boiled with water for half-an-hour.

The Oxidation and Titration.—The oxidation of the iodine to iodate may be carried out by potassium permanganate in alkaline solution (Grützner, *loc. cit.*; Pickworth, *loc. cit.*), the method adopted officially in the German Pharmacopoeia. As, however, the removal of excess of oxidising agent is a comparatively long process, it is generally preferable to use a halogen as oxidising agent.

When a nitrate has been used in the fusion, sodium bisulphite is added in order to prevent the loss of free iodine due to the nitrous acid formed on acidification, and the solution is then either neutralised to methyl orange, or acidified with phosphoric acid. The oxidation of the iodide to iodate is carried out by the addition of chlorine water (Schulek and Stasiak, *loc. cit.*), sodium hypochlorite (Hunter, *loc. cit.*), bromine (Kendall, *loc. cit.*; Kelly and Husband, *loc. cit.*), or bleaching powder solution (Kolthoff, *Z. anal. Chem.*, 1921, **60**, 403). Excess of halogen is removed by boiling, and the last traces by the addition of sodium salicylate or phenol. The solution is then titrated, after the addition of potassium iodide, with standard thiosulphate solution.

There is thus no agreement regarding the three essential factors of the determination—the degree of acidity of the solution, the nature of the oxidising agent, and the method for its removal.

The degree of acidity of the solution depends on the method used for the ignition. If nitrate has been used, the product will contain nitrite. On treatment with bromine, nitrate and bromide are formed, and both of these may, under certain

conditions, interfere with the determination. In order to avoid this, the acidity of the solution should be kept comparatively low by the use of phosphoric acid for the acidification. When no oxidising substance is added to the melt sulphuric acid may be used for the acidification (Pickworth, *loc. cit.*), and this gives a sharper end-point. The addition of sulphite to prevent loss of free iodine on acidification is unnecessary when no oxidising compounds are present.

Of the halogen oxidising agents mentioned above, chlorine water and solutions of hypochlorites cannot be relied on, as they often contain traces of chlorates, while even bromine water is open to suspicion unless freshly prepared. For a similar reason it is not permissible to add bromine to the alkaline solution before acidification, owing to the risk of producing traces of bromate which, though they may not cause serious error when comparatively large amounts of iodine are to be determined, destroy the accuracy of determination of such small quantities of iodine as are here in question. An advantage incidental to the use of bromine is that the approximate amount present may be judged by the colour of the solution, while it is also more easily removed by boiling than is chlorine.

After boiling, a relatively large amount of bromine may still be present in the solution, although no yellow colour can be detected. Prolonged boiling leads to loss of iodate, and it is, therefore, not advisable to attempt to remove all traces of bromine by boiling, but to use for this purpose a compound which combines with bromine but not with iodine. Salicylic acid has the disadvantage that, unless all but faint traces of bromine have been first removed, the end-point during the final titration with thiosulphate is less definite, owing to a tendency of the blue starch-iodide colour to return. The use of phenol is free from this disadvantage.

For the removal of the halogen it is usual to boil for a considerable time; this leads to loss of iodate, particularly if potassium nitrate has been used in the initial fusion, since the nitrous acid formed on acidification is oxidised by bromine, forming potassium bromide which, on prolonged boiling, reacts with the iodate and causes a reduction in the titre of the solution.

Although the latter source of error is absent when no oxidising agent is used in the fusion, I have found that the best results are obtained if the boiling is stopped while bromine is still just visible in the solution. After cooling, the excess is removed by the addition of phenol, conveniently added as a 25 per cent. solution of crystalline phenol in glacial acetic acid. Provided that at least two minutes are allowed between the addition of the phenol and of the potassium iodide, perfect blanks may be obtained, and there is no danger of low results due to prolonged boiling.

Experiments were also made with sodium cinnamate, maleic acid, or formic acid for the removal of traces of bromine. The objection to the first of these is the sparing solubility of cinnamic acid; maleic acid combines comparatively slowly with bromine; while with formic acid a large excess must be used.

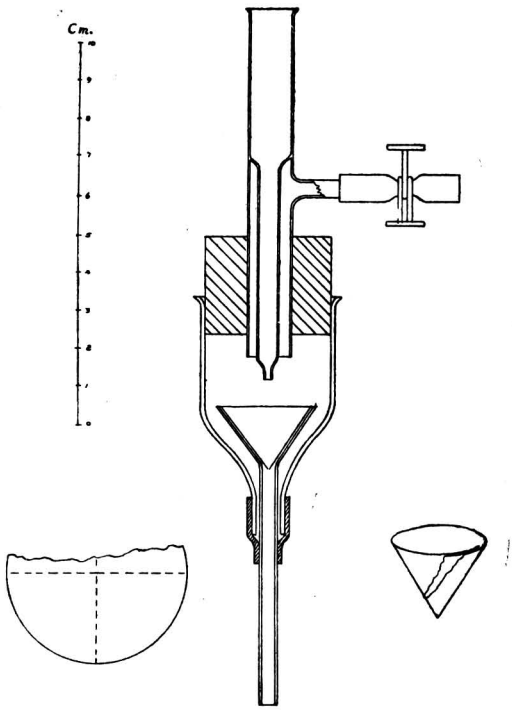
Kendall (*loc. cit.*) has paid considerable attention to the smooth boiling of the solution. Having tried and rejected sand, brick, glass, granite, pumice, capillary tubes, paper fibres, charcoal, porcelain, and coke, he finally used a piece of hard coal, which was removed from the solution before adding potassium iodide. The use of talc and similar powders is undesirable, as the end-point is less distinct in

a turbid solution, while porous substances are objectionable because they are liable to retain a little bromine. In a blank determination with 1 grm. of broken pipeclay, I obtained a reading of 0·04 c.c. of $N/100$ thiosulphate solution. The error, therefore, is not negligible if more than 0·1 grm. of this material is used. It may, however, be reduced by pouring off the solution from the pipeclay before adding potassium iodide. In place of adding substances of this nature it is better to facilitate boiling by passing a current of carbon dioxide through the hot liquid.

The details of the method recommended for the determination of total iodine (*vide infra*) have been based on the results of a large number of experiments carried out with definite quantities of standard solutions of potassium iodide and iodate. Proof of the accuracy of the method was obtained by determining the iodine in an acidified solution of sodium carbonate to which had been added 5·0 c.c. of a standard solution of potassium iodide containing 0·4 mgrm. per c.c. The titration results were 7·26, 7·20, 7·14, 7·14, 7·12, 7·14 c.c. of $N/100$ thiosulphate solution, respectively (theory 7·23 c.c.). Blank determinations made without the addition of iodide gave zero readings. To prove that no iodine was lost through its adsorption on the carbon produced in the ignition, 0·1 grm. of thyroxine was dissolved in 0·5 c.c. of N sodium hydroxide solution and made up to 10 c.c. with alcohol. Of this solution, 0·2 c.c. was dried with sodium carbonate and ignited as described. Another portion of 0·2 c.c. was dried with 0·5 grm. of a mixture in equal parts of casein and lactose, and the iodine in the mixture was determined. The titrations in the two cases were 6·15 and 6·18 c.c., respectively. Finally, three determinations were made of the total iodine of a sample of standardised thyroid, the titration readings obtained being 5·18, 5·22, 5·18 c.c. The results obtained by the proposed method are thus shown to be consistent and accurate.

Determination of Acid-insoluble Iodine.—For this purpose Harington and Randall (*Quart. J. Pharm.*, 1929, **2**, 501) employ Kendall's method of determining iodine, and calculate the difference between the total iodine of a solution obtained by boiling the thyroid with alkali and the iodine in the same solution after acidification and filtration. The errors inherent in Kendall's method have been discussed above. It is always undesirable to determine a quantity indirectly, especially when the original figures are subject to error. By igniting with sodium carbonate, as described above, the iodine may be determined directly on the acid-insoluble precipitate, only one experimental error, and that a very small one, being thus involved. In the method described below the essential details of the hydrolysis and the precipitation as given by Harington and Randall (*loc. cit.*), and now adopted in the *British Pharmacopoeia*, 1932, are retained, the precipitate being collected on a filter paper of 45 mm. diameter. To avoid the rather tedious process of continual filling of such a small filter, the apparatus shown in the figure has been devised. The liquid to be filtered is poured into the tube, and drops into the funnel at the same rate as the liquid filtering through, while the side tube and the spring clip allow of the release of air for filling the filter. It is essential that there should be no air-passage between the filter paper and the funnel; this is attained by tearing the paper, after it has been folded into quarters, as shown in the diagram, the wet paper being then fitted closely to the funnel. After the filtration, the filter paper and precipitate are dried at 100° C. The precipitate becomes tarry on

heating, some liquid being liberated, and it is therefore necessary to dry the paper and precipitate in the crucible in which they are to be ignited. When dry, the paper with the precipitate is ignited with sodium carbonate.



After the hydrolysis of thyroid with sodium hydroxide Harington and Randall filter the solution. This operation is rather troublesome, as the solution forms a jelly when cold. A determination of the iodine in the alkali-insoluble residue from 10 grms. of standardised thyroid gave a titration reading of 1·0 c.c. of $N/200$ thiosulphate solution, equivalent to 0·05 c.c. of $N/100$ thiosulphate solution on 1 grm. of material. Thus filtration of the alkaline solution does not appear necessary.

In order to check the method 10 grms. of thyroid were heated beneath a reflux condenser for 4 hours with 100 c.c. of N sodium hydroxide solution, the product was filtered as quickly as possible through a hot sintered-glass crucible, and the filtrate and washings were made up to 500 c.c. Fifty-c.c. portions of this solution were treated with acid, and the acid-insoluble iodine was determined as described below. The titration readings were 4·31, 4·30 c.c. of $N/100$ thiosulphate and 8·70 c.c. of $N/200$ thiosulphate solutions, respectively.

Details of Procedure.—*Total Iodine.*—The dried thyroid (0·5 grm.) is weighed into a nickel crucible of 26 mm. diameter and mixed with about 1 grm. of pure dry powdered sodium carbonate. The crucible is filled with the carbonate and inverted on a layer of sodium carbonate at the bottom of a large crucible of 36 mm. diameter, and more sodium carbonate is added to fill the space between the two crucibles. The two crucibles are then heated for twenty minutes so as to raise the outside of the larger crucible to a dull red heat. When cold, the contents of the larger crucible are tipped into about 100 c.c. of water and broken up immediately to avoid caking, and the larger crucible is rinsed out. After being boiled for 10 minutes the solution is filtered into a 600-c.c. conical flask through a hardened filter paper, or through a sintered-glass filter (1 G4 or 3 G4), and washed. The residue on the filter paper is washed back into the beaker by means of a jet of water, and the extraction is completed by boiling with another 100 c.c. of water for thirty minutes. The filtrate and washings are cooled, made up to about 400 c.c., and, after the addition of methyl orange,

50 per cent. sulphuric acid is introduced until the mixture is just acid. After the addition of 10 c.c. of 10 per cent. sulphuric acid in excess and a few drops of iodine-free bromine, the solution is heated; and when it begins to boil a current of carbon dioxide is passed into it to prevent bumping. The heating is stopped when the solution has only a slight yellow colour (after about 3 minutes' boiling), and the solution is cooled. About 0·2 c.c. of a 25 per cent. solution of crystalline phenol in glacial acetic acid is then added, and, *after the lapse of at least two minutes*, a few crystals of potassium iodide are added, and the mixture is titrated with $N/100$ thiosulphate solution, with starch as indicator.

Acid-insoluble Iodine.—One grm. of the thyroid is boiled for four hours beneath a reflux condenser with 10 c.c. of N sodium hydroxide solution. The solution is diluted to about 25 c.c., filtered as hot as possible through a previously-warmed sintered-glass filter, and the residue is washed with a little hot water. The filtrate is transferred to a beaker and acidified with 11 c.c. of N sulphuric acid; the reaction of the mixture is then adjusted, if necessary, to be slightly acid to congo red paper. After standing overnight the clear liquid is decanted, and the precipitate is collected on a 45 mm. filter paper (preferably with the aid of the filtration device shown in the diagram) and washed with water. The filter paper (with the precipitate) is transferred to a nickel crucible of 26 mm. diameter and dried. A little powdered sodium carbonate is then placed at the bottom of the crucible and in the filter, and the latter is completely embedded in the carbonate. The subsequent ignition in a double-crucible, extraction and treatment of the solution are exactly as described for the determination of the total iodine in the material.

STANDARDISATION OF THYROID.—The standardisation of thyroid on a basis of acid-insoluble iodine-content, determined by the method described, requires only one determination of iodine. It is neither practicable nor necessary to fix a second standard for total iodine-content. The determination of "inorganic" iodine (*i.e.* iodine compounds soluble in cold water) in order to detect adulteration by the addition of iodides no longer serves any useful purpose.

NOTE.—In a recent paper by Leland and Foster (*J. Biol. Chem.*, 1932, 105) there is described a method of determining thyroxine by shaking out into butyl alcohol after alkaline hydrolysis. These authors consider that the results obtained by the method of Harington and Randall are too high. Further investigation of the separation of thyroxine from thyroid is necessary before the problem of the chemical assay of thyroid can be considered fully solved. This investigation must be based on the iodine-content of different fractions; for this, the first requirement is an accurate and reliable method of determining the iodine in them. The methods described in this paper may, therefore, prove helpful in the final solution of the problem, even though the "acid-insoluble iodine" process may be found not to effect a sufficiently complete separation of thyroxine.

I wish to record my thanks to the Directors of The British Drug Houses, Ltd., for permission to publish this paper.

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The Iodine and Thiocyanogen Values of Irish Butter

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(*Read at the Meeting, May* 4, 1932)

The results recorded in this paper are taken from a series of observations made in connection with the investigation of the consistency of Irish butter—a property which depends, among other factors, on the content of unsaturated glycerides. Since the method devised by H. P. Kaufmann (Analyst, **1926**, **51**, 157, 264) for determining linolic, as well as oleic glycerides, in oils appeared to offer possibilities as a routine method for the examination of large numbers of samples, it was decided to attempt to apply it to butter fat, for the data hitherto obtainable by other and more elaborate methods have of necessity been comparatively few. It may be noted that in the present account, the terms "oleic" and "linolic glycerides" are only intended to indicate the combined acids in the glycerides, without reference to the mixed or simple character of the glycerides.

Iodine Value.—The unsaturated constituents of butter fat, of which this value gives a total measure, have been shown by Hilditch and Jones (Analyst, **1929**, **54**, 85), and by Hilditch and Sleightholme (*Biochem. J.*, 1930, **24**, 1096; **1931**, **25**, 507) to consist essentially of oleic and linolic glycerides. The main object of the present investigation was to study broadly the seasonal variations of these constituents in Irish butter. Some foreign butters were analysed for comparison.

The seasonal variations in the iodine value of Danish butter have been studied by Kilde and Winther (*Milchwirtschaftl. Forsch.*, Bd. **10**, Heft 3, 228), who found that the figures for mixed average samples varied from 29·9 in December, 1928, to 41·4 in August, 1929. Similarly, Haglund, Wode and Olsson (*Bull.* 387 [1930], *Centralanstalten*) found the average iodine values of Swedish butter to vary from 31·0 in January and February to 40·0 in August and September. The fall in iodine value during winter in these countries is regarded as undesirable, as it renders the butter too firm; this matter received some attention at the International Dairy Congress, held in Copenhagen in 1931, at which the following resolution was passed:—"In view of the importance for the production and the consistence of winter butter, of the amount of fat contained in oil-cakes and of their composition, the Congress recommends that the manufacturers of, and dealers in, oil-cakes should consider the possibility of bringing an oil-cake on the market which would be guaranteed to contain at least 5 to 6 per cent. of oil, and, if the oil-cakes are sold in mixture, of a mixture containing sunflower-seed cakes and rape cakes in at least the same quantity as coconut or palm-kernel cakes or soya meal." This resolution was largely the result of an extensive series of feeding experiments which were carried out under practical conditions during the three winter seasons, 1927–30, at the Danish State Experimental Dairy by A. P.

Hansen; an account thereof was published by the Congress as Paper No. 85, Section 3.

The seasonal variations in the iodine value of Danish butter are considered to be due wholly to the winter-feeding factor; there is no lactation factor to be considered, as is very probably the case with Irish butter. From the results now to be discussed, it will be seen that the iodine value of Irish butter is also affected by a winter-feeding factor, though to a modified extent; in this account the term "winter-feeding factor" is intended to include such concomitant factors as the influence of the change from outdoor to indoor life and seasonal variations in temperature, but not the influence due to the stage of lactation.

Table I shows the results obtained from iodine-value determinations carried out on mixed average samples each month; during the greater part of the year, the number of individual samples represented by each mixed average sample was about 400; the number decreased to a minimum of 50 in December.

TABLE I

MIXED AVERAGE SAMPLES OF BUTTER FAT

	Month	Iodine value (Wijs)	Reichert–Meissl value	Polenske value
1931	May	38·7	31·4	2·80
	June	39·3	30·8	2·75
	July	40·0	30·4	2·30
	August ..	41·0	28·6	2·15
	September ..	41·3	27·6	2·10
	October ..	43·0	26·5	1·90
	November ..	42·3	25·6	1·80
	December ..	41·3	24·7	1·75
1932	January ..	39·6	27·0	1·95
	February ..	39·5	27·7	1·95
	March	38·3	29·9	1·95
	April	38·3	30·0	1·90
	May	38·2	31·1	2·30
	June	38·1	31·9	2·85
	July	39·2	31·8	2·50
	August ..	41·4	30·4	2·10

In the case of Irish winter butter there are probably two factors—the winter-feeding factor, which tends to lower the iodine value, and the lactation factor, the effect of which is to raise the iodine value at times when the Reichert–Meissl value is decreasing, and conversely. The seasonal changes in the latter and in the Polenske value are generally recognised as lactation phenomena, and it is reasonable to suppose that the corresponding movements in the iodine value in the opposite sense, which occur regularly during the greater part of the year, are assignable under the same heading. It will be seen that in 1932 the minimum iodine value and the maximum Reichert and Polenske values occurred later than in 1931. This is attributable to the fact that the butter-making season was a late one in 1932.

The influence of the winter-feeding factor becomes evident in November, *i.e.* two months later than in the case of the Danish and Swedish butters; this

difference is, no doubt, due largely to climatic factors, and may also be partly due to the counteracting influence of the lactation factor which would tend to retard the fall in the iodine value during the latter half of the year. The fact that the extent of the variation between summer and winter iodine values is only about one-half of that experienced in the other countries mentioned may well be explained by the supposition that the two factors tend to counteract one another, especially during the spring and autumn months. Further, the difference between summer and winter feeding is not so pronounced in Ireland as in some other countries.

THIOCYANOGEN VALUE.—Kaufmann (*loc. cit.*) has shown that linolic acid or glyceride combines with thiocyanogen only at one of the two double bonds, so that a knowledge of the thiocyanogen absorption (generally calculated in terms of iodine) and of the iodine value makes possible the calculation of the linolic and the oleic glyceride content of a fat. For the preparation of the thiocyanogen solution, the method recommended by Kaufmann (ANALYST, 1928, **53**, 613) was used; the solvent was pure glacial acetic acid (99–100 per cent.) containing 10 per cent. of acetic anhydride, and the mixture was kept at least a week before use. Twenty-five grms. of carefully dried, pure lead thiocyanate were added to 500 ml. of the solvent, and 3 ml. of bromine to another 500 ml. The latter solution was added to the former, and the mixture was shaken until colourless, allowed to settle, and then filtered through a dry filter paper. On titration, Kaufmann has shown that this solution must be poured into an excess of potassium iodide solution, when iodine will be quantitatively displaced by thiocyanogen, and may be titrated by means of thiosulphate as usual. If the potassium iodide solution is poured into the thiocyanogen solution, a little loss of thiocyanogen by hydrolysis takes place. The solution, prepared as described, is approximately decinormal in strength; it should be kept in the dark and protected from access of moisture; its stability depends, to some extent, on the purity of the materials used in its preparation; during the first 3 or 4 days a loss in strength corresponding with not more than about 0·5 to 0·1 ml. per 25 ml. per day may usually be expected; for reasons stated below it is advisable to use the solution as fresh as possible.

The general outline of the determination of the thiocyanogen value is similar to that of the iodine value method according to Wijs. It would appear that the work hitherto carried out has been on oils or fatty acids which remain in solution in the acetic acid solvent; most solid fats, including butter fat, however, partly crystallise out of solution in 2 to 3 hours at 22° C., even when the 25 c.c. of thiocyanogen solution is diluted with twice its volume of the acetic acid and anhydride mixture, the amount of fat taken being 0·25 to 0·30 grm.

Some trials were made with carefully purified and dried carbon tetrachloride and petroleum spirit (ligroin) as fat solvents, as in iodine-value determinations, but it was found that the absorption of thiocyanogen was inhibited to a considerable extent in such cases; it was therefore decided to raise the temperature at which the absorption mixture was kept at 25 to 26° C. In order to study what effect this raising of the temperature might have on the accuracy of the results, some comparative trials were made at two temperatures, *viz.* 16 to 18° C. and 25 to 26° C.,

with olive and arachis oils. For the lower temperature, 0·2 grm. of the oil was dissolved in 25 ml. of the thiocyanogen solution, and for the higher temperature, the same weight of oil was first dissolved in 25 ml. of the acetic acid and anhydride mixture, after which 25 ml. of the thiocyanogen solution were added; the absorption mixtures were contained in well-stoppered 100-ml. bottles and kept at the respective temperatures together with blanks. The results were as follows:

Oil	Absorption time Hours	Thiocyanogen value (16 to 18° C.)	Thiocyanogen value (25 to 26° C.)
Olive ..	4	76·0	75·8
,, ..	5	75·9	75·6
Arachis ..	16	69·7	69·3

It will be seen that there is a tendency for somewhat lower thiocyanogen values to be obtained at the higher temperature than at the ordinary temperatures, and that this is accentuated with longer absorption periods. The latter point (*i.e.* the effect of the absorption time) can be verified from Kaufmann's results. As the thiocyanogen solution is not quite stable even when freshly made, there will always be a tendency for more loss of free thiocyanogen to occur by polymerisation in the blank tests than in the actual absorption tests; this will cause the blank values to be slightly low, and thus decrease the apparent absorption. At higher temperatures the rate of deterioration of the thiocyanogen solution is somewhat increased; thus in the olive oil experiments the blank values were 26·25 ml. and 26·1 ml., when working at the lower and the higher temperatures, respectively.

It thus follows that the error inherent in the method due to the deterioration of the thiocyanogen solution is increased with longer absorption periods and higher absorption temperatures. In the case of the more highly unsaturated oils, periods of twice to three times that necessary for butter fat are required; it may, therefore, be presumed that the results obtained for butter fat, in spite of the temperature error, will be comparable with those obtained for the oils as regards reliability. From what has been said, it will be understood that the linolic glyceride percentages given below in Table II may be somewhat high, possibly from one-third to one-half of a unit per cent., but they may, at any rate, be used for comparative purposes, such as in the study of seasonal variations.

Method of Determining the Thiocyanogen Value of Butter Fat.—Quantities of 0·25 to 0·30 grm. of the fat were weighed out in small tubes, which were introduced into 150-ml. conical flasks furnished with well-fitting glass stoppers. The flasks were gently warmed, just sufficiently to melt the fat, whereupon 25 ml. of the acetic acid and anhydride mixture at 38° C. were added, followed by 25 ml. of the thiocyanogen solution after the fat had been dissolved. The absorption flasks, with blanks, were placed in an incubator at 25 to 26° C. for 7 hours, this having been determined as the minimum time required for the maximum absorption by butter fat. For titration, the contents of the flasks were poured into 40 ml. of 5 per cent. potassium iodide solution, and the liberated iodine was titrated with $N/10$ thiosulphate and starch in the usual way; towards the end

of the titration, some of the solution was used to rinse out the remains from the absorption flask, and the titration was continued to the end.

The thiocyanogen values were expressed in terms of iodine absorbed, and the percentages of combined linolic and oleic acids were calculated. The difference between the iodine and thiocyanogen values, divided by the factor 0·905, gives the percentage of linolic acid.

The results of the determinations are given in Table II. The samples include Irish summer and winter butters and some foreign butters.

TABLE II

IODINE AND THIOCYANOGEN VALUES OF BUTTER

Date	Description	Iodine value	Thiocyanogen value	Linolic acid Per Cent.	Reichert–Meissl value	Polenske value	Mark creamery
Dec. 1930	Irish creamery	42·4	38·7	4·1	21·1	1·65	A
		43·0	39·6	3·8	22·7	1·55	B
		42·0	39·0	3·3	21·5	1·50	C
		43·2	40·0	3·5	22·2	1·60	D
		41·8	38·7	3·4	20·7	1·40	E
		42·1	38·2	4·3	21·8	1·40	F
		42·5	38·8	4·1	21·1	1·55	G
		43·0	39·2	4·2	22·2	1·35	H
		43·0	39·2	4·2	22·1	1·50	I
		42·6	38·8	4·2	22·2	1·50	J
	Average	42·56	39·02	3·91			
June 1930	Irish creamery	37·0	34·0	3·3	31·5	2·80	A
		38·3	34·2	4·5	31·3	3·15	B
		38·1	34·7	3·8	30·7	2·40	C
		37·1	33·6	3·9	32·2	3·25	D
		37·3	33·5	4·2	31·1	3·10	E
		37·1	32·9	4·6	30·9	3·10	F
		38·7	35·7	3·3	31·2	3·05	G
		37·5	34·5	3·3	31·6	2·75	H
		39·0	34·8	4·6	30·0	2·80	I
		38·0	33·8	4·6	32·1	2·90	J
		36·8	33·4	3·8	31·5	2·80	K
	Average	37·72	34·10	3·99			
Feb. 1931	New Zealand	32·6	28·8	4·0			
	Danish	31·9	28·0	4·3			
	,,	32·5	29·5	3·3			
	,,	30·6	27·6	3·3			
June 1931	Australian	34·5	31·2	3·6			
	Siberian	33·8	30·6	3·5			
	Argentine	39·4	35·7	4·1			

The variations in the combined linolic acid were from 3·3 to 4·3 per cent. for the Irish winter butter samples, from 3·3 to 4·6 for the Irish summer butters, and from 3·3 to 4·3 for the seven foreign butters.

Table III has been drawn up to show the composition of the unsaturated acids (assuming these to consist of oleic and linolic acids only) in the various classes of butter; where these were represented by more than one sample, the results were averaged.

TABLE III

Description of butter	Oleic acid in fat Per Cent.	Linolic acid in fat Per Cent.	Linolic acid in unsaturated acids Per Cent.	Number of samples represented
Irish winter ..	39·02	3·91	9·1	10
Argentine ..	35·2	4·1	10·4	1
Irish summer ..	33·54	3·99	10·6	11
Australian ..	30·8	3·6	10·5	1
Siberian ..	30·3	3·5	10·4	1
Danish ..	27·61	3·63	11·6	3
New Zealand ..	27·5	4·2	13·2	1

From the first column of figures it is seen that the results have been entered in order of descending percentages of oleic acid (obtainable from the fat); the third column of figures shows that there is a definite tendency for the percentage of linolic acid in the total unsaturated acids to increase as the percentage of oleic acid in the fat decreases. There is thus a tendency for the linolic glyceride-content to remain approximately constant, whilst the oleic glyceride-content is the variable factor. It is noteworthy that these relationships should be found between samples differing so widely in origin as those which have been examined.

In conclusion, I wish to express my thanks to the Department of Agriculture, Dublin, for permission to publish this paper.

BUTTER TESTING STATION,
HARCOURT TERRACE, DUBLIN

Tests for the Keeping Quality of Unsalted Butter

BY J. T. MINSTER, B.Sc., A.I.C.

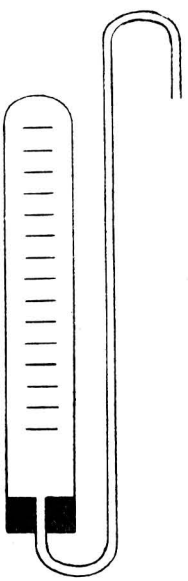
IT is a well-known fact that unsalted butter which has been kept for a month or two in cold store, *e.g.* during transport from the Dominions, very frequently deteriorates rapidly in flavour when it is again exposed to normal atmospheric conditions.

Possible causes of this deterioration are direct oxidation due to contact with the air, and the action of micro-organisms. The oxygen absorption was followed by a development of Issoglio's "oxidisability" method,[1] and it was found that this property cannot be correlated with the specific complaint under consideration, although it may have a bearing on changes which occur later when the butter is quite rancid. The tests here described are concerned essentially with the first stages in deterioration. The work on the oxygen absorption involved keeping the samples at 37 to 38° C. for several days, and it was observed that some tended

to putrefy, whilst others remained sweet, indicating that bacteriological development was proceeding along different lines in the various samples. The following tests for enzymes and micro-organisms were employed:

(1) THE CATALASE TEST.—True lactic acid bacteria do not give rise to the enzyme catalase, whereas putrefactive organisms very frequently do so. Other bacteria which are objectionable from the butter-making point of view, and many of which occur in dirty water, also develop catalase. Therefore, if a butter is found to be rich in catalase, it may be deduced that the conditions of its manufacture were imperfect, and that objectionable organisms have gained access to it.

The technique employed—practically that of Orla-Jensen,[2] with the use of Ling's apparatus, as modified for butter-testing by Hesse[3]—is as follows:—Weigh 100 grms. of butter into a wide-mouthed separating funnel with a stem about 1 cm. long. Close the end of the stem with a small rubber stopper, and stand the funnel in a water bath at 45° C. until the butter is melted. Add 40 c.c. of distilled water at 45° C., and mix thoroughly. When separation has taken place, run off the aqueous layer, and place 15 c.c. of this in a graduated test-tube of 20 c.c. total capacity. Add about 3·5 c.c. of hydrogen peroxide (10 vol.), and mix by inverting the tube three times. Fill the tube completely with hydrogen peroxide and insert the rubber stopper and delivery tube as shown in the figure. Keep in a water-bath at 37 to 38° C. for six hours. Read the volume of gas evolved, shaking to disperse foam, if necessary.



The results become more expressive when arranged in an arbitrary scale in which the result 0 c.c. has a value of 100. Very rarely is 8 c.c. exceeded, and this volume (or more) is taken as zero. As a matter of experience, it was found that the difference in quality between a sample giving, say, 4 c.c., and one giving 6 c.c., is very slight, whereas the difference in quality between 0·1 c.c. and 2·1 c.c. is very great. The important section is from 0 to 2 c.c., and the scale has, therefore, been arranged as a curve giving a drop of 100 to 50 for 0 to 2 c.c., and 50 to 0 for 2 to 8 c.c. The equation employed is $C = 100 - 35{\cdot}35\sqrt{V}$ where V c.c. represent the volume of gas, and C the catalase value.

(2) REDUCTASE TEST.—The time taken for milk coloured with methylene blue to become decolorised under standard conditions is a function of the number and type of organisms present, and affords a fair criterion of cleanliness and keeping quality (*cf.* ANALYST, 1926, **51**, 459; 1928, **53**, 106, 213, 532). It may be applied to the examination of butter in the following way:—Place 10 c.c. of the aqueous extract, as prepared for the catalase test in a test-tube, 0·5 inch in diameter. Add 0·5 c.c. of a 0·005 per cent. solution of methylene blue. Mix by inverting the tube with as little dispersal of air as possible. After a few minutes, to allow air bubbles to escape, pour a layer of melted petroleum jelly on to the surface. Place the tube in the water-bath at 37 to 38° C., and note the time taken to bleach the dyestuff.

An arbitrary system of recording results has again been adopted:

Bleached in 0·5 hour or less	0
If any blue is present after 6 hours, but none after 21 hours ..	90
,, ,, ,, 21 ,, ,, ,, 24 ,, ..	95
,, ,, ,, 24 hours	100

Between the points 0·5 hour = 0 and 6 hours = 90 the results lie on the straight line $R = 16{\cdot}36t - 8{\cdot}18$, where t hours = time required for decolorisation, and R = reductase value.

In order to obtain additional information regarding the organisms present in the butter, without going to the extent of plate counts, the following tests have been employed.

(3) CULTURE MEDIA TUBES.—Prepare the following culture media:

(*a*) *Litmus Milk.*—Skim milk, 1 litre; azolitmin, 1 grm.

(*b*) *Purple Salt Agar.*—Beef extract, 3; peptone, 5; agar-agar, 15; sodium chloride, 8; bromcresol purple, 0·025 grms., per litre. (Adjust p_H to 7·4.)

(*c*) *Purple Lactose Agar.*—Beef extract, 3; peptone, 5; agar agar, 10; lactose, 10; bromcresol purple, 0·025 grms., per litre. (Adjust p_H to 6·8.)

Place 10 c.c. of each medium in test-tubes, and plug and sterilise them in the usual way. Melt the contents of the tubes in boiling water and afterwards cool them to as low a temperature as possible consistent with the media remaining fluid. By means of a clean dry pipette place 1 c.c. of the aqueous butter extract in each of the three tubes. Mix well and cool, allowing the agar to harden to form a slant. Incubate at 37 to 38° C. for 16 hours.* At the end of this period award points according to the condition of the tubes, in accordance with the following scale:

(*a*) *Litmus Milk.*

Colour	Bleached	0	Curd	Bad curd (torn and liquefied)	0
	Part bleached (but at least 50 per cent. remaining unbleached) ..	1		Moderate curd (few holes and channels and little liquid in a mostly solid curd)	1
	Entirely unbleached ..	2		Good curd (uniformly solidified, or not even solidified)	2

Maximum for litmus milk, 4.

Note.—For types of curd see Orla-Jensen, *Dairy Bacteriology*, 1921, p. 164.

(*b*) *Purple Salt Agar.*

Colour below the slant	Yellow ..	0	Gas	Blown and distended with gas	0	Surface growth	Heavy .. 0
	Yellow-purple ..	1		Isolated individual bubbles	1		Moderate .. 1
	Purple ..	2		No gas ..	2		Trace or nil 2

Maximum for purple salt agar, 6.

* This test may be made more exact by observing the usual precautions to ensure sterility throughout the experiment.

Notes.—Colour : Organisms which form acid in the absence of lactose are particularly undesirable, as this indicates some action on the proteins akin to putrefaction.

Gas : Gas-forming organisms in butter indicate lack of cleanliness at some stage of the manufacture.

Surface growth : This may be regarded as heavy if more of the surface is covered with growth than is left clear: moderate, if there are more than two or three colonies, but the surface is at least half-free; and as a trace when there are only one or two isolated colonies.

(*c*) *Purple lactose agar.*

Colour below the slant	Yellow. If purple salt agar is purple	1
	" " " " " " yellow-purple ..	½
	" " " " " " yellow	0
	Purple	0
	Yellow-purple	1

Gas and surface growth as for purple salt agar.

Maximum for purple lactose agar, 5.

Note.—Colour : The presence of a few genuine lactic acid bacteria is regarded as beneficial, tending to keep down the growth of other (more objectionable) organisms. If purple salt agar has given the result "purple," it indicates that any acid-formers are of the true lactic acid bacteria type, and in these circumstances "yellow" in the purple lactose agar test counts higher than if some, at least, of the acidity is due to organisms acting on the proteins.

Add the totals for the three different tubes, multiply by 100/15 and regard the result as a percentage.

(4) THE INCUBATION TEST.—Incubation at 37 to 38° C. for four days has been employed as follows:—Fill a glass-stoppered jar from half to three-quarters full with the sample. Melt in a water-bath at 45° C., and shake well. Incubate for four days at 37 to 38° C. Shake daily, and observe the odour immediately *before* shaking each day.

The results of this test depend very largely on the judgment and experience of the observer, and, for this reason and others stated later, great reliance is not placed on it. Removing the jar of butter from the incubator and allowing it to cool increases the intensity of any unpleasant smell. This might be advantageous as a regular practice, but all the results given in this paper have been obtained by examining the samples while warm.

The following marking system has been adopted:

Very pleasant warm, sweet, butter-smell	100
Odourless	85
Slightly stale, musty or sour, but with no trace of putrefaction ..	70
Distinctly stale, musty or sour, or very faintly putrid	50
Distinctly putrid and unpleasant	30
Extremely offensive	0

The average of the figures for the four days is taken as the result of this test.

For the final evaluation of the butter the average of the four results—catalase, reductase, culture media tubes and incubation is taken, and, in general, the results are in agreement, a low catalase figure accompanying a low reductase figure, etc. This is not invariably the case, however, and the average figure has been found more trustworthy than any of the individual results. If one value is much below the average the butter frequently has some unusual characteristic. For example, butter number 13 (for which figures are given below) had a very rank flavour when sampled, but this became no worse on keeping for some weeks.

The table below shows typical figures for butter samples giving high and low results. A figure of 70 or more is taken as indicating good keeping quality.

TABLE I

Sample	No.	Catalase	Reductase	Culture media tubes	Incubation	Average
Good	1	100	100	100	65	91
,,	2	100	95	84	85	91
,,	3	100	100	71	80	88
,,	4	95	90	63	80	82
,,	5	85	86	63	76	77
,,	6	78	68	87	65	74
Poor	7	75	50	73	60	65
,,	8	53	40	63	60	54
,,	9	42	24	33	75	43
,,	10	34	50	41	40	41
,,	11	39	28	27	55	37
,,	12	17	30	25	50	31
Erratic-poor	13	19	90	100	44	63
,,	14	0	5	21	80	27

RESULTS IN PRACTICE.—Practical proof of the applicability of these results to the problem of keeping butter under ordinary domestic conditions has been obtained.

(1) Two samples of butter, one with high and the other with low figures, were packed in 1-lb. blocks, wrapped in vegetable parchment, and kept at room-temperature for four days. On inspection, the sample with the higher value was found to be still fresh and sweet, whereas that with the lower values had a very obvious stale smell and taste. The same determinations as before were then carried out.

TABLE II

		Catalase	Reductase	Culture media tubes	Incubation	Average
High	Original	95	100	53	80	82
	After 4 days	90	100	52	80	81
Low	Original	66	60	20	75	55
	After 4 days	13	5	5	75	25

The high values changed hardly at all during this period, but the low original values dropped considerably, with the exception of those given by the incubation test, which gave no indication of the difference in quality either between the two samples in their original or final state, or between the low-valued sample before and after the 4 days' storage.

In a similar experiment with entirely different samples, the following results were obtained:

TABLE III

		Catalase	Reductase	Culture media tubes	Incubation	Average
High	Original	87	88	57	55	72
	After 7 days	75	80	50	45	63
Low	Original	53	16	40	45	39
	After 7 days	12	11	37	45	26

The sample with lower values gradually deteriorated until, after two weeks, it was too unpalatable to use. The one with higher values was still in good condition, and remained so until some time afterwards.

(2) The tests were applied to several samples which had been kept until they showed the objectionable characteristics which this work was carried out to investigate. The following results, obtained with three of these, are typical:

TABLE IV

	Catalase	Reductase	Culture media tubes	Incubation	Average
1	0	0	20	75	24
2	3	9	20	73	26
3	21	10	9	80	30

The following points arise from the examination of the figures in Tables II, III and IV:

(*a*) Low catalase, reductase and culture media tube figures are characteristic of butter which has gone "off," and these low values develop most rapidly in butter which has original sub-normal values.

(*b*) It is questionable whether the incubation figure has much significance. Samples occasionally occur, however, which give very low results when tested in this way, all the other figures being normal.

TABLE V

	Catalase	Reductase	Culture media tubes	Incubation	Average
1	85	85	—	0	57
2	69	73	65	0	52
3	80	100	65	35	70
4	90	100	55	40	71
5	95	90	81	47	78
6	90	100	91	43	81

A sample which soon gives rise to an extremely objectionable odour at 38° C. must be expected to deteriorate more rapidly under ordinary atmospheric conditions than butter which remains perfectly sweet at the higher temperature.

(3) Many observers all over the country have regularly received samples of butter which have been tested by the methods described above. The samples have been kept under the most varied conditions, including all those met with under

ordinary domestic circumstances. Weekly reports have been received regarding the behaviour of the butter, as judged by the various observers, and the number of unfavourable reports regarding the keeping of the samples has been plotted, together with the results of the tests for keeping qualities. (Atmospheric temperature variations may be excluded; the average temperature was slightly higher when few adverse reports were made, and lower when the number of such reports increased.)

It is not suggested that either the actual determinations, or the methods of expressing the results have a very high standard of precision, and their interpretation must be made in a broad way. Distinction cannot be made between samples because of a difference of a few points, but the figures should be taken more as an indication of the class of butter to which the sample belongs.

SUMMARY.—(1) The onset of the first stages of deterioration of unsalted butter, when exposed to normal atmospheric conditions after a month or two in cold store, could not be correlated with the oxygen absorption.

(2) Methods of estimating the activity of the enzymes, catalase and reductase, are described, together with a simple method for the bacteriological examination of butter, with arbitrary scales for expressing the results.

(3) Incubation for four days at 37 to 38° C. and observation of the odour daily has been employed as a test, and a system of marking is described. Less reliance is placed on this test than on those summarised under (2).

(4) The average of the catalase, reductase, bacteriological and incubation tests has been found to be the most reliable guide to the keeping quality of a butter sample.

(5) Typical results for good and poor samples and for those already in process of deterioration are given. Low catalase, reductase and bacteriological test values are characteristic of butter which has gone "off," and these low values develop most rapidly in samples with original sub-normal values.

(6) The opinion of independent observers on the quality of the butter became more adverse with diminution in the average keeping-quality value.

I wish to thank the Directors of the Dominion Dairy Company, in whose laboratory this work was carried out, for their help and advice and for permission to publish this paper.

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DOMINION DAIRY CO., LTD.,
AYLESBURY, BUCKS.

The Separation and Determination of Traces of Lead in the Presence of Small Quantities of Iron

By J. HUBERT HAMENCE, M.Sc., A.I.C.

In the course of the examination of chemicals (particularly alkali and alkaline earth salts), dyes and foodstuffs, for objectionable impurities, it is often necessary to determine traces of lead which may range between one part and 300 parts per million of the solid under examination. For this purpose the colorimetric method, based upon the formation of a colloidal solution of black lead sulphide in an alkaline medium, is almost invariably employed.

The presence of traces of iron, however, introduces difficulties. Some provision is made for this by preliminary treatment of the solution with potassium cyanide (Teed, ANALYST, 1892, **17**, 141; Hill, *Chem. and Drugg.*, 1905, **77**, 388) and Wilkie (*J. Soc. Chem. Ind.*, 1909, **28**, 636) recommended treatment of the solution with sodium thiosulphate prior to the addition of potassium cyanide.

It has been found, however, that when the iron in solution exceeds a few mgrms. it is difficult to prevent (*a*) the precipitation of the iron as hydroxide or sulphide, or (*b*) the formation of a yellow solution which renders the assessment of the lead sulphide difficult.

ADSORPTION OF LEAD BY FERRIC HYDROXIDE.—The removal of the iron by precipitation as ferric hydroxide leads to very low results, owing to adsorption of the lead on the ferric hydroxide (Wilkie, *loc. cit.*). Three mgrms. of ferric hydroxide will adsorb 1 mgrm. of lead, and this lead is not liberated by re-precipitation of the ferric hydroxide.

By precipitating the iron in the presence of ammonium acetate and boiling the solution until it is just acid the adsorption error is largely reduced, but is not entirely avoided. Thus 3·3 mgrms. of ferric hydroxide were precipitated in a solution containing 2 grms. of ammonium acetate and 5·0 mgrms. of lead, and the liquid was boiled until acid. After being filtered off and washed well with hot water the adsorbed lead was determined by dissolving the ferric hydroxide in nitric acid and separating the lead by the thiocyanate method subsequently to be described; 0·20 mgrm. was found to be adsorbed. Similarly, with smaller amounts of lead, a small percentage always remained adsorbed on the ferric hydroxide.

The large adsorption produced by precipitating the ferric hydroxide in the presence of ammonium acetate and leaving the solution ammoniacal is not always fully realised. The following results were obtained by precipitating 3·3 mgrms. of ferric hydroxide in the presence of ammonium acetate and lead nitrate; after

precipitation the solution was heated to 80° to 90° C. in a water-bath for 20 minutes, and the lead was determined in the supernatant solution, which was still strongly ammoniacal.

Ammonium acetate in solution Grms.	Lead added Mgrm.	Lead adsorbed Mgrm.
0·5	1·00	0·96
5·0	1·00	0·72

Even in the presence of 5 grms. of ammonium acetate the adsorption is very considerable.

Errors due to the adsorption by filter paper when ammoniacal solutions of lead are filtered are also considerable and must be avoided.

The existing methods for the separation of lead and iron which are free from adsorption errors are all tedious, with the exception of the method recently described (Allport and Skrimshire, ANALYST, 1932, 440). Their method is based upon the extraction of the lead from an ammoniacal solution with a solution of diphenylthiocarbazone in chloroform, the lead diphenylthiocarbazone complex dissolving in the chloroform. The chloroform solution is subsequently evaporated, the organic matter is destroyed, and the lead in the residue is determined colorimetrically. This excellent method, which can be employed in the presence of many interfering substances, has been found to give good results, but it was thought desirable to find a method for the separation of a mixture of lead and iron which was even more rapid than that of Allport and Skrimshire.

SEPARATION AS THIOCYANATES.—The present method of separation depends upon the properties of lead and ferric thiocyanates. Lead thiocyanate was prepared by the double decomposition of equivalent amounts of ammonium thiocyanate and lead nitrate in aqueous solution. After repeated recrystallisations from hot water, washing with alcohol and ether, and drying at 100° C., a white crystalline product containing 64·05 per cent. of lead was obtained [theory for $Pb(SCN)_2$ = 64·09 per cent.]. This compound contained no water of crystallisation or moisture and gave not the slightest reactions for ammonia or nitrate. It was thus identical with the product obtained by Hall (*J. Amer. Chem. Soc.*, 1902, **24**, 570).

The solubility of lead thiocyanate in water at 12° C. was found to be 3·180 grms. per litre, and at 12° C. it was nearly zero in a mixture of equal volumes of ether and amyl alcohol. Ferric thiocyanate, however, is very soluble in this mixture, and may be extracted from aqueous solution by this solvent. This mixture of ether and amyl alcohol was found to be a far more efficient solvent for ferric thiocyanate than either ether or amyl acetate, in both of which it is soluble.

Since no precipitations are involved in this separation adsorption errors are no longer introduced.

PROCEDURE FOR ALKALI AND ALKALINE EARTH SALTS.—Five grms. of the salt are dissolved in 30 c.c. of water in a separating funnel. To this solution 5 c.c. of 10 per cent. nitric acid and 5 c.c. of saturated ammonium thiocyanate solution

are added. The solutions are mixed, 15 c.c. of amyl alcohol and 15 c.c. of ether are added, and the whole is vigorously shaken. When the two layers have separated (emulsions are not formed), the aqueous layer is drawn off and the lead present is determined colorimetrically by the sulphide method.

If the aqueous solution still shows a pronounced red colour, it must be extracted once more with 15 c.c. of ether and 15 c.c. of amyl alcohol. A trace of red coloration may be ignored, as it is readily bleached on the addition of ammonia and potassium cyanide solution, and the small amounts of iron that are now present in no way interfere with the determination of the lead.

When more than 0·2 mgrm. of lead is present the ethereal solution in the separating funnel should be washed once with a few c.c. of very dilute nitric acid; this serves the double purpose of removing any of the aqueous phase adhering to the sides of the separator and of extracting any minute traces of lead thiocyanate dissolved by the ethereal layer. A blank experiment should be made on the reagents employed and the result deducted from the lead found.

To test the efficacy of the process, 5 grms. of lead-free salts were taken, and known amounts of lead nitrate and ferric chloride solution were added. The results are shown in the following table.

The blank on the reagents for both one and two extractions with the ethereal mixture was 0·02 mgrm., and this amount has been deducted in each instance from the lead found.

Compound	Ferric oxide added Mgrms.	Lead added Mgrm.	Lead found Mgrm.	Number of extractions
No salts	—	0·10	0·10	1
,,	5·0	—	Nil	1
Potassium chloride	5·0	0·10	0·09	1
Potassium nitrate	2·5	0·20	0·20	1
Ammonium sulphate	5·0	0·30	0·29	1
Ammonium nitrate	5·0	1·00	0·98	1
Sodium sulphate	25·0	0·15	0·15	2
Potassium chloride	12·5	1·50	1·53	2
Calcium nitrate	15·0	0·30	0·30	1
Magnesium nitrate	10·0	0·50	0·48	1
Ammonium sulphate	5·0	1·00	0·50	1

It will be seen that with ammonium sulphate containing 200 parts per million of lead only half the lead was found. This was due to the precipitation of lead sulphate, that is, to the inability of the ammonium sulphate to dissolve the lead sulphate. This defect may be remedied by working on a much smaller quantity of the sample.

Basic compounds, such as carbonates and oxides, can be tested for lead by dissolving 5 grms. in the minimum quantity of dilute nitric acid, making the volume up to 30 c.c., and continuing the process as detailed for water-soluble substances. If only the minimum quantity of nitric acid has been employed to obtain solution, 5 c.c. of 10 per cent. nitric acid should be added, as for soluble

substances, but if excess of acid has been employed to obtain solution, the determination may be continued without the further addition of acid.

The process was tested in a similar manner to that used for water-soluble substances.

Compound	Ferric oxide added Mgrms.	Lead added Mgrm.	Lead found Mgrm.	Number of extractions
Sodium carbonate	15·0	0·20	0·19	2
Calcium hydroxide	5·0	0·10	0·10	1

APPLICATION TO ORGANIC COMPOUNDS.—After the destruction of a dyestuff or other organic substance by wet oxidation or by the Bang process, any iron in the resulting sulphuric acid solution may be removed by the following procedure:— The sulphuric acid solution is evaporated until only one or two c.c. of acid remain. This is diluted with 10 c.c. of water, 2 grms. of lead-free ammonium acetate are added, and then ammonia, until the solution is ammoniacal, and the liquid is boiled for 15 minutes or until it gives an acid reaction with litmus. Concentrated nitric acid is then added until the precipitated iron has re-dissolved, the solution is diluted with water to 30 c.c., and the thiocyanate separation process is employed. Where calcium phosphate is present in the solution after wet oxidation, 10 c.c. of the ammonium citrate solution described by Nicholls (ANALYST, 1931, **56**, 594) should be added after separation of the iron before applying the lead test; this will ensure the calcium phosphate remaining in solution during the test.

CALCIUM PHOSPHATE.—In the determination of lead in calcium phosphate by the process described by Nicholls (*loc. cit.*) the presence of traces of iron are objectionable, and Nicholls recommends preliminary treatment of the solution with sodium thiosulphate. The iron, however, may be separated by the thiocyanate method before the test for lead is applied: One grm. of calcium phosphate is dissolved in 3 c.c. of concentrated nitric acid and 17 c.c. of water, 5 c.c. of saturated ammonium thiocyanate solution are added, and the ferric thiocyanate is shaken out with ether and amyl alcohol. Thirty c.c. of Nicholls' ammonium citrate solution are then added, and the mixed solution is tested for lead by the sulphide method.

One grm. of iron-free calcium phosphate containing 30 parts per million of lead was mixed with 25 mgrms. of iron (added as ferric chloride) and 0·50 mgrm. of lead; the iron was completely extracted by two extractions, and the total amount of lead found was 0·5 mgrm.

FERROUS IRON.—Ferrous iron must be oxidised to the ferric state prior to the separation, by boiling the aqueous solution with 5 c.c. of 10 per cent. nitric acid; after cooling, the process is continued without further addition of acid.

By this process all the iron from a mixture of 0·350 grm. of ferrous ammonium sulphate, 5 grms. of sodium chloride and 0·30 mgrm. of lead was removed, without loss of lead, by two extractions.

INFLUENCE OF OTHER METALS.—Copper and bismuth, which are likely to occur as impurities in substances which may be tested by this process, yield coloured thiocyanates.

Copper.—Cupric ions yield a yellow thiocyanate which is partly extracted by a mixture of ether and amyl alcohol, but the colour remaining in the aqueous solution after extraction is instantly discharged on the addition of ammonia and potassium cyanide solution, and under these conditions the copper does not react with sulphide.

Bismuth.—Traces of bismuth in acid solution yield a characteristic yellow colour with ammonium thiocyanate, provided a large excess of this reagent is present. Bismuth thiocyanate may be extracted from an aqueous solution by the ether and amyl alcohol solvent, but, unfortunately, with the present mode of procedure the percentage of bismuth extracted depends largely on the nature of the salt under examination. Conditions for the separation, applicable to all compounds containing traces of bismuth, are in course of investigation.

I wish to thank Dr. A. M. Ward for his advice and helpful criticism.

THE SIR JOHN CASS TECHNICAL INSTITUTE,
LONDON, E.C.

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 FLUORESCENCE ANALYSIS OF WAXES

FOR the examination of the fluorescence of waxes a K.B.B. quartz mercury vapour lamp and a Hanovia quartz mercury vapour lamp were used. The filters in both lamps had the same spectral characteristics, *viz.* all visible light was absorbed and a strong band of ultra-violet light between the limits of 3000 and 4000 Å with a maximum at 3660 Å was obtained.

English, foreign and white beeswax, Japan wax, Chinese wax, carnauba wax, commercial paraffin wax, yellow and white ceresin all gave characteristic fluorescences. Those waxes which were white in daylight showed a white fluorescence with blue or violet tones when examined under the lamp; the coloured waxes showed a fluorescence colour very similar to their colour in daylight.

With 50 per cent. mixtures of other waxes with English beeswax only, paraffin wax and carnauba wax were detectable under the lamp. Solutions in chloroform showed no fluorescence, except in the case of paraffin wax and carnauba wax; 10 per cent. of paraffin wax gave a milky yellowish-green fluorescing solution, and 10 per cent. of carnauba wax gave a solution with a strong bluish-white fluorescence. So strong is the fluorescence of carnauba wax that the addition of 1 per cent. to English beeswax is easily detectable in chloroform solution. By making standard samples containing known amounts of carnauba wax and dissolving 0·2 grm. of each in 100 c.c. of chloroform a good estimate of the amount of carnauba wax present in a suspected sample may be made.

J. A. RADLEY

THE ANALYTICAL CHARACTERISTICS OF COCONUT TODDY

In the manufacture of coconut toddy the sap is drawn from an unopened flower spathe of the coconut palm and collected in an earthenware vessel, which is placed over the end of the spathe. Tapping is usually carried out twice daily, and at each visit the chatty is emptied, a thin shaving is removed from the end of the spathe, and the earthenware vessel is replaced. The sap rapidly becomes infected with bacteria and yeasts, and the sugar present is soon converted into alcohol and acetic acid.

If it is desired to use the juice as a source of sugar instead of alcohol, a little milk of lime is placed in the earthenware vessel at the first tapping. This inhibits fermentation, and the liquid is evaporated to produce the brown sugar locally known as "*gula malacca.*" The non-sugar substances average about 12·5 per cent. of the total solids.

The following formula for calculating the original solids (mainly sugar) in the original sap from the percentages of alcohol and acetic acid in the toddy gives results very close to the truth:

Original solids = (alcohol by weight) × 2 + (acid as acetic acid) × 1·5 + extract.

For morning toddies (*i.e.* tapped at 6.30 a.m.) the average value for original solids (S) was 18·7 per cent., with a variation from 17·0 to 20·1 per cent.; and for afternoon toddies (tapped at 2.30 p.m.), the average value was 18·3 per cent.

It was found that rainfall lowers the strength of toddy. For example, 96 per cent. of contractor's toddies taken in dry weather had an average strength (S) of 17·1 per cent.; whilst of those taken during a rainfall of less than 0·5 inch, the average strength was 16·7 per cent.

It is considered that the acidity of toddy cannot safely be regarded as a criterion of its age. Acidities of over 0·7 per cent. have been found in toddies which were undoubtedly fresh, and, on the other hand, toddies 66 hours old have given acidities of less than 0·6 per cent. In any case it would be expected that acidity would depend to some extent on the strength of the toddy, and should, therefore, be calculated as a percentage of the total original solids.

It is suggested that a better index of the age of a toddy is to be found in the extent to which fermentation has proceeded. In a stale toddy up to about 85 per cent. of the original solids may have fermented. In fresh toddies the degree of fermentation should not average more than 60 to 70 per cent., and in no individual sample should it have proceeded beyond 75 per cent., that is to say, at least 25 per cent. of its original solids should be unchanged.

Recommendations.—The present standards for toddy agreed upon by chemists in Malaya are based upon analyses made by Marsden in Kuala Lumpur in 1926. These standards require a minimum of 14 per cent. of original total solids, and an acidity calculated as acetic acid of less than 0·6 per cent. The present investigation has been carried out chiefly in order to discover what quality of toddy might reasonably be expected to be supplied to Government by a contractor, but it will also serve to provide a standard for toddy sold in non-government (or licensed) shops. It is suggested, therefore, that toddy drawn in dry weather from a considerable number of trees should, over a period of some months, reach an average strength of 17 per cent. of original total solids, and in no individual sample should this figure fall below 16 per cent. On the other hand, it may rise as high as 20 per cent.

Rainfall may have a considerable effect on the strength of toddies, and it is believed that this is due to water mechanically entering the collecting vessels. Where the rainfall during the period of collection does not exceed 0·5 inch its diluting effects are slight, and should not amount to more than about 5 per cent.

In such weather the average strength of toddies should not be less than 16·5 per cent., and no individual sample should fall below 15·5 per cent.

It is recommended that, so far as is possible, the sampling of toddy should be undertaken immediately after delivery by the contractor, and if it cannot be analysed immediately, it should be preserved by the addition of copper sulphate solution. According to experiments made in the Trade and Customs Laboratory in Kuala Lumpur, 20 drops of a saturated solution of copper sulphate will keep 300 c.c. of toddy unchanged for six months.

If samples of toddy must be taken some hours after delivery by the contractor, the degree of fermentation should not exceed 80 per cent.

It is further recommended that the collecting vessels should be of earthenware, and sufficiently wide in the mouth to be readily cleaned. They should be brushed out with a stiff wire brush after each tapping, and 5 to 10 per cent. of them should be replaced daily by vessels previously cleaned by immersion in boiling water. All other vessels, such as those used for carrying and mixing, should be of glazed earthenware or enamelled metal. Gourds should not be used.

It is clear that, if the above recommendations are to be carried out, toddy estates must be under continual supervision.

J. C. COWAP
F. H. GEAKE

GOVERNMENT ANALYST'S DEPARTMENT,
STRAITS SETTLEMENTS

EXAMINATION OF UNREFINED SUGARS ON SALE IN VARIOUS HEALTH FOOD SHOPS

THE samples purchased were apparently raw sugars or sugars boiled from unrefined syrups. Five samples, the colours of which ranged from yellow to dark brown, were tested.

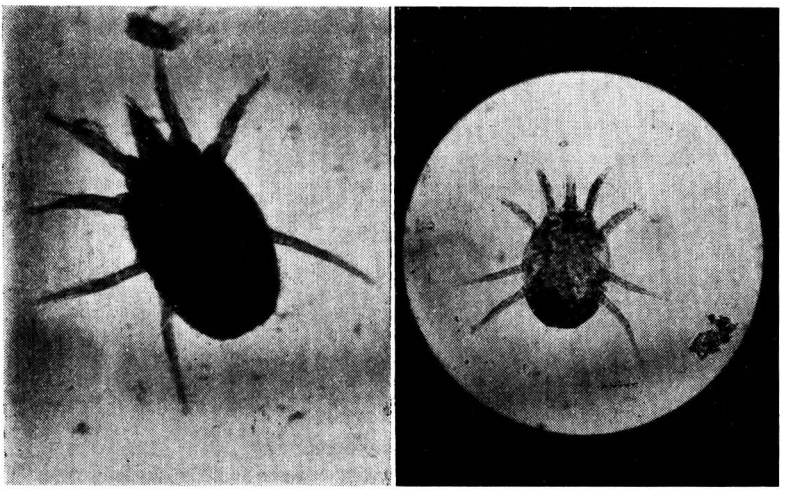
	1	2	3	4	5
Moisture, per cent.	3·83	1·51	3·86	3·86	3·53
Ash, per cent.	2·34	1·36	2·29	2·29	2·38
Insoluble in boiling water, per cent. ..	0·98	0·82	0·64	0·82	0·96
Ash of insoluble matter, per cent. ..	0·56	0·40	0·32	0·44	0·56
Arsenic, parts per million	free	free	0·1	0·1	free
Sulphur dioxide, per million	240	60	200	200	200
Bacterial counts per grm. on agar media	2140	680	1860	1930	not tested

As the analyses show, these are very dirty raw sugars containing unusually high proportions of insoluble matter. The bacterial counts are very high, as are also the sulphite-contents. For some of these samples the content of sulphite is in excess of that legally permissible.

Another sample of unrefined sugar, purchased at a different shop, was found to be swarming with an incredible number of sugar acari. When about 10 grms. were shaken with about 200 c.c. of water until dissolved, and then allowed to stand, a film of wriggling acari, visible to the naked eye, was left on the surface. One sample of sugar contained 40 per grm.

Apart from these impurities, the high moisture-content makes these sugars unsound and most unfit for feeding children, for which purpose they are apparently recommended. Even if they have been dried, the coating of molasses on the sugar crystals renders them hygroscopic, and, in consequence, water is absorbed from

the atmosphere as soon as an opportunity is found, and the packing material is unable to prevent it.



Viewed from above × 66. Underside × 28.

Sugar lice (*Carpoglyphus anonymus*) commonly but erroneously termed *Acarus sacchari*

H. C. Prinsen Geerligs in *Cane Sugar and its Manufacture* (p. 297–298) comments on this, and points out that the development of micro-organisms is thus promoted. Sugar is refined not solely for colour, as some would have us believe, but to make it a non-hygroscopic, sound food of exceptional chemical and bacteriological purity.

H. C. SIEGFRIED DE WHALLEY

Official Appointments

THE Minister of Health has approved the following appointments:

ADRIAN J. C. LICKORISH, F.I.C., as a Public Analyst for the County of Bedford, in addition to J. Kear Colwell (August 11th).

FREDERICK DIXON, B.Sc., F.I.C., as Public Analyst for the County of Stafford, in place of A. E. Johnson, and in addition to E. V. Jones (September 30th).

The Minister of Agriculture and Fisheries has approved the following appointments:

FREDERICK DIXON, B.Sc., F.I.C., as Deputy Agricultural Analyst for the County of Stafford, in place of A. E. Johnson (October 5th).

ERIC VOELCKER, F.I.C., as Deputy Agricultural Analyst for the County Borough of Oxford (October 5th).

Report of the Milk Products Sub-Committee to the Standing Committee on Uniformity of Analytical Methods

MILK PRODUCTS. REPORT No. 3

THE ANALYSIS OF SWEETENED CONDENSED MILK IN WHICH THE SUCROSE HAS ALTERED DURING STORAGE

THIS Sub-Committee was convened by the Standing Committee, and consists of the following members:—

Nominated by the Government Chemist: A. More, A.R.C.S., F.I.C.

Nominated by the London Chamber of Commerce: E. R. Bolton, F.I.C., M.I.Chem.E., A. L. Bacharach, B.A., F.I.C., *Ir.* W. J. Pelle, and J. Tavroges, B.Sc., A.I.C.

Nominated by the Manufacturing Confectioners' Alliance: T. Macara, F.I.C.

Nominated by the Society of Public Analysts and Other Analytical Chemists: G. D. Elsdon, B.Sc., F.I.C., E. Hinks, M.B.E., B.Sc., F.I.C. (*Chairman*), E. B. Hughes, M.Sc., F.I.C. (*Hon. Sec.*), A. E. Parkes, F.I.C., and J. D. Roberts, B.Sc.

In previous reports (*Milk Products: Report No.* 1, p. 2, ANALYST, 1927, **52**, 403; *Report No.* 2, "The Determination of Sucrose in Sweetened Condensed Milk," p. 10, ANALYST, 1930, **55**, 120) it was recommended that for the purpose of the Public Health (Condensed Milk) Regulations the percentage of total milk solids should be determined by subtraction of the percentage of sucrose from that of total solids.

Such a procedure is admissible only when no material alteration has taken place in the sucrose, and the recommendation in *Report No.* 2 contained the proviso "it being understood that the sample is a product prepared from milk and sucrose only, and that it is in sound normal condition." If any of the sucrose has been hydrolysed, or has undergone other change, total milk solids will be in error, any non-volatile derivative of sucrose being wrongly credited to milk solids.

The present Report (No. 3) deals with certain decomposition products of sucrose, their influence, if present, upon the determination of total milk solids as described in *Report No.* 2, and the means to be adopted in order to obtain the correct figure for total milk solids. As will appear later, the total milk solids figure is not the only one to be affected by breakdown of the sucrose: if decomposition has proceeded in a certain direction, the figure obtained for the remaining sucrose, as determined by the polarisation process, is also affected.

Work on the matter which is the subject of this Report was begun in March, 1930. The problem to be solved was found to require the devising of new processes rather than the examination of established processes and selection of the best.

The work carried out by the Committee can be divided into three stages:

(i) Investigation of methods of determining small amounts of invert sugar in sweetened condensed milk.

In the course of this work it was discovered that the change which occurs in the sucrose on keeping sweetened condensed milk does not usually result in the presence of invert sugar, as such, but that the laevulose fraction appears, in part, as laevan.

(ii) The second stage of the work involved the study of this change, and the elaboration of methods for determining the products of the change, so that the sucrose originally present could be calculated.

(iii) The third stage developed into a simplification of Stage (ii), *i.e.* the development of a direct method which admits of a determination of the amount of sucrose originally present, without carrying out the more lengthy procedure of Stage (ii), which, however, remains essential as affording an explanation of the change, and providing, if necessary, a confirmation of the result obtained by the simplified process.

(I) GENERAL

The problem was viewed at first as being one solely of the detection and determination of invert sugar, and processes were developed accordingly. During the course of this work, however, the observation was made that in a sweetened condensed milk, in which the sucrose had been altered by prolonged storage, the "apparent lactose," as determined by chloramine-T-iodide oxidation,[1] was higher than when determined by copper reduction; if invert sugar had been present the reverse should have been the case, since chloramine-T-iodide does not oxidise laevulose. The only explanation of this observation appeared to be that, assuming dextrose and laevulose to be present, the dextrose exceeded the laevulose in amount, but at the time there was no means of confirming this.

Later, on application of the Hinton and Macara process for the direct determination of laevulose[2] to a sweetened condensed milk, which had undergone extensive alteration on keeping, it was found that, although between 8 per cent. and 9 per cent. of sucrose had disappeared, only about 0·5 per cent. of laevulose was present, whilst chloramine-T-iodide oxidation again pointed to excess of dextrose over laevulose.

These perplexing results were eventually found to be due to the formation of laevan.

Nascent laevulose from sucrose is known to be condensed to laevan, a gum,[10] by the action of the levanase of certain micro-organisms, 9 parts by weight of laevan being formed from 10 parts of laevulose. From a sweetened condensed milk which, after storage, exhibited this excess of dextrose over laevulose, a laevan-forming organism was isolated and grown in pure culture. A preparation of laevan was made from a sucrose-peptone solution in which this organism was allowed to grow. This laevan was precipitated by 86 per cent. alcohol, washed with alcohol and ether, and dried. It was free from dextrose and laevulose, and its $[\alpha]_D^{20}$ was found to be $-50°$, calculated on the dry ash-free material.

Laevan does not reduce cupric reagents and is less laevo-rotatory than laevulose. On treatment with acids (as, for instance, in the inversion process for the determination of sucrose), laevan is hydrolysed quantitatively to laevulose.

Formation of laevan thus introduces considerable complication into the analysis of a sweetened condensed milk. In the first place, the copper-reducing power of the serum is diminished proportionately to the amount of laevan formed; secondly, the rotatory power of the serum is affected; and, thirdly, when the serum is inverted, for the determination of sucrose, the laevulose is re-constituted from the laevan and exhibits the same rotatory power and the same copper-reducing power as it would have done if no condensation to laevan had taken place.

The optical rotation and the copper-reducing power of the "inverted" serum are, therefore, the same whether invert sugar has, or has not, been formed from the sucrose, and whether laevan has, or has not, been formed either directly from the sucrose or from the laevulose fraction of invert sugar; they are the same as would have been shown by the inverted serum of the freshly prepared milk before any alteration in the sugars had taken place, provided that formation of invert sugar,

or of laevan, laevulose and dextrose accounts for the whole of the altered sucrose. The optical rotation and copper-reducing power of the "uninverted" serum are, on the contrary, affected according to the extent of laevan formation. Thus, when laevan is formed, not only is the determination of altered sucrose affected, but the method for the determination of the remaining sucrose recommended by the Committee (*Report No.* 2) is invalidated, as would be any method depending upon acid inversion, whether by polarisation or by copper reduction.

It is clear, therefore, that it is of prime importance to ascertain, in any given case, first, whether any alteration of the sucrose has taken place; and secondly, whether the altered sucrose appears in the form of invert sugar or in that of a mixture of dextrose, laevulose and laevan.

From the examination of a number of old sweetened condensed milks (which had been kept in their original sealed containers) it would appear that formation of dextrose and laevulose, with condensation to laevan of a considerable proportion of the laevulose, is the usual direction in which sucrose is altered in those cases where any alteration at all has taken place. The analogous formation of dextran, by condensation of dextrose, has not been observed, though such a possibility should be borne in mind. It is important to note that no evidence was found of any alteration in the lactose, even in an extreme case in which nearly 9 per cent. of sucrose, as such, had disappeared, with the formation of much laevan.

The possible presence of invert sugar, of dextrose and of laevan, must, then, be taken into account in any critical analysis of sweetened condensed milk.

In considering a scheme for such an analysis, it is convenient to approach the more complicated issue of laevan through the simpler one of invert sugar.

In a mixture of lactose, sucrose and invert sugar, all these should be determinable by a combination of polarimetric and suitable copper-reduction methods. The heating of milk, however, has a considerable effect upon the specific rotation, and some effect on the copper-reducing power, of lactose. Though the temperature to which the milk is exposed during the manufacture of *sweetened* condensed milk does not appear to be high enough to have any significant effect, the uncertainty attaching to this renders the determination of doubtful accuracy for small amounts of invert sugar.

(II) COLORIMETRIC DETERMINATION OF INVERT SUGAR

Some success was obtained with a direct qualitative and quantitative colorimetric process based upon the destruction of lactose by the method of Kruisheer[3] and the colorimetric method of Kolthoff.[4] It was found that by chloramine-T-iodide oxidation of the serum (prepared by phosphotungstic acid clarification) lactose and dextrose could be eliminated and the laevulose determined colorimetrically by an alkaline 3-5-dinitro-salicylic acid reagent. This method was abandoned in favour of more satisfactory methods devised later.

(III) THE BARFOED REAGENT

Monier-Williams,[5] in a Report published by the Ministry of Health, has worked out an application of Barfoed's solution (copper acetate and acetic acid) to the detection and determination of invert sugar in sweetened condensed milk. This method has been fully studied by the Committee, and in a modified form has supplied the basis of one of the processes recommended in this Report.

(IV) MODIFICATION OF THE BARFOED REAGENT AND PROCESS

When a mixture of Barfoed's solution and serum prepared from sweetened condensed milk is heated, reduction readily takes place if invert sugar is present. Even in the absence of invert sugar, however, a noticeable amount of reduction takes place, increasing steadily with the time of heating. Monier-Williams

attributes this to hydrolysis of the sucrose by the acetic acid, but the Committee finds that the lactose in the serum has a considerable reducing action upon Barfoed's solution.

In the method described in the Ministry's Report[5] a fresh milk serum, containing sucrose, is used as a control, the reaction in the two serums—the one under examination and the control—being stopped when the control begins visibly to reduce the copper. The lactose effect is, however, sufficiently powerful to necessitate equal concentrations of lactose in the serum under examination and in the control serum. Variation in the proportion of lactose in condensed milk, and in fresh milk, may be so large as to render any one fresh milk uncertain as a control for a given condensed milk.

Attempts were made to destroy the invert sugar in one portion of the condensed milk serum by preliminary heat treatment with the Barfoed reagent, and to use the resulting serum (after filtration) as the control, thus ensuring an approximately equal concentration of lactose in the control. This procedure did not give satisfactory results, as the reducing power of the serum was greater after the preliminary treatment than before.

In order to overcome these defects in the Barfoed method under discussion, experiments were made with the object of minimising the reducing action of lactose, and so avoiding the necessity for the fresh milk control.

It was found that by modifying the composition of the copper acetate solution, by reducing the temperature, and by limiting and fixing the time for the reduction, the reducing action of lactose, and of a milk serum free from invert sugar, could be rendered negligible, while at the same time the reducing action of invert sugar could be maintained.

First, the addition of sodium acetate to the Barfoed reagent effects an improvement and causes the reduction to become more nearly a linear function of the amount of invert sugar present. This effect is not due to buffering of the reagent by the sodium acetate, for determination of its neutralisation curve shows that true buffering does not occur.

Secondly, the rate at which sugars reduce cupric solutions depends, for a given type of reagent, upon the acidity or alkalinity of the solution and also upon temperature. Application of these principles enabled further improvement in the selective action of a Barfoed reagent to be effected.

Moreover, laevulose and dextrose are not equally reactive to Barfoed's solutions, the reduction by laevulose being the greater. By varying the proportion of acetic acid (in the presence of sodium acetate) the relative reducing powers of these two sugars may be altered. Diminishing the acetic acid causes their reducing powers to approach equality, but when this point is reached the lactose effect becomes unduly high. A compromise has, therefore, to be adopted.

The reduced copper is best determined by the Bertrand procedure (solution in acid ferric sulphate solution and titration of the reduced iron with standard permanganate solution), and is conveniently expressed in terms of $N/10$ potassium permanganate solution.

Table I shows the effect of varying the proportions of sodium acetate and acetic acid. All the reagents contained about 50 grms. of crystallised copper acetate per litre, the crystallised sodium acetate and acetic acid being varied as shown in this table. 70 ml. of the reagent and 25 ml. of a sweetened fresh milk serum,* alone and also with the addition of 1 ml. of a 2·5 per cent. solution of dextrose or laevulose, were immersed in a bath at 80° C. for 20 minutes.

* In these and subsequent experiments a sweetened fresh milk serum was prepared to simulate the serum of sweetened condensed milk by zinc acetate and potassium ferrocyanide clarification, as described in Appendix I, 100 grms. of fresh milk and 16 grms. of sucrose being used instead of the 40 grms. of sweetened condensed milk.

TABLE I

Reagent	Sodium acetate Grms. per litre	Acetic acid Ml. per litre	Serum alone	Serum *plus* 25 mgrms. of dextrose	Serum *plus* 25 mgrms. of laevulose
			N/10 potassium permanganate solution		
			Ml.	Ml.	Ml.
1.	40	2	3·1	10·2	11·3
2.	40	4	1·0	10·3	11·35
3.	40	8	0·2	2·8	7·2
4.	80	4	0·75	10·15	11·25
5.	80	6	0·2	8·8	10·65
6.	80	8	0·1	6·6	9·3
7.	80	12	0·15	1·15	6·9
8.	60	6	0·15	8·7	10·7
9.	60	8	0·1	7·3	10·0
10.	60	10	0·15	5·25	8·75

Reagent 8 was adopted as being the most suitable; at 80° C. the lactose effect is negligible, whilst the dextrose and laevulose effects are maintained at a high level. This reagent is hereinafter referred to as *Modified Barfoed reagent.*

The reaction, at the point at which the reducing action of lactose is negligible but at which laevulose and dextrose react sufficiently rapidly, is very sensitive to temperature, and, to a less extent, to time. The effect of temperature on the extent of the reduction by sweetened fresh milk serum is shown in Table II.

TABLE II

70 ml. Modified Barfoed solution *plus* 25 ml. of sweetened fresh milk serum. Time of heating: 20 minutes.

Temperature ° C.	*N*/10 potassium permanganate solution Ml.
78	0·1
80	0·2
82	0·8

The following curves show the reducing actions of laevulose and dextrose on the Modified Barfoed reagent at various concentrations and at three temperatures. The monose sugar was dissolved in 25 ml. of sweetened fresh milk serum.

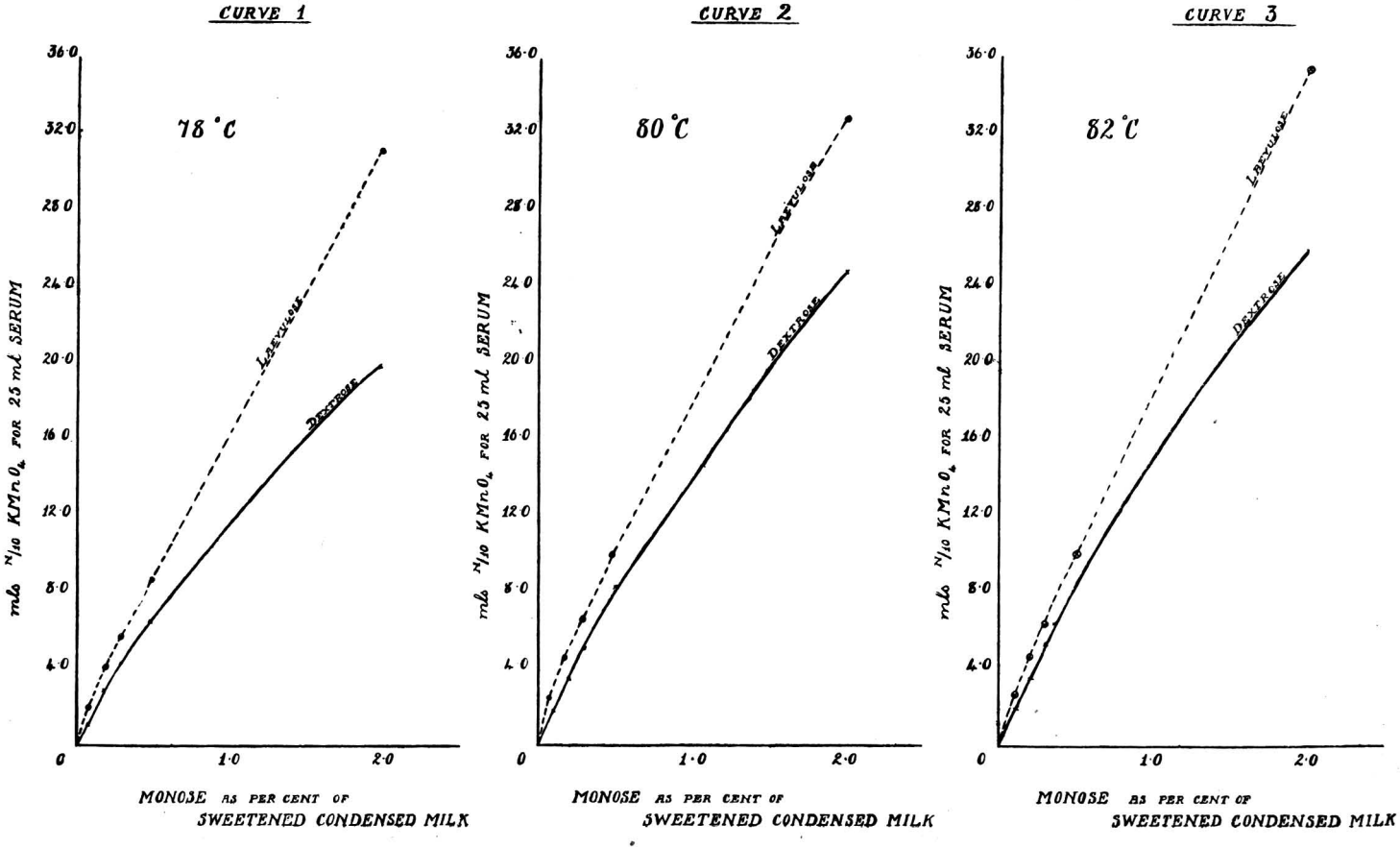
In Table III are given the results obtained with the Modified Barfoed reagent for laevulose, dextrose and invert sugar, the sugars being expressed as percentages of a sweetened condensed milk of ordinary composition; for the experiment these reducing sugars actually were added to 25 ml. of a sweetened fresh milk serum.

TABLE III

Temperature 80° C.; time of heating: 20 minutes.

Monose sugar as per cent. of condensed milk Per Cent.	*N*/10 potassium permanganate solution for Laevulose Ml.	Dextrose Ml.	Invert sugar Ml.
Nil	0·15 (blank)	0·15 (blank)	0·15 (blank)
0·1	3·50	2·7	2·8
0·2	5·50	4·75	4·25
0·3	7·35	6·0	6·15
0·5	11·55	8·75	9·85
1·0	19·50	15·45	18·0
2·0	34·05	25·35	29·45

MODIFIED BARFOED REAGENT



WHICH THE SUCROSE HAS ALTERED DURING STORAGE

The most favourable conditions are:—

(i) Heating in a large water-bath maintained at 80° C.
(ii) Allowing the reaction to proceed for 20 minutes.

Under these conditions a sweetened fresh milk serum has a negligible reducing action. Owing, however, to the sensitiveness of the reaction to temperature, and also to a slight difference in reactivity of different preparations of the reagent, precise quantitative relations cannot be assumed from the above tables and curves. For accurate work it is necessary to standardise the reagent by using control serums containing approximately the same proportions of sugars as the serum under examination, the controls being heated in the same bath as the serum being tested.

By the use of this Modified Barfoed solution as little as 0·1 per cent. of invert sugar, of dextrose or of laevulose in a condensed milk may readily be detected. Obviously the process will not indicate which of these sugars is present, and a knowledge of the amount of either the laevulose or dextrose is necessary in order to interpret, quantitatively, the reduction obtained.

The process has been of great service in establishing the nature of the changes occurring in the sucrose of sweetened condensed milk (as will appear later), and it takes its place in the recommendations of the Committee in this Report, as providing a means of detecting whether or not any significant change has taken place in the sucrose.

(V) INVERT SUGAR BY DIRECT DETERMINATION OF LAEVULOSE

Hinton and Macara[2] have developed a process for the determination of laevulose in sweetened condensed milk. It was intended to make this process the basis for the determination of invert sugar, and, if invert sugar were the only hydrolysis product of sucrose present, the process would be perfectly valid.

The application of this and of the chloramine-T and the Modified Barfoed processes to samples of altered sweetened condensed milk, however, demonstrated that the products of the altered sucrose were not dextrose and laevulose in equal proportions, the dextrose being invariably in excess, and it is, therefore, inadmissible to infer the amount of dextrose from a determination of the laevulose. Further investigation, by means of the polarimetric readings combined with chloramine-T titrations on the serum before and after inversion, indicated that some substance was present which was neither dextrose nor laevulose, but which was converted into one of these during the inversion process. This substance, as stated earlier, proved to be laevan. Consequently, neither the Modified Barfoed nor the Hinton and Macara process can be used by itself to determine the amount of altered sucrose. Nevertheless, the determination of laevulose by the latter process is necessary in the analysis of these altered samples when their detailed composition is required. This process has, therefore, been closely studied by the Committee.

In the following table are given the results obtained in a series of analyses by this process by members of the Committee (A–H), using sweetened condensed milk from various sources, with the addition of known quantities of invert sugar.

TABLE IV

Laevulose (added as invert sugar) as per cent. of condensed milk	Laevulose found Per Cent.							
	A	B	C	D	E	F	G	H
0·05	0·06	0·06	0·06	—	0·06	0·06	0·03	0·06
0·1	0·10	0·10	0·10	0·10	0·11	0·11	0·11	0·11
0·25	0·26	0·27	0·26	0·26	0·26	0·25	0·26	0·26
0·5	0·52	0·55	0·50	0·52	0·52	0·54	0·51	0·53
0·6	—	—	—	—	0·60	—	—	—
1·0	1·00	1·00	0·99	1·10	1·02	1·06	0·91	1·03

This process has been much used by the Committee during their investigations, and is recommended as quite satisfactory for the determination of laevulose. Full details of the process are given in Appendix III.

(VI) SUGARS AND LAEVAN BY THE MODIFIED BARFOED REAGENT

As it appeared that, in the altered sweetened condensed milks examined by the Committee, a portion of the sucrose had been changed to dextrose, laevulose and laevan, it was, at first, thought to be necessary, in order to ascertain the proportion of total milk solids, to determine the various sugars and laevan. This was first attempted by use of the Modified Barfoed reagent, in combination with the direct determination of laevulose and the determination of the specific rotation before and after inversion. The application of this method is best explained by means of an example.

The amount of laevulose in the condensed milk serum (20 per cent.) was determined by the Hinton and Macara process, and the serum was tested against a standard prepared by adding dextrose and laevulose to sweetened fresh milk serum. The amount of laevulose so added was arranged to be the same as that present in the condensed milk serum, and the dextrose added was in the neighbourhood of the amount expected in the unknown.

The mixtures tested by the Modified Barfoed procedure were:—

(1) 4 ml. condensed milk serum (this contained 0·0120 grm. laevulose).

(2) 4 ml. sweetened fresh milk serum containing 0·0120 grm. laevulose and 0·0380 grm. dextrose.

(3) 4 ml. sweetened fresh milk serum.

The titrations of the reduced copper were:—

(1) 16·95 ml.; (2) 16·75 ml.; (3) 0·3 ml. of $N/10$ permanganate solution.

Hence the net amounts due to laevulose and dextrose were:—(1) 16·65 ml.; (2) 16·45 ml.

The laevulose in both of these solutions was the same in amount; hence, since the titrations were so close, the relative proportions of the dextrose and laevulose must have been very similar in both cases, and it is sufficiently accurate to assume that the *total* amounts of the two sugars were directly proportional to the respective titrations. [Had the titrations lain further apart, then for strict accuracy it would have been necessary to repeat the test with a more suitable amount of added dextrose in (2).]

The total dextrose and laevulose in (2) being 0·0500 grm., the total in (1) is therefore

$$0{\cdot}0500 \times \frac{16{\cdot}65}{16{\cdot}45} = 0{\cdot}0505 \text{ grm.}$$

But of this, 0·0120 grm. is laevulose; therefore the amount of dextrose is 0·0385 grm., and the percentage of dextrose in the sample is

$$0{\cdot}0385 \times \frac{100}{4} \times \frac{100}{20} \times 0{\cdot}9714^{*} = 4{\cdot}67 \text{ per cent.}$$

The laevulose is 1·45 per cent. (direct determination). Hence, the laevulose, converted into laevan, is 4·67 − 1·45 = 3·22 per cent., and the laevan so formed is 3·22 × 0·9 = 2·90 per cent.

* Volume of precipitate correction; the specific rotations given in this example are corrected for volume of precipitate.

The amount of sucrose present as such can obviously be determined if we subtract from the direct polarisation reading the rotations due to the laevan, laevulose and dextrose, and, from the invert polarisation reading, that due to the inversion products derived from these substances.

The values taken in this example for the specific rotations of dextrose, laevulose and laevan are corrected, as far as is possible from available data, for concentration, effect of acid and salts, etc.

The part of the direct $[\alpha]_D^{20}$ due to dextrose	$=+52{\cdot}7\times\frac{4{\cdot}67}{100}$	$=+2{\cdot}46°$
„ „ „ „ „ „ „ „ laevulose	$=-93{\cdot}0\times\frac{1{\cdot}45}{100}$	$=-1{\cdot}35°$
„ „ „ „ „ „ „ „ laevan	$=-50\ \times\frac{2{\cdot}90}{100}$	$=-1{\cdot}45°$
	Net effect	$-0{\cdot}34°$

The actual direct $[\alpha]_D^{20}$ was + 29·75°, and the direct $[\alpha]_D^{20}$ thus amended is, therefore, +30·09°.

In the inverted solution, the part of the $[\alpha]_D^{20}$ due to these three constituents is the rotation of an amount of invert sugar equal to twice the amount of the dextrose, that is,

$$-20{\cdot}85\times\frac{9{\cdot}34}{100}=-1{\cdot}95°$$

The actual invert $[\alpha]_D^{20}$ was −1·59°, and the amended invert $[\alpha]_D^{20}$ is, therefore, +0·36°.

The sucrose present is then given by the change of $[\alpha]_D^{20}$ on inversion, divided by the inversion divisor factor 0·8825; thus sucrose

$$=\frac{30{\cdot}09-0{\cdot}36}{0{\cdot}8825}=33{\cdot}68 \text{ per cent.}$$

The analysis thus gives:—

Sucrose	..	33·68 per cent.
Laevulose	..	1·45 „ „
Dextrose	..	4·67 „ „
Laevan	..	2·90 „ „

(VII) SUGARS AND LAEVAN BY CHLORAMINE-T TITRATION

On inversion of the condensed milk serum, laevan is hydrolysed to laevulose, whilst dextrose and laevulose are unaffected. By determination of the specific rotation and of the chloramine-T titration, both before and after inversion, combined with a direct determination of the laevulose, the amounts of all sugars and laevan can be calculated. This method, again, is best explained by an illustrative example.

The particular milk, the analysis of which is here recorded, was at least two years old. The analysis was conducted as follows:—

Polarisation by the Committee's *Report No.* 2 method, with zinc clarification.

$[\alpha]_D^{20}$ *Direct* + 29·75° (corrected for volume of precipitate)
Invert − 1·59° („ „ „ „ „)

Laevulose by direct determination.

1·45 per cent. (corrected for volume of precipitate).

Chloramine-T titrations. A 20 per cent. serum, prepared by phosphotungstic acid clarification, was diluted to 4 per cent.

Direct. Twenty ml. of this 4 per cent. serum taken for titration. Chloramine consumed (as $N/20$ thiosulphate) **23·67 ml.**

Invert. Forty ml. of 4 per cent. serum inverted, neutralised and made up to 100 ml.; 20 ml. of this taken for titration. Chloramine consumed (as $N/20$ thiosulphate) **22·25 ml.**

CALCULATION OF TRUE LACTOSE AND ORIGINAL SUCROSE.*

From the "invert" titration:

The correction for the effect of inversion, etc., on lactose and other milk constituents (see Section VIII) = 0·15 ml. in this case.

Hence the iodine equivalent to the lactose *plus* inverted sucrose, is

$$(22{\cdot}25 - 0{\cdot}15) \times 0{\cdot}006346 = 0{\cdot}14025 \text{ grm.},$$

or, expressed as per cent. of the sample, 42·89 per cent. (corrected for volume of precipitate).

The iodine equivalents of lactose hydrate and invert sugar under the conditions of this titration (see Tables XIII and X of the original paper[1]) are 0·701 and 0·705, respectively.

If L and S be the percentages of lactose hydrate and of sucrose in the milk before any alteration, then for the fully inverted solution:

$$0{\cdot}701\,\text{L} + (0{\cdot}705 \times 1{\cdot}053\,\text{S}) = 42{\cdot}89$$

Also, from the invert polarisation:

$$0{\cdot}524\,\text{L} - 0{\cdot}2195\,\text{S} = -1{\cdot}59,\dagger$$

and from these two equations

$$\text{S} = 43{\cdot}47, \text{ and } \text{L} = 15{\cdot}15.$$

Sucrose present at the time of analysis.—The "invert" chloramine titration, corrected as above mentioned, is 22·10 ml. Then the sucrose present is calculated from the difference in thiosulphate titrations before and after inversion, thus

$$\frac{\left(22{\cdot}10 \times \frac{100}{40} - 23{\cdot}67\right) \times 0{\cdot}006346}{0{\cdot}741} \times \frac{100}{0{\cdot}8} \times 0{\cdot}9786 = 33{\cdot}09$$

(0·9786 being the volume of precipitate correction, $\frac{100}{0{\cdot}8}$ the factor converting 20 ml. of the solution titrated into 100 grm. of sample, and 0·741 the sucrose factor taken from Table XV[1]).

Note.—The dilutions and amounts taken for the chloramine titrations are not identical with those used for unchanged condensed milk as in the published process[1]; they had to be arranged so as to give suitable titrations in view of the extra reducing sugar present. It is desirable to have the "direct" not very different from the "invert" titration. It sometimes happens, as in this case, that the titrations fall somewhat outside the range of the tables of factors given in the published paper; slight extrapolation is then required, and is permissible.

* The term "original sucrose" means the sucrose in the condensed milk before any alteration had taken place.

† See Section (VIII) formula (2).

Apparent Lactose.—From the "direct" titration a small correction has to be deducted for the slight absorption of iodine by the sucrose (see Table XIV of the original paper). In the present case this is 0·09 ml., and the corrected titration difference is thus 23·58 ml.

Then the "apparent lactose" is

$$\frac{23{\cdot}58 \times 0{\cdot}006346}{0{\cdot}700} \times \frac{100}{0{\cdot}8} \times 0{\cdot}9786 = 26{\cdot}15 \text{ per cent.}$$

Dextrose.—The difference between the true and apparent lactose (26·15—15·15 =11·00) represents the dextrose, expressed as lactose hydrate. Since dextrose has just twice the iodine-reducing power of lactose hydrate, the actual percentage of dextrose is, therefore, 5·50.

Laevan.—The difference between the dextrose and laevulose gives the amount of the latter which has been condensed to laevan, which is, therefore,

$$0{\cdot}9 \times (5{\cdot}50 - 1{\cdot}45) = 3{\cdot}65 \text{ per cent.}$$

The full analysis thus gives:

	Per Cent.
Sucrose	33·09
Lactose (anhydrous) ..	14·39
Laevulose	1·45
Dextrose	5·50
Laevan	3·65

It will be observed that this scheme of analysis gives the lactose, and is thus a more complete scheme for the determination of all sugars present than that described under Section VI, though the latter has the advantage of providing a direct demonstration of the presence of reducing sugars other than lactose.

As the result of various analyses carried out as above, it became clear that by polarimetric and chloramine-T determinations, using inverted serum, it was possible to arrive at the amount of sucrose originally present in the milk. This being so, a detailed determination of the various sugars and laevan is not necessary for the purpose of determining the percentage of milk solids. A simplified method of determining the original sucrose is given in the following Section (VIII), but where a determination of the various sugars and laevan is desired, the most suitable scheme available is that described in this section (VII).

(VIII) DETERMINATION OF ORIGINAL SUCROSE* BY POLARIMETER AND CHLORAMINE-T-IODIDE OXIDATION

When sucrose, which has become partly altered to dextrose, laevulose and laevan (or to invert sugar), is inverted, the product is invert sugar only, and in the same quantity as would have been given by the original sucrose, had none undergone alteration. Consequently, after inversion of the sucrose of a sample of sweetened condensed milk the sugars in solution are lactose and invert sugar; and these two may obviously be determined by calculation from the results of two independent quantitative measurements of these two sugars, *e.g.* by polarisation and measurement of reducing power towards copper or iodine (using chloramine-T). Examples of analyses by polarisation and copper-reducing power of the inverted serum are given later (see Table VII). The Committee found, however, the most suitable and reliable reduction method to be the chloramine-T method.

This method was studied by the Committee during the preparation of *Report No.* 2, and was then found to give slightly higher results for sucrose than those obtained by the polarimetric method recommended in that report, the accuracy of

* See footnote, Section VII, page 639.

which, in the absence of hydrolysis products, was fully established. It has since been found that this error is due to a slight absorption of iodine by non-sugar constituents of the *inverted* milk serum, as shown by experiments carried out on the following lines:—

Sweetened fresh milk serum* containing a known amount of sucrose and, also, sucrose mixed with clarified fresh milk, were subjected to the chloramine-T process before and after inversion. In the absence of any effect produced by the milk serum constituents, the amount of reagent absorbed in the inverted solution should be the sum of the amount used in the uninverted solution, due to lactose, *plus* the amount, calculated from the tables in the paper[1], due to the inverted sucrose. This, however, was found not to be the case, the results obtained from the inverted solutions being invariably higher by a small amount. The discrepancy was found to vary directly with the proportion of milk solids in the serums, the absorption of iodine by the non-sugar, non-precipitated milk constituents being found to be equivalent to 2·2 ml. $N/20$ thiosulphate solution per 1 grm. of fat-free milk solids.

Hence, it is possible to apply a correction to the titration results on the inverted serum to compensate for this effect. In the analysis of a condensed milk by the chloramine-T method, the amount of fat-free milk solids represented by the quantity of serum taken in the test is approximately 0·07 grm., and the correction to be applied to the titration is, therefore, 0·15 ml. $N/20$ thiosulphate solution. If other dilutions are taken, or abnormal samples (as regards fat-free solids) are in question, this figure can be adjusted to accord with the amount of fat-free solids corresponding to the volume of serum taken.

When this correction is applied, the chloramine-T method gives the true sucrose. In the following table is given the analysis of a fresh sweetened condensed skim milk:

TABLE V

	Per Cent.
Sucrose by chloramine-T process	43·40
Lactose (anhydrous)	14·88
Dextrose	0·01
Laevulose	0·02

Direct $[\alpha]_D^{20}$ calculated + 36·96°
Do. $[\alpha]_D^{20}$ found + 37·05°.

Sucrose by Committee's *Report No.* 2 method, 43·40.

Directions for the process are given in Appendix IV. The derivation of formulae there employed is as follows:—The serum prepared by zinc acetate and potassium ferrocyanide clarification is a 20 per cent. solution of the condensed milk; this becomes a 16 per cent. solution after inversion. A suitable dilution of this serum is 20 ml. made up to 250 ml., of which 25 ml. is taken for chloramine-T titration. The phosphotungstic clarification is, however, to be preferred for the chloramine-T process, and for this a 5 per cent. solution of condensed milk is clarified; 25 ml. of this are inverted and made up to 100 ml. Twenty-five ml. of this dilution are taken for the chloramine-T titration (in both cases the dilutions must be neutralised as described in Appendix IV).

Let R = $[\alpha]_D^{20}$ of the inverted solution (zinc clarification);
E = grms. of iodine consumed by 100 grms. of sample after inversion;
n = titration-difference in ml. $N/20$ thiosulphate.
V = volume in ml. to which the sample is diluted before filtration;
v = correction in ml. for volume of precipitate produced during clarification.

* See footnote, page 633.

(The titration-difference (n) has to be corrected for absorption by non-sugars; this correction, as shown above, is usually −0·15 ml.)

$$\text{Then E (zinc serum)} = (n - 0{\cdot}15) \times 0{\cdot}006346 \times \frac{250}{25} \times \frac{100}{20} \times \frac{100}{16} \times \frac{V-v}{V}$$

$$= (n - 0{\cdot}15) \times \frac{63{\cdot}46}{32} \times \frac{V-v}{V}$$

and E (phosphotungstic acid serum)

$$= (n - 0{\cdot}15) \times 0{\cdot}006346 \times \frac{100}{25} \times \frac{100}{25} \times \frac{100}{5} \times \frac{V-v}{V}$$

$$= (n - 0{\cdot}15) \times 63{\cdot}46 \times 0{\cdot}032 \times \frac{V-v}{V}$$

Now, with the dilutions suggested, the factors for lactose hydrate and invert sugar (1·053 × sucrose), in grms. of iodine per 1 grm. of sugar, are 0·702 and 0·706, respectively.

Hence, if L and S be the percentages of lactose hydrate and of original sucrose, respectively, we have

$$0{\cdot}702\,L + 0{\cdot}706 \times 1{\cdot}053\,S = E. \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

The specific rotations $[\alpha]_D^{20}$ in the inverted zinc serum of lactose hydrate and of inverted sucrose are 52·4° and −21·95°, respectively. Hence

$$0{\cdot}524\,L - 0{\cdot}2195\,S = R. \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

From equations (1) and (2)

$$S = 0{\cdot}964\,E - 1{\cdot}29\,R \text{ and } L = 0{\cdot}404\,E + 1{\cdot}37\,R$$

Thus from the two operations of determining the specific rotation and the chloramine-T titration of the inverted solution the percentage of original sucrose is obtained.

Chloramine-T titrations obtained on the zinc clarified serum tend to be a little low. This appears to be connected with the precise conditions of alkalinity. As this difficulty does not occur with the phosphotungstic clarified serum, the latter is advised, though the zinc clarified serum can be used as a preliminary if desired.

Tests of the Process on Old Condensed Milks.—*Report No.* 2[6] (Tables II and III) contains the results of analyses by members of the Committee of samples of specially prepared condensed milk. These analyses were made in November and December, 1929. One tin of each of these batches was reserved, and in March, 1932, was analysed by the above method, with the following results:

A. *Table II Milk.*

Appearance on opening: Normal.

Polarisation. Direct $[\alpha]_D^{20}$ on sample + 34·28°.
Invert $[\alpha]_D^{20}$,, ,, − 3·15°.

Original sucrose from invert polarisation and chloramine-T (both on zinc serum)	42·39 per cent.
Average figure as published in *Report No.* 2 for sucrose	42·54 per cent.

Note.—Very little alteration of the sucrose had taken place in this milk.

B. *Table III Milk.*

Appearance on opening: Showed brownish discoloration and thickening.

Polarisation. Direct $[\alpha]_D^{20}$ on sample + 33·93°.	
Invert $[\alpha]_D^{20}$,, ,, — 2·32°.	
Original sucrose from invert polarisation (zinc clarification) and chloramine-T (phosphotungstic clarification)	44·56 per cent.
Original sucrose from invert polarisation and chloramine-T (both zinc clarification)	44·16 per cent.
Average figure as published in *Report No.* 2 for sucrose	44·52 per cent.

Note.—The polarisation in March, 1932, points to an alteration of about 4 per cent. of sucrose.

Six tins of another preparation which had been kept for a considerable time and which, on opening, exhibited varying signs of alteration, the milk being discoloured and thickened, were mixed and distributed to members of the Committee. The samples were analysed in the various laboratories by this method, with the following results:

TABLE VI

Laboratory	Original sucrose Per Cent.
A	42·80
B	42·94
C	42·68
D	42·90
E	42·63
F	42·99
G	42·73
Mean	42·81
Maximum deviation from the mean	0·18
Mean deviation from the mean	0·11

As mentioned at the beginning of this section, original sucrose can be determined by combination of polarimetric measurement of the inverted solution and the reducing action of the inverted solution towards copper solution or chloramine-T. The chloramine-T method is the one recommended, but by the use of copper solution satisfactory results have been obtained. The Lane and Eynon method[7] was used for determining the copper-reducing power, solutions containing lactose and invert sugar in approximately the proportions which would be obtained from a normal condensed milk in similar dilution being used as a control, thus giving a comparison for reference to Lane and Eynon's tables. It is not necessary to describe the method in detail, but the results obtained by it are included in Table VII with those obtained by the chloramine-T and Modified Barfoed methods. The analyses recorded in this table are those of old sweetened condensed milks, more or less altered during storage, and they afford confirmation of the view that dextrose, laevulose and laevan formation is the usual, and in the experience of the Committee the only, change which takes place in the sucrose. In these analyses the laevulose was determined in all cases by the Hinton and Macara process, and in the analyses with Modified Barfoed reagent the lactose figure is taken from the chloramine-T analyses.

TABLE VII

Method	Milk A		Milk B	
	Chloramine-T and polarisation after inversion Per Cent.	Modified Barfoed and polarisations before and after inversion Per Cent.	Chloramine-T and polarisation after inversion Per Cent.	Modified Barfoed and polarisations before and after inversion Per Cent.
Sucrose	33·09	33·68	30·83	29·41
Lactose (anhydrous)	14·39	14·39	14·94	14·94
Dextrose	5·50	4·67	7·15	7·21
Laevulose	1·45	1·45	1·72	1·72
Laevan	3·65	2·90	4·89	4·94
Direct				
$[\alpha]_D^{20}$ calculated ..	+29·60°	+29·93°	+28·38°	+27·44°
found ..	+29·75°	+29·75°	+27·21°	+27·21°
Original sucrose calculated from the above figures	43·54	42·55	44·42	43·11

	Milk C		Milk D	
Sucrose	33·29	33·25	35·40	35·95
Lactose (anhydrous)	11·21	11·21	11·44	11·44
Dextrose	4·33	4·03	2·75	2·09
Laevulose	1·66	1·66	1·86	1·86
Laevan	2·40	2·13	0·80	0·21
Direct				
$[\alpha]_D^{20}$ calculated ..	+27·79°	+27·74°	+29·11°	+29·41°
found ..	+27·63°	+27·63°	+29·26°	+29·26°
Original sucrose calculated from the above figures	41·51	40·91	40·63	39·92

	Milk E	
	Chloramine-T and polarisation after inversion Per Cent.	Copper reduction (Lane and Eynon) and polarisation after inversion Per Cent.
Sucrose	31·80	32·08
Lactose (anhydrous)	11·92	11·99
Dextrose	4·68	4·51
Laevulose	0·57	0·57
Laevan	3·70	3·55
Direct $[\alpha]_D^{20}$ calculated	+27·75°	+27·95°
found	+27·71°	+27·71°
Original sucrose calculated from the above figures	40·69	40·65

Method	Milk F: Chloramine-T and polarisation after inversion Per Cent.	Milk F: Lane and Eynon and polarisation after inversion Per Cent.	Milk F: Modified Barfoed and polarisation before and after inversion Per Cent.
Sucrose	32·26	33·04	32·65
Lactose (anhydrous) ..	14·46	14·40	14·46
Dextrose	6·15	5·60	5·60
Laevulose	1·67	1·67	1·67
Laevan	4·03	3·54	3·54
Direct $[\alpha]_D^{20}$ calculated ..	+29·04°	+29·48°	+29·26°
found	+29·14°	+29·14°	+29·14°
Original sucrose calculated from the above figures ..	43·95	43·68	43·29

Milks A, B and F were of the same brand, and probably from the same batch. Milks C, D, and E were of another brand, and were probably of the same batch. E was analysed in January, 1931, C in September, 1931, D and A in December, 1931, B and F in January, 1932. It is interesting to note that a sample of the same make, and again probably of the same batch, as A and F, was analysed in September, 1929, before some of the processes used later had been devised; the analytical results obtained then have been recalculated, as far as possible, in the light of the more recent work, and are as follows:

	Per Cent.	
Sucrose	37·61	from chloramine-T and invert polarisation determinations.
Lactose (anhydrous) ..	15·02	Do.
Dextrose	3·18	
Laevulose	0·44	from Lane and Eynon method.
Laevan	2·47	
Direct $[\alpha]_D^{20}$ calculated ..	+33·25°	
found ..	+33·22°	
Original sucrose calculated from the above figures ..	43·66	

The analyses show that in September, 1929, about 6 per cent. of sucrose had broken down, and that the action continued (in the original sealed tins) until, by January, 1932, between 11 and 12 per cent. of sucrose had disappeared, with progressive formation of larger and larger quantities of dextrose, laevulose and laevan.

During the course of this work many sweetened condensed milks of commerce have been examined. It would seem that only when freshly prepared are these free from hydrolytic products of sucrose; amounts of these products corresponding with 0·2 to 0·5 per cent. of altered sucrose are common, although the milk is apparently quite sound.

(IX) DETERMINATION OF TOTAL MILK SOLIDS IN ALTERED SWEETENED CONDENSED MILK

It has been shown that after extensive alteration of the sucrose, the percentage of sucrose originally present can be determined. The total solids, however, will be determined with the altered sucrose present. In so far as sucrose is converted into invert sugar, the total solids will be higher than the original total solids,

1 part of sucrose giving 1·053 part of invert sugar. On the other hand, in so far as the laevulose is condensed to laevan, there is a loss of total solids, 1 part of laevulose giving 0·9 part of laevan. If all the laevulose is converted into laevan, the loss by condensation is equal to the gain by hydrolysis. In any of the cases investigated by the Committee there would have been, on balance, a slight increase of total solids, resulting in a slightly higher figure for total milk solids if these are obtained by subtraction of the "original sucrose" from total solids as determined. The error, giving a slight credit to total milk solids, may be neglected.

RECOMMENDATIONS

That for the purpose of the Public Health (Condensed Milk) Regulations (it being understood that the sample is a product prepared from milk and sucrose only):

I. Sweetened condensed milk should be examined by the Modified Barfoed process described in Appendix II to this report;

II. If no significant alteration is disclosed by this examination, the percentage of total milk solids should be determined by subtracting the percentage of sucrose determined by the method recommended in *Report No.* 2 from the percentage of total solids determined by the method described in *Report No.* 1;

III. If significant alteration is disclosed, the percentage of total milk solids should be determined by subtracting the percentage of original sucrose, determined as described in Appendix IV to this Report, from the percentage of total solids determined by the method described in *Report No.* 1.

APPENDIX I

PREPARATION OF SERUMS

PREPARATION OF SAMPLE.—Mix the sample as in the manner prescribed in *Report No.* 1[8].

ZINC ACETATE — POTASSIUM FERROCYANIDE SERUM*

REAGENTS.—*Zinc Acetate Solution.*—21·9 grms. of crystallised zinc acetate, $Zn(C_2H_3O_2)_2$, $2H_2O$, and 3 ml. of glacial acetic acid, in water, made up to 100 ml.

Potassium Ferrocyanide Solution.—10·6 grms. of crystallised potassium ferrocyanide in water made up to 100 ml.

Concentrated Ammonia Solution, nominal S.G. 0·880.

Dilute Ammonia Solution.—10 ml. of concentrated ammonia solution diluted with water to 100 ml.

Dilute Acetic Acid Solution approximately equivalent to the dilute ammonia solution.

PROCEDURE.—Transfer to a 100 ml. beaker an accurately weighed quantity, approximately 40 grms., of the well-mixed sample; add 50 ml. of hot distilled water (80°–90° C.); mix; transfer to a 200 ml. measuring flask, washing in with successive quantities of distilled water at 60° C., until the total volume is from 120 to 150 ml. Mix; cool to air temperature, and then add 5 ml. of the dilute ammonia solution. Again mix, and then allow to stand for 15 minutes. Add a sufficient quantity of the dilute acetic acid solution to neutralise the ammonia added (the exact equivalent is determined beforehand by titration), and again mix.

* This is the same serum as is used for the determination of sucrose according to Report No. 2. The procedure for determining the specific rotation of the inverted serum (Appendix IV) is identical with that described in that Report.

Add, with gentle mixing, 12·5 ml. of zinc acetate solution and mix, followed in the same manner by 12·5 ml. of potassium ferrocyanide solution. Bring the contents of the flask to 20° C., and add distilled water (at 20° C.) up to the 200 ml. mark.

Up to this stage all additions of water or reagents should be made in such a manner as to avoid formation of air bubbles, and, with the same object in view, all mixings should be made by rotation of the flask rather than by shaking. If bubbles are found to be present before completion of dilution to 200 ml., their removal can be assisted by temporary attachment of the flask to a vacuum pump, and rotation of the flask.

Close the flask with a dry stopper and mix thoroughly by shaking. Allow to stand for a few minutes, and then filter through a dry filter paper, rejecting the first 25 ml. of filtrate.

This serum is used for

(*a*) Modified Barfoed method. (Appendix II.)

(*b*) Determination of $[\alpha]_D^{20}$ of inverted solution. (Appendix IV.)

(*c*) If desired, for approximate chloramine-T titration. (Appendix IV.)

(*d*) Determination of laevulose. (Appendix III.)

Phosphotungstic Acid Serum

Reagent.—*Phosphotungstic Acid Precipitant.* 50 grms. of crystalline sodium tungstate, $Na_2WO_4,2H_2O$, and 6 grms. of crystalline disodium phosphate are dissolved in about 200 ml. of distilled water, and 220 ml. of 2 *N* hydrochloric acid solution (or the equivalent amount of acid of other normality) are added slowly with stirring. The solution is diluted to 500 ml. and filtered. The acidity of the reagent should be so adjusted that 20 ml. require approximately 16·0 ml. of *N*/2 sodium hydroxide solution when titrated with methyl orange as indicator, and the p_H of the reagent, diluted to five times its volume with water, is approximately 1·3.

Procedure.—Transfer to a 200 ml. measuring flask an accurately weighed quantity of 10 grms. of the well-mixed sample by successive quantities of distilled water at about 60° C., using about 120 ml. of water in all. Mix; cool to air temperature, and add, with gentle mixing, 10 ml. of the phosphotungstic acid reagent. Bring the contents of the flask to 20° C., and add distilled water (at 20° C.) up to the 200 ml. mark.

Up to this stage all additions of water or reagents should be made in such a manner as to avoid formation of air bubbles (see under zinc acetate and potassium ferrocyanide serum, above).

Close the flask with a dry stopper and mix thoroughly by shaking. Allow to stand for 10 minutes, and then filter through a dry filter paper, rejecting the first 25 ml. of filtrate.

(In the case of small samples the full quantity given above may not be available. If the prescribed quantity of 10 grms. is departed from, the amount of the phosphotungstic acid reagent must be varied so that its volume in ml. is numerically equal to the weight of sample taken in grms. The calculation in Appendix IV [ii] for the phosphotungstic acid serum holds only when this relationship is maintained and for a 5 per cent. serum.)

This serum is used for the chloramine-T titration.

APPENDIX II

MODIFIED BARFOED PROCESS

Reagent.—*Copper Solution.*—Dissolve 60 grms. of crystallised sodium acetate in water, add 105 ml. of *N*/1 acetic acid and make up to 1 litre with water.

Transfer to a dry bottle, add 52 grms. (or more) of finely powdered crystallised copper acetate and shake to saturation. Filter.

Ferric Sulphate Solution.—Dissolve 50 grms. of ferric sulphate in about 400 ml. of water, to which 109 ml. of concentrated sulphuric acid has been added. Make up to 1 litre with water and filter. Before use this solution should be treated with *N*/10 permanganate until the colour of the latter ceases to be discharged.

N/10 *Potassium Permanganate Solution.*

PROCEDURE.—Introduce 25 ml. of serum (zinc serum: Appendix I) into a thin-walled boiling tube (internal measurements 8 × 1¼ inches); add 70 ml. of the copper solution; mix; cover the tubes with a watch-glass and immerse, to the level of the liquid in the tube, in the water in a large water-bath maintained at 80° C. for 20 minutes. Remove; cool in running water; filter on asbestos by suction and wash the tube and filter containing the cuprous oxide rapidly a few times with freshly boiled distilled water, rejecting the filtrate and washings. Dissolve the cuprous oxide (including any remaining in the tube) in 20 ml. of the ferric sulphate solution; wash the asbestos pad with cold freshly-boiled distilled water, adding the washings to the ferric sulphate filtrate, and titrate with *N*/10 permanganate to faint permanent pink.*

Note.—A convenient asbestos filter may be prepared by inserting a loosely fitting glass bead into the neck of a Allihn filter tube; above the bead is placed a layer of glass wool, and on this is laid the asbestos, which is added as a wet pulp and drawn on to the glass wool by suction. Solution of the cuprous oxide is rapidly effected by pipetting the ferric sulphate solution into the reduction tube, transferring to the Allihn tube and thoroughly mixing the surface asbestos layers with the sulphate solution by means of a flat-ended glass rod, leaving the lower asbestos layers and the glass wool undisturbed.

APPENDIX III

HINTON AND MACARA PROCESS FOR DETERMINATION OF LAEVULOSE[2]

REAGENTS.—*Sucrose Solution.*—Approximately 9 grms. per 100 ml. (freshly prepared).

Iodine Solution.—13 grms. of iodine and 15 grms. potassium iodide per 100 ml.

Mixed Alkali Solution.—Equal parts of 2 *N* sodium carbonate and 2 *N* sodium hydroxide.

Sulphuric Acid.—Approximately 5 *N*.

Sodium Sulphite Solution.—20 per cent. w/v.

Dilute Sodium Sulphite Solution.—2 per cent. freshly prepared; or diluted from the 20 per cent. solution.

Luff's Solution.—Dissolve 25 grms. of crystallised copper sulphate in 100 ml. of water; 50 grms. of citric acid in 50 ml. of water; 388 grms. of $Na_2CO_3, 10H_2O$ in 300 or 400 ml. of luke-warm water. Add the citric acid solution to the sodium carbonate solution, and then add the copper solution. Mix, cool, make up to 1 litre, and filter.

Note.—This solution should be accurately prepared, and 10 ml. of the finished solution should require approximately 45 ml. of *N*/2 sulphuric acid for neutralisation to methyl orange.

* If the titration exceeds about 15 mls. *N*/10 $KMnO_4$ the test should be repeated with a suitable quantity of serum made up to 25 ml. For specially accurate work this dilution should be made with a serum prepared from fresh milk and sucrose.

Iodate-iodide Solution.—2·7 grms. of potassium iodate, 30 grms. of potassium iodide, and 10 ml. of $N/2$ sodium hydroxide solution per litre.

Potassium Oxalate Solution.—A saturated aqueous solution.

Sodium Thiosulphate Solution.—$N/20$.

Soluble Starch Solution.—Approximately 2 per cent.

Control Serum.—Prepared from fresh milk, using 100 ml. of milk with the same quantities of ammonia and acetic acid and precipitants as for 40 grms. of condensed milk, made up to 200 ml. and filtered.

PROCEDURE.—(i) *Oxidation of the Aldose Sugars.*—Pipette 10 ml. of the prepared condensed milk serum (zinc serum, Appendix I) and the same amount of the control serum into 250 ml. conical flasks, ensuring that the liquid does not flow on to the sides of the flasks. To the condensed milk serum add 10 ml. of water, and to the control serum 10 ml. of the sucrose solution.

Note.—Some workers find it preferable to add 2 ml. of a 0·5 per cent. solution of invert sugar to each serum so as to ensure a perceptible reduction in the control; this is termed "sensitising invert." Such addition makes no difference to the subsequent procedure.

To each, then add exactly 5 ml. of the iodine solution and exactly 6 ml. of the 2 N mixed alkali solution; mix gently, and allow the flasks to stand for 10 minutes at from 18° C. to 20° C. Acidify with 1·6 ml. of 5 N sulphuric acid, and remove the liberated iodine, first, with 20 per cent. sodium sulphite solution, and, finally, after adding 6 drops of soluble starch solution, with the 2 per cent. sulphite solution. (This operation should have the precision of a titration, though the quantities of sulphite solution needed are not measured; it should be conducted as rapidly as possible.) When all free iodine is eliminated, immediately add one drop of methyl orange solution and neutralise with the 2 N mixed alkali solution.

Note.—The time elapsing between acidifying with 5 N sulphuric acid and neutralising with the mixed alkali should not exceed 2 minutes, to avoid the danger of inversion of the sucrose.

(ii) *Treatment with Luff's Solution.*—To the contents of each flask add 20 ml. of Luff's solution; cover with a watch-glass and heat the contents to boiling on a plain wire gauze over a burner regulated so that boiling takes place in 2 minutes; impinging of the flame or hot gases on the sides of the flask should be prevented by an asbestos sheet with central hole of suitable dimensions placed in contact with the wire gauze. When boiling takes place, transfer the flask to an asbestos-covered gauze already heated by a small Bunsen flame, attaching a reflux condenser, and maintain gentle ebullition for exactly 10 minutes.

Remove from the flame and cool in running water for four or five minutes.

Titration of the Reduced Copper.—Add exactly 25 ml. of the iodate-iodide solution and 20 ml. of saturated potassium oxalate solution. Acidify carefully, while swirling, with 20 ml. of 5 N sulphuric acid. Shake round (with some care, as frothing occurs) until the precipitate of cuprous oxide (which is partly converted into white cuprous iodide) has dissolved, and titrate with $N/20$ thiosulphate. No further addition of starch should be required. The end-point is distinguished by a sharp change to a fine light blue (the colour of the cupric salt).

Calculation of the Laevulose.—The difference between the titrations of the sample serum and the control serum, as ml. of $N/20$ thiosulphate solution, multiplied by 0·064, gives the percentage of laevulose in the sample, uncorrected for the volume of the clarification precipitate. (This factor is strictly correct only for a 20 per cent. serum, *i.e.* if exactly 40 grms. of condensed milk was diluted to 200 ml. in the preparation of the serum.) The correction for volume of precipitate, if required, is calculated as in Appendix IV, i.

APPENDIX IV

THE DETERMINATION OF "ORIGINAL SUCROSE"

(i) Specific Rotation of the Inverted Serum

Reagent.—*Hydrochloric Acid Solution* = 6·34 times normal.

Inversion.—Pipette into a 50-ml. measuring flask 40 ml. of the filtrate obtained by zinc clarification (see Appendix I); add 6 ml. of 6·34 *N* hydrochloric acid. Immerse the entire bulb of the flask for 12 minutes in a water-bath maintained at 60° C., mixing by rotatory movement during the first three minutes, in which time the contents of the flask should have attained the temperature of the bath. Cool, and make up to the 50 ml. mark at 20° C., with distilled water; mix, and allow to stand for one hour.

Invert Polarisation.—Determine the rotation at 20° C.

Calculation.

W = weight of sample taken in grms.
F = percentage of fat in the sample,
P = ,, ,, protein (N × 6·38) in the sample,
V = volume (in ml.) to which the sample is diluted before filtration,
v = correction in ml. for volume of precipitate produced during clarification,
I = observed invert polarimeter reading,
l = length in dm. of polarimeter tube,
*R = specific rotation of the inverted serum ($[\alpha]_D^{20}$),

* *Note.*—If the mercury green line is used instead of the D line, the reading should be multiplied by 0·847 to convert to rotation for the D line.

then $v = \frac{W}{100}[(F \times 1{\cdot}08) + (P \times 1{\cdot}55)]$,

and $R = (\frac{5}{4} \times I) \times \frac{V-v}{V} \times \frac{V}{l \times W}$

(ii) The Chloramine-T Titration

Reagents.—*N*/20 *Chloramine-T Solution.*—Containing 7·04 grms. per litre, freshly prepared, and protected from light.

Standard Sodium Thiosulphate Solution.—Preferably rather stronger than *N*/20, so that 50 ml. of the chloramine-T solution can be titrated without refilling a 50 ml. burette.

Note.—The thiosulphate must be accurately standardised against pure potassium dichromate by the method of Popoff and Whitman.[9] The procedure is as follows:

To 25 ml. of *N*/10 potassium dichromate solution add 20 ml. of 10 per cent. potassium iodide solution and 10 ml. of 2 *N* hydrochloric acid; stopper the flask, and allow to stand in the dark for 10 minutes; then titrate with the thiosulphate.

N/2 *Sodium Hydroxide Solution.*

N/10 *Sodium Hydroxide Solution.*

Soluble Starch Solution.—Approximately 2 per cent.

PROCEDURE.—*Inversion.*—Pipette 25 ml. of the phosphotungstic acid serum (see Appendix I) into a 100-ml. measuring flask, add 15 ml. of distilled water and 5 ml. of 6·34 *N* hydrochloric acid. Immerse for 12 minutes the entire bulb of the flask in a water-bath maintained at 60° C., mixing by rotatory movement during the first 3 minutes, in which time the contents of the flask should have attained the temperature of the bath. Cool, add *N*/2 sodium hydroxide solution, continually mixing, until neutral, carrying the addition of alkali to the point of a definite turbidity (no internal indicator should be used). Cool and make up to the 100 ml. mark at 20° C. with distilled water.

Titration of inverted and neutralised Serum.—Into one of two 250 ml. flasks or bottles pipette 25 ml. of the inverted neutralised serum which has been diluted to 100 ml.; into the other pipette 25 ml. of water (as a blank). To each add 3 ml. of *N*/10 caustic soda solution, followed by 20 ml. of 10 per cent. potassium iodide solution, then, into each, pipette accurately 50 ml. of the *N*/20 chloramine-T solution; close the flasks or bottles and leave in the dark for 1½ hours at a temperature of from 17° to 18° C. At the end of this time add to each flask 10 ml. of 2 *N* hydrochloric acid and titrate at once with the thiosulphate solution, with starch solution as indicator.

Note.—All measuring flasks, pipettes and burettes must be accurately calibrated, special attention being paid to drainage of the burette, which should be calibrated in the manner in which it is used for the titration. Readings of the burette are made to 0·01 ml.

CALCULATION.

S = percentage of "original sucrose" in the sample,
E = iodine absorbed per 100 grms. of sample,
W = weight of sample taken in grms.,
F = percentage of fat in the sample,
P = percentage of protein (N × 6·38) in the sample,
V = volume (in ml.) to which the sample is diluted before filtration,
v = correction in ml. for volume of precipitate produced during clarification,
n = difference between the inverted serum and the blank titrations in ml. *N*/20 thiosulphate solution.

Then, for calculation of the original sucrose,

$$v = \frac{W}{100}[(F \times 1{\cdot}08) + (P \times 0{\cdot}74) + 3{\cdot}75] \text{ ml.}$$

$$E = (n - 0{\cdot}15^{*}) \times 63{\cdot}46 \times 0{\cdot}032 \times \frac{V-v}{V}$$

and $$S = 0{\cdot}964\ E - 1{\cdot}29\ R.$$

Addendum.—If it is desired to determine the chloramine-T figure on the zinc serum, the procedure is as follows:—Pipette 20 ml. of the inverted serum (which has been used for the determination of $[\alpha]_D^{20}$ invert) into a 250-ml. flask, add *N*/2 sodium hydroxide solution, with continual mixing (approximately 31 ml. will be required) until neutral, neutrality being shown by a distinct permanent turbidity.

* This is the correction for absorption by non-sugars, and applies only to the quantities prescribed and for a usual proportion of non-fatty solids in the milk.

Make up to the 250-ml. mark at 20° C. with distilled water and mix. 25 ml. of this solution are taken for the chloramine-T titration.

Then $$E = (n - 0{\cdot}15) \times \frac{63{\cdot}46}{32} \times \frac{V-v}{V}$$

and, as before, $S = 0{\cdot}964\ E - 1{\cdot}29\ R.$

(These formulae for the zinc serum apply strictly only to a 20 per cent. serum, *i.e.* if exactly 40 grms. of condensed milk was diluted to 200 ml. in the preparation of the serum.) As explained in the body of the Report, results obtained on the zinc serum are approximate only, and slightly low.

Note.—The volume of precipitate corrections are based upon the data recorded in *Report No.* 2 (THE DETERMINATION OF SUCROSE IN SWEETENED CONDENSED MILK).

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For and on behalf of the Sub-Committee,

(*Signed*) E. HINKS (*Chairman*),
E. B. HUGHES (*Hon. Secretary*)

July, 1932

Erratum.—*Report No.* 2, p. 2, line 33 (ANALYST, **1930, 55, 112**, line 33), *for* "sufficiently" *read* "not sufficiently."

Notes from the Reports of Public Analysts

The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.

CITY OF SALFORD

REPORT OF THE CITY ANALYST FOR THE YEAR 1931

THE 1445 samples of food and drugs examined comprise 574 formal and 871 informal samples (47 adulterated).

BOTTLED MILK.—A type of cap which is very common in U.S.A. covers the whole of the top of the bottle, and is secured beneath the welted rim of the neck. It is to be regretted that this form (which prevents contamination of the rim) is not in use in this country.

The advent of bottled milk has presented a new difficulty in dealing with adulterated samples. Very few retailers have warranties from wholesalers for the quality of the milk they get from them, the argument being that it is not fair that the wholesaler should be held responsible for milk which has passed out of his hands. The crux of the matter is the question of "control," and, as the Salford Stipendiary observed during the hearing of a recent case, milk in bottles may well be held to be under the control of the bottlers until the seal is removed.

It was suggested to the local Milk Dealers' Association that bottlers of milk should adopt a form of warranty to be printed on the cap in some such terms as the following:—"The milk in this bottle is warranted pure and to contain all its natural fat and milk solids, by . . . (name and address of bottler)," and that the retailer should be given a written continuing warranty. The Association was not prepared to adopt this suggestion, although one large wholesaler has done so. During the past two or three years there have been several cases in which bottled milk sold by a retailer has been found to be below standard, and in which enquiry has proved that the retailer could not be held responsible for the deficiency. In future, deficiencies in bottled milk cannot be passed over, and retailers would be well advised to obtain warranties from the bottlers of the milk they sell.

FREEZING POINT OF MILK.—The extreme range in the freezing points of 257 samples of genuine milk, determined by means of the Hortvet apparatus, was −0·535° to −0·572° C., with an average of −0·551° C. One sample froze between −0·566° and −0·570° C. and one between −0·571° and −0·575° C.

"MARGARINE BLENDED WITH BUTTER."—An informal sample, labelled "Margarine blended with Butter," contained about 3·5 per cent. of butter, and a formal sample contained 5 per cent. of butter. In view of the decision in the case of *Anness* v. *Grivell* (in which the vendor sold a substance containing 4·5 per cent. of butter as "a very good mixture of margarine and butter"), it was considered that a prosecution would have little chance of success. It was not pointed out to the judges, however, in the case of *Anness* v. *Grivell*, that, although the Food and Drugs Act prohibits the sale of mixtures containing more than 10 per cent. of butter, the proviso applies to margarine, and the Act expressly provides that any mixture of butter and margarine must be sold simply as "margarine."

"HOUSEHOLD TURPENTINE."—Of 16 samples of turpentine examined, 8 were found to be adulterated with "white spirit," in amounts varying from 30 to 100 per cent. In several of these cases the article was described as "household turpentine," and was labelled "Not to be used for medicinal purposes." This raised a difficulty in instituting proceedings under the Food and Drugs Act, as the defence would have been that the sample could not be called a "drug" within the meaning of the Act. Proceedings were, therefore, taken under the Merchandise Marks Act, both the packers and the sellers being summoned. A conviction was secured, and each was fined £15, with £5 5s. costs.

H. E. MONK

Straits Settlements

ANNUAL REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1931

IN his Annual Report the Government Analyst (Mr. J. C. Cowap) points out that the greater portion of the routine work is provided by the Monopolies Department, and is concerned with the protection of revenue. It consists in the examination of opium, chandu, imported or locally distilled liquors, toddy and deleterious drugs.

LIQUORS REVENUE ORDINANCE.—Of the 68 samples of brandy analysed for classification under the Liquors Revenue Ordinance, 1927,* 43 were passed as brandy; the remaining 25 were either refused admittance as brandy or the importers were asked to produce evidence that the liquor actually was brandy. In no case was such evidence produced.

Definition of Brandy.—Brandy is defined as a potable spirit manufactured from fermented grape juice and from no other materials. Brandy containing less than 60 grms. of esters in 100 litres of absolute alcohol is deemed to be adulterated unless satisfactory evidence is forthcoming from the place of origin that it is genuine. In the case of French brandies, practically the only ones imported, the "acquit blanc," or "acquit jaune d'or," issued by the French Government, is alone accepted. (Note: "Arrack" is ordinary rectified spirit; practically the whole of that imported is distilled in Java from sugar waste.)

DELETERIOUS DRUGS.—Thirty-six exhibits consisted chiefly of medicines for the cure of various diseases, including opium smoking. Many of them contained opium. One seizure of 9 bottles, labelled morphine hydrochloride, contained boric acid with a layer of quinine hydrochloride at the top of each bottle.

MEDICAL DEPARTMENT.—Samples examined for this department included water and sewage, poisons and toxicological exhibits, milk and other foods and drugs.

Tuba Root.—Among the poisons detected was tuba (or derris) root, a well-known Malay fish poison. The root is bruised in water with lime, and the milky emulsion is thrown into a stream to stupefy the fish. When used in sufficient quantity the emulsion can cause death in human beings. The root contains about 25 per cent. of resin soluble in acetone and alcohol, and this (and also the aqueous extract) is exported as an insecticide in increasing quantities from Singapore to Europe and U.S.A. The action of the resin on small fish is characteristic.

Milk.—The standards are 3·25 per cent. of fat and 8·5 per cent. of solids-not-fat.

POLICE DEPARTMENT.—Toxicological exhibits, counterfeit coins, fireworks and explosives were examined for this department.

Counterfeit Coins.—The total number of coins examined in 28 cases was 14,600. The hands and clothing of coiners frequently show traces of silver nitrate and nitric acid. These are recognised by the ordinary tests, *e.g.* mercuric chloride or potassium cyanide for silver stains. As the coins are trimmed with a small file, filings of metal are often found under the finger nails of coiners. These are scraped out and examined under the microscope. The metal is then dissolved in acid while still on the microscope slide, and the solution is touched with drops of appropriate reagents, *e.g.* mercuric chloride for tin. In several cases evidence was thus obtained.

IDENTIFICATION OF STARCH PASTE.—A starch of unusual type (East Indian arrowroot) used for pasting up seditious leaflets, was found to be identical with

* According to the Straits Settlements Liquors Revenue Ordinance, 1927, the following duties (*inter alia*) are imposed:—Arrack, brandy, $14.00 per proof gall.; brandy imported in bottle and not exceeding 81 per cent. of proof spirit, $10.50; brandy consigned from Great Britain or the British Dominions, $9.50; whiskey, rum and gin, $13.00; Japanese saki and Chinese samsu, $12.00; bitters and liqueurs not exceeding 100 per cent. of proof spirit, $13.00; sparkling wines, $6.00; do. from Great Britain or the British Dominions, $5.50; still wines (including medicated wines) exceeding 26 per cent. but not exceeding 42 per cent. of proof spirit, $4.50; do. from Great Britain or the Dominions, $4.20; still wine not exceeding 26 per cent. of proof spirit, $1.50; do. from Great Britain and the Dominions, $1.30; ale, beer, stout, porter, cider, and perry, $1.30; do. from Great Britain or the Dominions, $1.20 per gallon.

The preferential rates specified will not be applicable unless 25 per cent. of the brandy, wine, ale, etc., is the result of labour within the United Kingdom of Great Britain and Northern Ireland, the British Dominions within the meaning of the Statute of Westminster, 1931, or any Malay State under British Protection.

that in certain stains on the hands of a suspect. This starch, in spite of its name, is not in general commercial use, and is usually obtainable only from European shops; it is never used for bill posting. Unfortunately for himself, the accused mixed the starch with cold water, so that the granules on his poster and on his hands were easily recognised.

FACE POWDERS CONTAINING LEAD.—The discovery of lead in face powders, mentioned in the 1930 Report (ANALYST, 1931, **56**, 812), has led to the prohibition of the sale of such powders. Of 8 samples of Chinese origin examined in Penang Municipality, 2 contained an appreciable quantity of lead carbonate.

DOPING OF RACE-HORSES.—A number of samples of sweat and saliva of race-horses were examined for alkaloids in general, and caffeine and strychnine in particular, by the methods described by Lander (ANALYST, 1930, **55**, 474). It has never been suggested that horses in Singapore are doped to make them lose a race, but only to assist them to win.

Fertilisers and Feeding Stuffs Act, 1926

REVISION OF REGULATIONS

STATUTORY RULES AND ORDERS, 1932, No. 658*

THE Ministry of Agriculture and Fisheries and the Department of Agriculture for Scotland announce that, in pursuance of the powers conferred on them by Section 23 of the Fertilisers and Feeding Stuffs Act, 1926, they have made new Regulations entitled "The Fertilisers and Feeding Stuffs Regulations, 1932 (Statutory Rules and Orders, 1932, No. 658), to replace the Fertilisers and Feeding Stuffs Regulations, 1928." The new Regulations will come into operation on September 1st, 1932.

The principal matters in the Regulations affected by the revision may be grouped, for convenience, under two heads:—(*a*) the methods of sampling and analysis, and (*b*) alterations to the Schedules to the Fertilisers and Feeding Stuffs Act, 1926.

As regards (*a*), the provisions relating to the manner of taking samples have been rearranged and amended in certain respects, mainly in order to facilitate the work of the officials concerned. The methods of analysis have been amended by the adoption of the British Standard Test Sieve, Mesh No. 100, in place of the present prescribed sieve, for the determination of the fineness of grinding of basic slag, etc.; and also by the inclusion of a method of analysis for the determination of citric-soluble phosphoric acid.

As regards (*b*), the fresh amendments are confined to the First, Second and Fourth of the Schedules to the Act.

It may be explained that the First and Second of the Schedules to the Act include the classes of articles in respect of which the seller is required under the Act to give to the purchaser a statutory statement, which has effect as a warranty, containing certain prescribed particulars. In the Fourth Schedule to the Act the names of the more commonly used fertilisers and feeding stuffs are defined, and the use in a statutory statement of any of these names constitutes a warranty that the article accords with the relative definition.

With one exception, the present amendments to these Schedules relate to feeding stuffs, the exception being "Lime mixtures," which, under the New Regulations, is now included amongst the fertilisers in the Second Schedule.

Alfalfa meal has been added to the Second Schedule, and has been defined in the Fourth Schedule as "Alfalfa (Lucerne) as grown, dried and ground, to which no other matter has been added."

The entry "Barley Meal" in both the First and Fourth Schedules is now replaced, in the New Regulations, by two entries, namely, "Barley Meal" and "Barley Meal Grade II." These names are defined for the purpose of the Fourth Schedule, as follows:—

Barley Meal.—The meal obtained by grinding barley, as grown, which shall be the whole grain together with only such other substances as may reasonably be expected to have become associated with the grain in the field; the meal to contain not less than 96 per cent. of pure barley.

* Copies of the new Regulations can be obtained from H.M. Stationery Office, Adastral House, Kingsway, W.C.2, and branches. Price 10d. exclusive of postage.

Barley Meal (Grade II).—The meal, other than barley meal, as defined above, obtained by grinding barley, as grown, which shall be the whole grain together with only such other substances as may reasonably be expected to have become associated with the grain in the field; the meal to contain not less than 90 per cent. of pure barley.

The definitions, in the Fourth Schedule, of Bean Meal and Pea Meal have been amended to read as follows:—

Bean Meal.—The meal obtained by grinding commercially pure beans of the species (1) *Vicia faba* (synonym, *Faba vulgaris*), or any of its varieties, commonly known as "horse bean," "field bean," or "broad bean"; or (2) *Phaseolus vulgaris*, the "true haricot bean," or any of its varieties, white or coloured.

Pea Meal.—The meal obtained by grinding commercially pure peas, as grown, of varieties of *Pisum sativum* or *Pisum arvense*.

The entries in the Schedules relating to Feeding meat and bone products have been varied, the chief point, perhaps, being that the definitions of Feeding meat meal and Feeding meat and bone meal in the Fourth Schedule have been amended with the object of fixing minimum percentages of albuminoids (protein) in these articles. The new definitions read as follows:

Feeding Meat Meal.—"The product, containing not less than 55 per cent. of albuminoids (protein) and not more than 4 per cent. of salt, obtained by drying and grinding animal carcases or portions thereof (excluding hoof and horn), to which no other matter has been added."

Feeding Meat and Bone Meal.—"The product, containing not less than 40 per cent. of albuminoids (protein) and not more than 4 per cent. of salt, obtained by drying and grinding animal carcases or portions thereof (excluding hoof and horn) and bone, to which no other matter has been added."

MINISTRY OF AGRICULTURE AND FISHERIES,
10, WHITEHALL PLACE, LONDON, S.W.1,
29th June, 1932

United States Department of Agriculture

FOOD AND DRUG ADMINISTRATION*

NEW AND REVISED DEFINITIONS AND STANDARDS FOR FOOD PRODUCTS

MAYONNAISE, mayonnaise dressing, mayonnaise salad dressing, is the semi-solid emulsion of edible vegetable oil, egg yolk, or whole egg, a vinegar, and/or lemon juice, with one or more of the following: Salt, other seasoning commonly used in its preparation, sugar and/or dextrose. The finished product contains not less than 50 per cent. of edible vegetable oil.

Milk bread is the product, in the form of loaves or smaller units, obtained by baking a leavened and kneaded mixture of flour, salt, yeast, and milk or its equivalent (milk solids and water in the proportions normal to milk); with or without edible fat or oil, sugar and/or other fermentable carbohydrate substance. It may also contain diastatic and/or proteolytic ferments, and such minute amounts of unobjectionable salts as serve solely as yeast nutrients.† The flour ingredient may include not more than 3 per cent. of other edible farinaceous substance. Milk bread contains, one hour or more after baking, not more than 38 per cent. of moisture.

The definition for rye bread has been deleted.

Farina macaroni, farina spaghetti, farina vermicelli, are plain alimentary pastes in the preparation of which farina is the only farinaceous ingredient used and are distinguished by their characteristic shapes.

Ginger ale is the carbonated beverage prepared from ginger ale flavour, harmless organic acid, potable water, and a syrup of one or more of the following: Sugar, invert sugar, dextrose, with or without the addition of caramel colour.

Ginger ale flavour, ginger ale concentrate, is the beverage flavour in which ginger is the essential constituent, with or without aromatic and pungent ingredients, citrus oils, fruit juices, and caramel colour.

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* *Service and Regulatory Announcements, Food and Drug No.* 2 (Second Revision), Supplement No. 1.

† The propriety of the use of minute quantities of oxidising agents as enzyme activators is reserved for future consideration and without prejudice.

Sarsaparilla is the carbonated beverage prepared from sarsaparilla flavour, potable water and a syrup of one or more of the following: Sugar, invert sugar, dextrose; with or without harmless organic acid, and with or without the addition of caramel colour.

Sarsaparilla flavour is the beverage flavour prepared from oil of sassafras and methyl salicylate (or oil of wintergreen or oil of sweet birch) with or without other aromatic and flavouring substances and caramel colour. It derives its characteristic flavour from oil of sassafras and methyl salicylate.

Root beer is the carbonated beverage prepared from root beer flavour, potable water, and a syrup of one or more of the following: Sugar, invert sugar, dextrose; with or without harmless organic acid, and with or without the addition of caramel colour.

Root beer flavour, root beer concentrate, is the beverage flavour in which oil of sassafras and methyl salicylate (or oil of wintergreen or oil of sweet birch) are the principal flavouring constituents, and contains other flavouring substances, with or without the addition of caramel colour.

Birch beer is the carbonated beverage prepared from birch beer flavour, potable water, and a syrup of one or more of the following: Sugar, invert sugar, dextrose; with or without harmless organic acid, and with or without the addition of caramel colour.

Birch beer flavour, birch beer concentrate, is the beverage flavour in which methyl salicylate (or oil of sweet birch or oil of wintergreen) and oil of sassafras are the principal flavouring constituents, with or without other flavouring substances, and with or without caramel colour. The flavour of methyl salicylate predominates.

Cream soda water, "cream soda," is the carbonated beverage prepared from cream soda water flavour, potable water, and a syrup of one or more of the following: Sugar, invert sugar, dextrose; with or without harmless organic acid, and with or without the addition of caramel colour.

Cream soda water flavour, cream soda water concentrate, is the beverage flavour prepared from vanilla, tonka, vanillin, or coumarin, singly or in combination, together with other flavouring substances; with or without the addition of caramel colour.

Issued December, 1931

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs Analysis

Detection of Washed, Abraded, and Oiled Eggs. P. F. Sharp. (*Ind. Eng. Chem.*, 1932, **24**, 941-946.)—If dirty eggs which have or have not been cleaned are sold, they may go into immediate consumption before any bacteria present cause marked spoilage. If, however, such eggs are kept either at a comparatively high temperature or for an appreciable time, bacterial deterioration may occur in from 3 to 50 (or even more) per cent. of them, according to the amount of the dirt on the shell and to the treatment they have undergone. Of eggs which have never been dirty and have been properly kept, only 2 or 3 per cent. will show bacterial development.

Attempts to recognise washed eggs by testing for the removal of protein from the shell proved unsuccessful, but micro-precipitation tests of an extract of the shell for potassium and chlorides gave positive results with unwashed, and negative results with washed, eggs. A drop of water was placed on the clean upper surface of the shell and left for 5 minutes, after which it was removed from the inverted egg to a microscope slide, to be tested for either chloride or potassium. With an unwashed egg, addition of a drop of 0·1 *N* silver nitrate solution to the water drop yields a precipitate, usually corresponding in amount with that obtained

with one drop of 0·005 *N* sodium chloride solution. The precipitate varies in appearance with different eggs, and is best viewed under strong lateral illumination and with a magnification of twenty to forty diameters. For the potassium test, the following reagent is used: 20 grms. of cobaltous nitrite, $Co(NO_2)_2$, $6H_2O$, 25 grms. of sodium nitrite, 65 c.c. of distilled water, and 10 c.c. of glacial acetic acid are mixed, heated to boiling after violent evolution of fumes ceases, left to cool for 24 hours, made up to 150 c.c., and filtered. When a drop of this solution is mixed, by means of a fairly stiff wire, with the drop on the slide, crystallisation of the potassium cobaltinitrite occurs usually within five minutes, but sometimes only after about 15 or 20 minutes. In over 1100 cases, the test for chloride gave erroneous results in about a dozen instances, while the potassium test failed or gave uncertain indications with two only out of over 500 eggs.

Eggs washed with egg albumen solution are recognisable by the heavy precipitate given by the shell extract with Millon's solution, and those washed with soap solution by their feel. Treatment of eggs with oil to retard evaporation and escape of carbon dioxide does not interfere with the tests for potassium and chloride. The greatest interference with this test for washed eggs occurs with storage eggs. During the storage the potassium chloride largely disappears from the surface of the shell; the time required for the disappearance of this salt diminishes as the degree of humidity of the store increases, but probably lies between 4 and 8 months for eggs kept at 0° C. and under conditions otherwise normal. Usually the potassium chloride has not entirely vanished, and tests made show that an experienced operator can nearly always distinguish between washed, unwashed, and storage eggs in uniformly-treated lots.

If the dirty spots on eggs have been cleaned by scraping with emery paper, sand paper, steel wool, etc., the protein and the potassium chloride are removed from the scraped regions. If a sand-blast has been used, the eggs generally yield an extract which gives no test for potassium or chloride. When, however, an abraded egg is immersed in the solution of a dye, such as methylene blue, malachite green, safranine, etc., capable of staining the protein material on the surface of the shell, the scraped parts, being unstained, show up quite clearly. The eggs, in a metal tray or basket, are immersed, first in water for 4 or 5 seconds (to prevent the dye from penetrating too deeply), and then in a solution of 1 grm. of rosaniline hydrochloride per litre for 30 seconds. After observation, the eggs are placed for about 1 minute in a 2 per cent. solution of sodium bisulphite to reduce the dye, and are afterwards washed in water; they are then in saleable condition.

Treatment of eggs with a sand-blast, either to remove dirt or to "deprocess" eggs which have been oiled, cannot be detected under the microscope, the scarifying effect being less than might be expected. Oil-treated eggs can usually be recognised by the appearance of the shell, especially if a whole case is examined, but it is sometimes difficult to ascertain in this way if individual eggs have been oiled. Such eggs can, however, be detected by dipping the end of the egg into ether for a second; in a good light an oily ring can then be seen at the edge of the ether-treated part. Removal of the oily appearance of the eggs by sand-blasting is often practised, but eggs thus treated still show the oily ring left by the ether.

T. H. P.

Determination of Total Solid Matter and Density of Egg White by means of the Refractometer. H. J. Almquist, F. W. Lorenz and B. R. Burmester. (*Ind. Eng. Chem., Anal. Ed.*, 1932, **4**, 305–306.)—Since egg white may be considered as protein, mineral matter and water only, and the mineral part is a small and usually constant fraction of the total, the system behaves as a binary mixture, and either the index of refraction or the specific gravity, but more conveniently the former, may be measured in order to find the total solids. The graphs may be constructed from the following points taken accurately from the plotted curves:

Refractive index $n_D^{25°}$	Total solids Per Cent.	Specific gravity at 25°/25° C.
1·3520	10·55	1·0312
1·3600	14·55	1·0442

The method cannot be used for egg yolk or whole egg, since these mixtures contain more than two major components. D. G. H.

Rye Germ Oil. A. W. Stout and H. A. Schuette. (*J. Amer. Chem. Soc.*, 1932, **54**, 3298–3302.)—The rye germ oil was obtained by extracting rye embryo (containing 11 per cent. of oil) with sulphur-free petroleum spirit. The embryo was obtained by repeated sifting and air-cleaning of so-called rye germ stock, a milling bye-product. The oil was of a deep golden-brown colour, and semi-drying, plastic at −60° C. It has the following characteristics: Sp.gr., at 20°/20° C., 0·9229; $n_D^{20°}$, 1·4779; saponification value, 176·8; iodine value (Wijs), 139·9; Reichert–Meissl value, 0·05; Polenske value, 0·3; free fatty acids (as oleic), 2·9 per cent.; unsaponifiable matter, 7·28 per cent.; acetyl value, 20·19; soluble acids (as butyric), trace; insoluble acids (Hehner value), 84·45; iodine value of insoluble fatty acids, 133·57; and thiocyanogen value, 85·05; unsaturated acids, corrected, 77·46 per cent. (with iodine value 158·9, and saponification value 172·6); saturated fatty acids (corrected), 10·12 per cent. The unsaturated acids were separated in form of bromine addition compounds, and the saturated acids by separation of the methyl esters. The percentage composition of the oil was calculated to be: myristin, 2·33; palmitin, 8·11; stearin, 0·18; olein, 31·92; linolin, 44·05; linolenin, 4·99; unsaponifiable matter, 7·28; and undetermined, 1·14. The lecithin equivalent of a hypothetical di-oleyl derivative ($C_{44}H_{86}O_9NP$), was calculated from the phosphorus-content of the oil and its unsaponifiable matter, and was 1·33 per cent. for the former and 3·05 for the latter. A pronounced blue coloration is formed on addition of antimony chloride to the oil, and a still more pronounced coloration if the extracted unsaponifiable matter is used. (*Cf.* Croxford, ANALYST, 1930, **55**, 735.) D. G. H.

Quince Seed Mucilage. A. G. Renfrew and L. H. Cretcher. (*J. Biol. Chem.*, 1932, **97**, 503–510.)—The quince seed gum was prepared by extraction of the seeds with alcohol and ether, followed by separation of the mucilage with water, and precipitation with alcohol. A neutral gum soluble in water and containing 6 per cent. of ash resulted. By precipitation in slightly acid solution, faintly acidic gum insoluble in water and with less ash was obtained; this contained: uronic acid, 27·8; pentose, 33·0; ash, 2·0; and cellulose, 33·0 per cent. When

hydrolysed with 0·5 *N* sulphuric acid, this gum yielded arabinose, a mixture of methylated and unmethylated aldobrionic acids and a cellulosic fraction. After further hydrolysis of 14 grms. of the calcium salts of the aldobrionic acids for 20 hours with 600 c.c. of 0·96 *N* sulphuric acid, 21 per cent. of the uronic acid had been destroyed. Calcium sulphate was removed, the concentrated solution was treated with 2 and with 6 volumes of alcohol, and about 2·3 grms. of unhydrolysed aldobrionic acid were recovered. The supernatant liquors were concentrated to dryness, and crystalline xylose was isolated from the portion soluble in alcohol. An 8 per cent. solution of this had a specific rotation of 19·1° after 2 hours, and this remained constant for 24 hours. D. G. H.

Composition of Iraq Dates. M. M. Cleveland and C. R. Fellers. (*Ind. Eng. Chem., Anal. Ed.*, 1932, **4**, 267–268.)—The edible portion of two samples of Iraq dates had the following percentage composition:

	Moisture	Ash	Protein	Fat (ethereal extract)	Reducing sugars (as invert sugar)	Total carbohydrates (other than crude fibre)	Crude fibre	Sucrose
Hallowi	19·0	2·22	1·72	1·90	73·50	73·67	2·17	None
Sayer	18·0	1·59	2·16	0·31	—	76·14	1·90	—

The percentage composition of the ash was as follows:

	Potassium oxide	Sodium oxide	Calcium oxide	Magnesium oxide	Ferric oxide	Phosphoric anhydride	Alumina	Copper oxide	Chlorine	Sulphuric anhydride	Manganese oxide	Silica
Hallowi	42·7	2·47	4·51	5·86	0·26	9·50	0·48	0·015	13·33	6·44	0·21	7·24
Sayer	40·8	2·65	6·96	6·77	0·23	7·47	—	0·014	16·64	7·62	0·30	—

The alkalinity of the ash (in terms of c.c. of *N* hydrochloric acid) was 16·7 and 15·9. D. G. H.

Identification of White Wine made from Red Grapes. G. Graff. (*Chem.-Ztg.*, 1932, **56**, 704.)—White wine prepared by decolorising red wine with charcoal gives a fairly stable pink coloration when 10 c.c. of the sample are treated with 3 to 5 drops of 10 per cent. hydrochloric or sulphuric acid, unless a very large excess of the decolorising charcoal has been used, which is exceptional. In any case, a pink coloration in this test indicates that the wine was made wholly or in part from red grapes expressed while still white, or from bleached red wine.

Changes in Fats during Frying. F. R. Porter, H. Michaelis and F. G. Shay. (*Ind. Eng. Chem.*, 1932, **24**, 811–813.)—When animal fat, vegetable oil, or hydrogenated vegetable oil is heated at 176° to 232° C., there is a slight formation of fatty acid, an increase in viscosity, particularly at the higher temperature, and some formation of gummy substances, probably due to polymerisation. If water is present, the breaking-down of the fat, with the production of acidity, is a direct result of the action of the water. At first the action is slow, but when about 0·75 per cent. of acid (calculated as oleic acid) has been produced, it proceeds more rapidly. The percentage quantity of free fatty acid in a used fat is a fairly reliable measure of the objectionable property of the fat of imparting an unpleasant flavour to food fried therein; this unpleasant flavour appears when the fat contains about 2 per cent. of free fatty acids. W. P. S.

Iodine Value and Refractive Index of Perilla Oil. C. A. Lathrap. (*Ind. Eng. Chem.*, 1932, **24**, 826–827.)—Six samples of perilla oil, cold-pressed from hand-picked seeds, had iodine values (Wijs) from 192·0 to 208·6, and refractive indices (n_D^{25}) from 1·4800 to 1·4820. One hundred samples of imported perilla oil, considered to be genuine, had iodine values ranging from 193·3 to 207·0, and refractive indices from 1·4802 to 1·4818. In the case of twenty of these oils, the refractive index was higher than 1·4815. W. P. S.

The Component Glycerides of Partially Hydrogenated Fats. T. P. Hilditch and E. C. Jones. (*J. Chem. Soc.*, 1932, 805–820.)—Olive oil was chosen for investigation on account of its comparatively simple structure and the fact that it contains a large amount (at least 60 per cent.) of tri-unsaturated glycerides, and cottonseed oil because of its content of saturated acids (over 20 per cent., almost all of which is palmitic). The experimental results of the investigation, which are collected in two tables, show the iodine values, the component fatty acids present in the whole fat (either calculated from observed fall in iodine value in conjunction with the analysis of the mixed fatty acids of the original oils, or found), the percentage of fully saturated glycerides, and the component fatty acids present in the latter. The figures are given as percentages by weight as determined and as molar percentages, owing to the varying molecular size of the component acyl radicals. The limits within which the molar percentages of the three classes of glycerides containing unsaturated radicals may vary are also given for each hydrogenated fat. The sequence of action involved in the conversion of a molecule of triolein into tristearin, and the experimental results (discussed in detail) lead to the conclusion that only one olein group undergoes hydrogenation at each effective contact with the catalyst, the semi-hydrogenated glyceride then leaving the catalyst and requiring fresh adsorption before further addition of hydrogen occurs. Also, hydrogenation of the different classes of unsaturated glycerides is definitely selective, and trioleins are more readily attacked than dioleoglycerides, and the latter more so than the mono-oleo compounds. Further, the data obtained indicate that a more delicate kind of selective hydrogenation goes on throughout the process, namely, the preferential attack on mixed glycerides containing palmitic groups over those containing only stearic residues. The experimental part of the paper gives the conditions of hydrogenation. The determination of the component fatty acids was made by the method described in several papers, *e.g.* ANALYST, 1929, **54**, 80. In the determination of the fully-saturated glycerides a preliminary separation of part of these was possible from olive oil, by cooling the ethereal solution of the whole products of oxidation to 0° C., and removing the solids by filtration. With cottonseed oils the separation of sodium salts from ether was omitted, owing to the relatively large quantities of palmitic glycerides, and most of the acidic products formed during oxidation were washed from the ethereal solution with dilute aqueous potassium carbonate solution, followed by water. D. G. H.

Effect of Storage on Pyrethrum Flowers. C. B. Gnadinger and C. S. Corl. (*Ind. Eng. Chem.*, 1932, **24**, 901–903.)—Commercial pyrethrum flowers are harvested in May and June, and begin to arrive in the United States in August

and September. Consequently, the pyrethrum crop of a given year cannot be used as an insecticide until the following year; flowers are, indeed, often carried over for a second year before reaching the user. It has been found that freshly-ground pyrethrum flowers, when stored in containers of different kinds, lose from 30 to 43·6 per cent. of their pyrethrin-content within the first year, and the toxicity towards flies diminishes to approximately the same extent. Examination of powdered and ground flowers stored in tightly closed tins showed that the loss of pyrethrins continues for more than two years, but at a diminishing rate. The pyrethrin content of whole Japanese flowers decreases as the age of the flowers increases. T. H. P.

Corrosion of Bronzes by Vinegar. E. M. Mrak and J. C. Le Roux. (*Ind. Eng. Chem.*, 1932, **24**, 797–799.)—All the bronzes tested corroded considerably under the conditions employed (immersion, aeration and spraying), and the vinegar used became highly contamined with heavy metals. Tin, copper and lead were also attacked by the vinegar, the maximum corrosion being attained in the case of lead, when, in a spraying test, 8826 mgrms. of metal per sq. dm. of surface were dissolved in 100 hours. Lead decreased the resistance of bronze to the action of vinegar, and a bronze containing 25 per cent. of lead lost 2607 mgrms. in weight per sq. dm. in 100 hours when sprayed with vinegar. W. P. S.

Lead from Earthenware Vessels. A. Gronover and E. Wohnlich. (*Z. Unters. Lebensm.*, 1932, **63**, 623–633.)—Restrictions are under consideration against the importation into Germany of earthenware vessels the glaze of which contains lead which can be dissolved under certain specific conditions by hot 4 per cent. acetic acid in excess of the following limits: for vessels of 0·5 litre capacity and upwards, 2 mgrms. of lead for each litre of capacity; for vessels of less than 0·5 litre capacity, 1 mgrm. of lead. The following method for the determination of the lead is based upon the results of numerous tests of the action of 4 per cent. acetic acid on glazed vessels under different conditions:—The vessel is thoroughly washed out with hot water, and then filled with hot water and placed on a water-bath until it is thoroughly heated throughout. The water is then poured out and the vessel is filled with 4 per cent. of boiling acetic acid, after which it is covered with, for example, a glass plate, and heated for half-an-hour on, or in, a bath of boiling water. The acid liquid is transferred to a flask, and the lead in an aliquot part is determined by Winkler's colorimetric method (*Z. Unters. Lebensm.*, 1923, **45**, 361). If the amount of lead is too large to be suitable for colorimetric determination, a part of the liquid is evaporated to dryness, and the lead is determined by the chromate method (*Arb. Kaiserl. Gesundheitsamt*, 1910, **33**, 240). Vessels of more than 3 litres capacity are filled to three-quarters of their volume with the acetic acid. If the amount of lead found is in excess of the limit, it is advisable to make a check determination in the same manner on the same vessel 24 hours later. S. G. C.

Biochemical

Effects of Carotene and of Vitamin *A* on the Oxidation of Linolic Acid. B. R. Monaghan and F. O. Schmitt. (*J. Biol. Chem.*, 1932, **96**, 387–395.)—In the course of work to determine the possible significance of carotene in the metabolic processes of nerve, certain experiments were carried out *in vitro* to test the effect of carotene on the absorption of oxygen by unsaturated fatty acids. It is shown that carotene, the precursor of vitamin *A* in the animal body, greatly inhibits the absorption of oxygen by linolic acid; in fourteen experiments the average degree of inhibition for the first hour was 51 per cent. Oxidised carotene, on the other hand, slightly accelerates the absorption; in twelve experiments the average acceleration in the first hour was 28 per cent. In two experiments, carotene bleached by heating at 105° C. for 3 hours failed to accelerate the absorption of oxygen by linolic acid, and even inhibited it somewhat; thus this substance behaves differently from carotene which has been allowed to bleach at room temperature in caproic acid. Vitamin *A* in small concentrations may completely inhibit the absorption for some hours. As in the case of carotene, this inhibition ceases when the vitamin is destroyed by oxidation. The unsaponifiable matter of cod-liver oil was the source of the vitamin *A* used in these experiments. There remains the possibility that these effects are due to the presence of some anti-oxidant other than the vitamin, but associated with it and subject to oxidation in a similar manner. The possibility that vitamin *A* may be concerned with phospholipid metabolism is discussed. Mattill (*J. Biol. Chem.*, 1931, **90**, 141; ANALYST, 1931, **56**, 200), Oleovich and Mattill (*J. Biol. Chem.*, 1931, **91**, 105; ANALYST, 1931, **56**, 409) and Olcott and Mattill (*J. Biol. Chem.*, 1931, **93**, 59, 65) studied the effect of a number of substances on the induction period of unsaturated fats. The experiments of the authors are concerned only with the period of rapid oxygen consumption following the induction period, and the results obtained by them with carotene and cod-liver oil extract are not in accord with the interpretations of Mattill and his co-workers. The authors consider that, in measuring the pro- or anti-oxidant activities of a substance which itself is subject to oxidation, it is essential to distinguish between the effects of the original substance and those of the oxidised form. P. H. P.

Stimulation of Yeast Growth by Thallium, A "Bios" Impurity of Asparagine. O. W. Richards. (*J. Biol. Chem.*, 1932, **96**, 405–418.)—When different brands of asparagine are used in the culture medium of Williams (*J. Biol. Chem.*, 1920, **42**, 259), the yield of yeast obtained, conditions otherwise being constant, may vary by 65 per cent. An opportunity occurred to investigate the differences between different asparagines. The differences in the yields of yeast obtained are shown to be due to thallium present as an impurity. Thallium was identified spectroscopically in certain of the asparagines. Asparagine which is further purified by recrystallisation gives less growth of yeast. When the asparagine sold by Eimer and Amend is used in the medium, the addition of 0·001 mgrm. of thallium per c.c. of medium gives an increase of 80 per cent. in the yield of yeast. Greater concentrations are toxic and smaller concentrations give

less growth. Concentrations of about 10 mgrms. per c.c. inhibit the growth of the yeast almost completely. Thallium should not be added to the medium when the yeast is to be used as a food for man, because of the extreme toxicity of this element to mammals. Inspection of the literature indicates that thallium may be one of the growth stimulants for yeast that have been referred to as "bios." Elvehjem (*J. Biol. Chem.*, 1931, **90**, 111) showed that the yeast-growth is very poor without the presence of iron and copper. The addition of 0·001 mgrm. of iron per c.c. to a medium containing etiolated yeast gave an increase in growth of nearly 400 per cent. The addition of the same amount of copper increased the yield further, but proportionally less. The inorganic salts and sugar of reagent grade used by the author contain enough iron and copper, as impurities, because on addition of 0·001 mgrm. per c.c. of iron, and of copper in the form of chlorides, there was no increase with the added iron, and slightly less growth when the copper was added. It is believed that attempts to isolate and identify complex organic materials alleged to stimulate the growth of yeast will be premature and unsuccessful until the inorganic food requirements of yeast are known to be supplied completely by culture media. The importance of inorganic foods for the growth of yeast is stressed, and it is suggested that the conflicting observations found in the literature on the growth of yeast are due partly to inadequate culture media and inconstant conditions of growth. P. H. P.

Toxicological and Forensic

Reagent facilitating the Formation of Haemin Crystals from Blood. G. Bertrand. (*Ann. Chim. Anal.*, 1932, **14**, 353–354.)—This reagent is made by dissolving 1 grm. of crystallised magnesium chloride in 1 grm. of water, adding 5 grms. of glycerol (30 per cent.), and then 20 grms. of glacial acetic acid, and mixing; it keeps unchanged in a corked vessel. A small quantity of residue obtained by evaporating the haemoglobin solution is treated with a drop of the reagent and covered with a microscope cover-glass. To examine blood which has dried on a support from which it cannot be completely detached, a fine particle is transferred to a slide and a drop of the reagent is added. The preparation is warmed for a few seconds over a small flame. Microscopic examination then usually reveals crystals of haemin, the number of these increasing if the slide is again heated. Dried blood gradually swells under the action of the reagent, and the first haemin crystals appear in the outside layers. T. H. P.

Organic Analysis

Application of Chromic Oxidation to Certain Alcohols. L. Semichon and M. Flanzy. (*Compt. rend.*, 1932, **195**, 254–256.)—In the study of this oxidation, 5 c.c. of solution, containing 1 mgrm.-molecule of the alcohol were treated at 15° C. with a solution of 0·68 grm. of potassium dichromate in 20 grms. of water and 10 c.c. of sulphuric acid (sp.gr. 1·71). Methyl alcohol is oxidised completely to carbon dioxide and water after 1 hour. Ethyl and other normal

alcohols (propyl, butyl, amyl, hexyl, and heptyl) are all oxidised quantitatively to the corresponding acids in periods varying from 10 minutes (ethyl) to 90 minutes; the acids formed are not attacked further, even after 20 hours. Isopropyl and secondary butyl alcohols, and methylpropylcarbinol give the corresponding ketones quantitatively within 1 hour; acetone is not attacked in 2 hours, and the butyl ketone undergoes very slight attack in 90 minutes. When 5 c.c. of solution containing 0·076 grm. of acetone are heated at 100° C. for 1 hour with a solution of 0·525 grm. of chromic oxide in 5 grms. of water and 5 c.c. of sulphuric acid (sp.gr. 1·71) the acetone is transformed completely into acetic acid. The homologous ketones undergo similar conversion into acetic acid under these conditions. To determine the primary and secondary alcohols present in a wine, this is oxidised in the cold, and the fatty acids and ketones are distilled off in a current of steam; the distillate is then neutralised and the ketones are distilled off alone.

Trimethylcarbinol is not completely oxidised in the cold in 125 hours, but, in concentrated solution, it is transformed quantitatively into acetic acid at 100° C., isobutyric acid, acetone, and propionic acid being formed as intermediate products in the order given. At 15° C., ethylene, propylene and butylene glycols are oxidised (similarly to the primary alcohols) to the corresponding acids. Chromic oxidation of glycerol, erythritol, mannitol, dextrose, laevulose, sucrose, dextrin, or starch yields appreciable amounts of formaldehyde. With the weak oxidising mixture (see above), glycerol is converted quantitatively into carbon dioxide and water in less than 1 hour, and with other polyhydric alcohols containing no methyl group, the oxidation also proceeds as far as carbon dioxide and water. In general, if an alcohol molecule contains one or more methyl groups, the stronger oxidising mixture converts it quantitatively into acetic acid in less than 1 hour at 100° C. If methyl groups are absent, but the molecule contains several CH_2 groups linked together, succinic or a homologous acid is formed quantitatively under these conditions. T. H. P.

Analytical Reactions of Alkyl Mercaptans in Benzene Solution. J. R. Sampey and E. E. Reid. (*J. Amer. Chem. Soc.*, 1932, **54**, 3404–3409.)—In determining mercaptans by iodimetry (*J. Amer. Chem. Soc.*, 1921, **43**, 119) in benzene solutions it is necessary to allow 3 hours in the case of normal mercaptans for the reaction to go to completion, and for long-chained secondary mercaptans 24 hours' contact in the dark is necessary. An intermediate addition-compound between the iodine and mercaptan is apparently formed, but this question is being further investigated. Modifications of this method were studied: (1) *Hydriodic acid method.*—Fifty c.c. of a benzene solution of the mercaptan are left to stand with excess of iodine solution until oxidation is complete; the excess of iodine is removed with thiosulphate solution, the aqueous and benzene layers are separated, the latter is washed with 60 c.c. of water in three portions, and the hydriodic acid in the aqueous layer and washings is titrated with 0·025 *N* sodium hydroxide solution with bromcresol purple or purified litmus as indicator. The method is not quite as accurate as the iodine method. (2) *Mercuric chloride method.*—Fifty c.c. of a benzene solution of the mercaptan are shaken vigorously for 3 minutes with 25 c.c. of 1 per cent. mercuric chloride solution. If no

(or very slight) precipitation occurs, the hydrochloric acid is washed from the benzene layer with three 25-c.c. portions of water, and the acid in the aqueous layer and washings is titrated with 0·025 *N* sodium hydroxide solution, methyl orange or red being used as indicator. If a heavy precipitate is formed the alkali is slowly added to the mixture without separation into layers, with frequent shaking, methyl orange being used as indicator. Results are on the low side, but a correction factor may be found by standardising a volume of sodium hydroxide approximately the same as that required for the titration against 0·025 *N* alkali with the same indicator. Both these methods may be used for mercaptans in naphtha solution which has been freed from unsaturated hydrocarbons, but they break down in the presence of unsaturated hydrocarbons. D. G. H.

Determination of Lactic Acid in Vegetable Tan Liquors. J. H. Highberger and D. L. Youel. (*J. Amer. Leather Chem. Assoc.*, 1932, **27**, 343.)—The method consists in boiling the tannin-free liquors with acid and potassium permanganate, by which means any lactic acid is oxidised to acetaldehyde and, after being distilled off, is titrated with standard iodine solution. The oxidation takes place in a 500-c.c. Kjeldahl flask, the stopper of which has two holes, through one of which passes a thistle-funnel for adding the permanganate solution, whilst the other is for an internally-cooled reflux condenser. The latter is connected through a small receiving flask with an absorption-tower, the top of which leads to a trap and water pump.

Ten c.c. of the tan liquor, diluted to 50 c.c., are thoroughly shaken with a small quantity of basic lead carbonate. Ten to 15 c.c. of 10 per cent. lead acetate solution are slowly added to the settled mixture, and the whole is again shaken, allowed to subside and filtered. The residue is washed five or six times with hot water, and the filtrate and washings are saturated with calcium hydroxide (about 0·75 grm.), and treated with about 0·3 grm. of pulverised copper sulphate crystals. After 20 minutes the precipitate is filtered off and washed with hot water, and the solution and washings are transferred to the Kjeldahl flask, together with 25 to 30 c.c. of 10 *N* sulphuric acid containing 76 grms. of manganous sulphate per litre. The solution is gently boiled, while slight suction is applied through the empty receiving flask for at least 3 minutes. The suction is stopped, the burner is removed, and the receiving flask is replaced by one containing 20 c.c. of 0·15 to 0·20 *M* sodium bisulphite solution. Gentle suction and boiling are renewed, after which 0·1 *N* potassium permanganate solution is run in at such a rate that the solution in the Kjeldahl flask is kept just free from becoming pink. As soon as brown manganese dioxide separates, addition of permanganate is stopped, and the boiling and aspiration are continued for 5 to 10 minutes, after which the absorption-tower is washed down. Starch indicator is added to the receiving flask, and 0·1 *N* iodine solution is run in until the end-point is approached, the final adjustment being made with 0·0222 *N* iodine solution. About 0·5 grm. of solid sodium bicarbonate is gradually added, discharging the blue colour, and the final titration is carried out with the dilute iodine solution (1 c.c. = 0·9 mgrm. of lactic acid).

By this method interfering substances which yield bisulphite-binding materials

on oxidation are removed; oxidisable substances not so removed yield no bisulphite-binding material on oxidation and occasion no loss of lactic acid. The results give the total lactic radical, rather than lactic acid only. Rocker liquors were found to contain 0·15 to 0·4 grm. per 100 c.c. of lactic acid radicle. R. F. I.

Comparison of Chestnut Bark and Chestnut Wood Extracts. A. Ponte. (*J. Inter. Soc. Leather Trades Chem.*, 1932, **16**, 394).—Extracts of chestnut wood contain only a small amount of catechol tan [about 4·3 per cent. of the total tan as determined by the formaldehyde and hydrochloric acid method of Ponte and Gualdi (*Boll. Uff. R. Staz. Sper. Ind. Pelli.*, Sept., 1931)], whereas bark extracts contain nearly 25 per cent. The latter contain more mineral matter, iron, copper and calcium, than the former. Thus, solutions (25° Bé) gave the following results:

	Ash Per Cent.	Calcium oxide Per Cent.	Iron Per Cent.	Copper Per Cent.	p_H of solution
Bark	1·55	0·43	0·013	0·144	3·7
Wood (de-barked)	0·27	0·08	0·007	0·008	2·7

The high copper-content is attributed to the acidity of the liquors in the autoclaves and, to a less extent, in the evaporators. The fact that more copper is present in the less acid material is explained by assuming that the presence of more mineral ash is indicative of a higher content of salts of oxyacids, the action of which on the autoclave is intensified by the oxygen of the air. The low p_H value of the wood extract is attributed to acetic acid formed by fermentation in the wood stacks before autoclaving. The general conclusion is that chestnut woods should be de-barked before being extracted. R. F. I.

Quantitative Determination of Cotton, Wool, Silk and Artificial Silks in Mixed Textiles. P. Krais and H. Markert. (*J. Text. Inst.*, 1932, **23**, 213.)—The material under examination must first be freed from any non-fibrous matter, such as oil, fat, finishing or waterproofing material, by treatment with ether, alcohol, enzymes, dilute acids, or dilute ammonia, followed by 24 hours' exposure in an atmosphere of 60 to 65 per cent. relative humidity. The general type of fibres present is ascertained by the use of the microscope. The quantitative separation of the various fibres is carried out as follows:—The acetate rayon may be removed by treatment with acetone. Five grms. of the sample are boiled with 150 c.c. of 2 per cent. sodium hydroxide solution for half-an-hour under a reflux condenser, filtered on to a fine bronze sieve, washed, dried, conditioned at 60 to 65 per cent. relative humidity, and weighed. Wool and silk are dissolved; cotton, flax and ramie are undissolved. Five grms. of the sample are placed in a glass-stoppered flask, 150 c.c. of 80 per cent. sulphuric acid are added, and the flask is shaken vigorously for 15 minutes, and then every quarter of an hour during 3 to 4 hours. The mixture is filtered, washed and dried as before. All vegetable fibres are removed, but wool and silk remain. Two hundred c.c. of calcium thiocyanate solution (1000 grms. per litre) are placed in a wide-mouthed flask (fitted with a stirring device), and warmed to 70° C., and 1·5 grm. of the sample is added. The whole is heated for 1 hour in a boiling water-bath, with constant stirring, after

which the mixture is filtered, etc., as before. This treatment removes silk, cellulose and acetate rayons, but not cotton or wool. A table is given showing the correction factors which should be taken into account with each treatment. R. F. I.

Drying Rates of Synthetic Resins with Drying Oils. I. China Wood Oil. C. A. Thomas and P. E. Marling. (*Ind. Eng. Chem.*, 1932, **24**, 871–873.)—The drying of commercially-pure China wood oil is accelerated by additions of small quantities of β-naphthol if the drying is carried out under mercury-arc light, or in sunlight, or at high temperatures. The presence of synthetic resins markedly influences the rate of drying of the oil, the rate of drying of China wood oil varnishes depending largely on the type of the resin used. With varnishes prepared with natural or synthetic resins, the drying may or may not be retarded by β-naphthol. In general, however, the drying of neutral resin varnishes is accelerated by addition of the naphthol, and this effect is cumulative when β-naphthol is used in conjunction with metal dryers. T. H. P.

Determination of Small Amounts of Methyl Chloride in Air. F. A. Patty, H. H. Schrenk and W. P. Yant. (*Ind. Eng. Chem., Anal. Ed.*, 1932, **4**, 259–262.)—The method is very similar to that used for the determination of total sulphur in fuel gases. The air containing the methyl chloride is mixed with natural gas and burned in a micro-burner, and the halogen products are made to combine with ammonia from ammonium carbonate cubes round the burner, and also with ammonium hydroxide formed by the ammonia from the ammonium carbonate and the water in the products of the combustion of the gas. The chlorides, some of which are deposited on the upper part of the trumpet tube surrounding the special burner, and the rest from the marble-filled absorption tower, are collected and determined by the Volhard method. The silver chloride is removed by filtration prior to the thiocyanate titration. With amounts of methyl chloride ranging from 20 to 30 mgrms. the accuracy of the method is mostly about 0·5 per cent., and always within 1 per cent., and with amounts as low as 12 mgrms. it is mostly about 1 per cent., and always within 2 per cent. Owing to the solubility of methyl chloride in water, water should not be used as the displacing medium for the most accurate work, although for practical health investigations it may be used if not agitated a great deal or left in contact with the gas-laden air. The same apparatus was also used to determine dichlorofluoromethane and dichlorodifluoromethane in concentrations of 2 and 20 per cent., samples being taken over mercury and diluted with air before burning. D. G. H.

Inorganic Analysis

[Determination of] Small Amounts of Sulphur Dioxide in the Atmosphere. S. W. Griffin and W. W. Skinner. (*Ind. Eng. Chem.*, 1932, **24**, 862–867.)—Various modifications in the iodimetric process, and also a field-testing outfit, are described. Volumes of the air (measured in a meter) are passed through scrubbers or absorbers which contain very dilute iodine solution (about 0·00003 *N*) in aqueous potassium iodide solution, together with a small amount of

starch (25 c.c. of 0·2 per cent. soluble starch solution per litre of the absorbing solution). One scrubber removes over 90 per cent. of the gas from air containing sulphur dioxide in the concentrations usually encountered, and this is regarded as sufficient, the result being corrected for the known efficiency of the particular scrubber under the prevailing conditions.

The air is passed through 100 c.c. of the starch-iodine solution at the rate of 7·5 litres per minute for four minutes, the volume of the air passed being corrected for temperature and pressure. The solution is at once transferred to a 250-c.c. flask, and, after addition of a pinch of sodium bicarbonate, titrated with about 0·0013 *N* sodium thiosulphate solution until a predetermined pale blue colour appears. This standard blue colour is obtained by dissolving commercial dyes, such as Diamond dyes, in water, and adding a trace of Indian ink to produce turbidity similar to that caused by the colloidal starch particles; the colour of such a solution is quite fast, and the standard may be used in the field for some weeks.

The absorbing solution is somewhat unstable towards light and loses a proportion of its iodine when pure air is bubbled through it. As this proportion depends on a number of factors, correction is impracticable, and it is essential that blank tests be carried out either at the same time as, or interspersed with, the actual tests. In these blank tests the air passing to the iodine absorbent is first passed through a tower, 12 inches in height and 1·5 inch in diameter, packed with loose, friable soda-lime; all glass and rubber connections used should be at least 0·5 inch in diameter, as narrow connections interfere with the accuracy of flow-meter readings.

For the field tests, air-suction is provided by either: (1) the intake manifold of the engine of an automobile or the suction tube leading to the wind-screen wiper, or (2) a rotary blower, worked electrically or by hand. In cold weather, the absorbing and measuring apparatus may be placed inside the car and the air drawn in through a glass or tin tube reaching some distance away from the machine. Under usual field conditions the method can detect 0·02 parts of SO_2 per million of air. Mechanically the method is limited by the accuracy of the measurement of the volume of air, namely, $\pm 2{\cdot}5$ per cent. The method is specific for the determination of sulphur dioxide from smelting furnaces, as these do not normally yield hydrogen sulphide. Sulphur trioxide in traces has no effect on the iodine solution. When dust or pollen appear to be present in appreciable amounts in the air, the air-inlet of the flow-meter should be protected by a filter, which is suitably prepared by sucking a thin mat of asbestos or cotton linters on to a fritted glass base or funnel. The first few readings made when such a filter is inserted should be discarded. The method has been used in connection with fumigating tests with sulphur dioxide. T. H. P.

Determination of Silver in Cyanide Solutions. A. Wogrinz. (*Z. anal. Chem.*, 1932, **89**, 120–121.)—The measured solution in a conical flask is diluted and treated with 3 grms. of potassium hydroxide and a pinch of oil-free aluminium bronze powder; foaming and hydrogen evolution indicate complete precipitation of the silver. The precipitate is collected on a porous crucible, carefully washed, returned to the flask, and dissolved in chlorine-free nitric acid. The silver solution

is titrated with thiocyanate (Volhard). The author finds that gravimetric determination of silver in cyanide solution, *i.e.* precipitation with hydrochloric acid and ignition of the precipitate in hydrogen, does not give pure silver, the results being always high. W. R. S.

Separation of Arsenic Trichloride and Germanium Tetrachloride. E. R. Allison and J. H. Müller. (*J. Amer. Chem. Soc.*, 1932, **54**, 2833–2840.)—Germanic chloride is practically insoluble, whilst arsenious chloride is very soluble, in strong hydrochloric acid. The authors studied the distribution of arsenious chloride between germanic chloride and hydrochloric acid, and succeeded in freeing germanic chloride from arsenic by repeated shaking with strong hydrochloric acid in a separating funnel. Antimonic, stannic, and titanic chlorides can be separated from germanic chloride by a single extraction with hydrochloric acid, which is explained by the formation of the complex chloro-acids. Arsenious chloride does not form a compound of this type. W. R. S.

Gravimetric Determination of Tellurium. O. E. Clauder. (*Z. anal. Chem.*, 1932, **89**, 270–282.)—Tellurium, as usually obtained in analysis, is an amorphous precipitate, the easy oxidisability of which complicates its determination. Accurate results are obtained if the element is precipitated in a crystalline condition; this can be achieved by the use of hypophosphorous or sulphurous acids, salts of hydrazine, semicarbazide, or hydroxylamine, or titanous chloride, provided the tellurium solution contains 15 to 20 per cent. of hydrochloric acid, with other conditions as follows: Absence, or almost complete absence, of sulphuric and organic acids; the reducing agent should be added in dilute (5 to 10 per cent.) solution all at once, and in moderate excess, to the cold tellurium solution (bulk, 100 c.c.), which is then warmed by being placed in boiling-hot water. The crystalline precipitate can be dried in a very short time, no oxidation taking place. Potassium hypophosphite is specially recommended as precipitant; 20 c.c. of a 5 per cent. solution were used for 0·02 to 0·3 grm. of tellurium. The cold solution (50 c.c.), placed in hot water, is agitated for 45 minutes by a brisk current of air, and diluted to 100 c.c. The precipitate is collected in a porous glass crucible and washed in succession with 50 c.c. of *N* hydrochloric acid, 25 c.c. of water, and 10 c.c. of alcohol; air is aspirated for 10 minutes through the crucible, which is then dried for one hour at 130° C.

If bismuth is present, the tellurium should be washed with 25 c.c. of a stronger (3 *N*) acid; the quantity of bismuth present should be less than 1·5 grm. Antimony should not exceed 0·75 grm.; the washing is conducted as for bismuth. Copper in quantities below 0·5 grm. is not precipitated by hypophosphite at the prescribed acidity. Lead as chloride contaminates the precipitate; in such a case the precipitate is dissolved in nitric acid, the solution is evaporated twice with hydrochloric acid, and the precipitation is repeated. Iron, cobalt, and nickel do not interfere.

The precipitation of tellurium dioxide by the method of Browning and Flint requires practice, as the precipitate is soluble in acids, as well as in ammonia. The author recommends the use of hexamethylenetetramine for the adjustment of the p_H. The solution of tellurous acid, containing 1 grm. at least of ammonium

chloride, is treated with ammonia to permanent cloudiness, then with 10 c.c. of *N* hydrochloric acid, diluted to 100 c.c., and boiled. It is precipitated while boiling, with 10 c.c. of a 20 per cent. solution of the base, added drop by drop. After standing overnight the precipitate is collected in a porous glass crucible and washed with 50 c.c. of water, followed by 5 c.c. of alcohol; it is dried at 130° C. for an hour, then by suction with air dried over calcium chloride for 5 minutes, and weighed as TeO_2. The method effects a separation of tellurium from selenium.

W. R. S.

Determination of Cobalt in Magnet and High-Speed Tool Steels. J. I. Hoffman. (*Bur. of Standards J. Research*, 1932, **8**, 659–668.)—In this method the bulk of the iron is removed from a solution of the steel by means of ether; the cobalt is separated from chromium, etc., by precipitation with sodium hydroxide and peroxide; copper and the remaining iron, etc., are separated from the cobalt by precipitation with cupferron; the cobalt is finally precipitated with α-nitroso-β-naphthol. *Method.*—A 1-grm. sample contained in a 400-c.c. beaker is decomposed by heating with 20 c.c. of dilute hydrochloric acid (2:1); 2 c.c. of dilute nitric acid (1:1) are added, and the liquid is boiled for 2 to 3 minutes and then evaporated to a volume of 5 c.c. Any separated tungstic acid is ignored. The liquid is transferred, with the aid of dilute hydrochloric acid (1:1), to a separating funnel (the total volume of the liquid should not exceed 35 c.c.), and cooled to about 5° C.; 50 c.c. of ether are added, and the mixture is shaken for 1 minute. The lower layer is run off in to another separating funnel; the ethereal extract is washed with 10 c.c. of dilute hydrochloric acid (1:1), and the washing liquid is run off into the second funnel. The contents of this funnel are extracted with a further 50 c.c. of ether, the ethereal layer being washed as before; the aqueous liquids are collected in the original beaker, heated gently to expel dissolved ether, and finally evaporated almost to dryness. Fifteen c.c. of dilute nitric acid (2:1) are added, and the whole is evaporated just to dryness; the residue is dissolved in 50 c.c. of dilute hydrochloric acid (5 per cent.). An aliquot portion of this solution, containing 0·1 grm. or less of cobalt, is poured into 150 c.c. of sodium hydroxide solution (5 per cent.), to which have been added 2 grms. of sodium peroxide; the mixture is heated on a steam-bath for half-an-hour to destroy the excess of peroxide, allowed to cool, and the precipitate is filtered off and washed with hot water. The paper and precipitate are digested with 15 c.c. of hot dilute hydrochloric acid (2:1) until the precipitate has dissolved; the paper is stirred to a pulp and 150 c.c. of water are added. The solution is neutralised with ammonia (with litmus as indicator); 5 c.c. of concentrated hydrochloric acid are added, the solution is cooled to about 10° C., and a slight excess of cupferron is added (a total of 10 c.c. of 6 per cent. solution is normally sufficient). The precipitate is filtered off after 10 minutes, washed with a cold, very dilute solution of cupferron in 1 per cent. hydrochloric acid, and rejected. The filtrate is diluted to about 400 c.c., and 15 c.c. of concentrated hydrochloric acid are added, followed by an excess of a solution of α-nitroso-β-naphthol in acetic acid. The liquid is heated to 60°–70° C. for 20 minutes and filtered; the precipitate is washed with hot dilute hydrochloric acid (1:3), and, finally, with hot water, dried, "burnt off" together with the filter

paper, ignited to constant weight at 750–850° C., and weighed as Co_3O_4. In all accurate work in which more than 0·01 per cent. of cobalt is involved, the oxide must be reduced in hydrogen and the cobalt weighed as metal. When more than 0·1 per cent. of copper is present in the steel, the cobalt metal thus obtained may contain a few tenths of a mgrm. of copper; a correction may be made for this by dissolving the cobalt in hydrochloric acid, diluting the solution and precipitating with hydrogen sulphide, the precipitate of copper sulphide being then ignited, the residue dissolved in nitric acid, and the copper determined colorimetrically with ammonia. The results of test experiments were very good when the cobalt was determined as the metal, but were slightly low when calculated from the weighing of Co_3O_4. S. G. C.

Determination of Titanium in Alloy Steels. J. Arend. (*Z. anal. Chem.*, 1932, **89**, 96–100.)—The drillings (10 grms.) are dissolved in 150 c.c. of hydrochloric acid (1:5 water) in a current of carbon dioxide, the bulk of the acid is neutralised with sodium carbonate, and the solution is diluted to 300 c.c. and treated with barium carbonate emulsion in slight excess, all in an atmosphere of carbon dioxide. After half-an-hour's settling, the precipitate is collected and washed with acetic acid (1:2 water). The precipitate is ignited and fused with carbonate-nitrate mixture, borax being added if alumina is present. The melt is extracted with water, and the solution (containing a little silicate, chromate, vanadate, tungstate, phosphate) is filtered.. The washed residue is ignited and again fused with carbonate The product is dissolved in strong hydrochloric acid, and a little water is gradually added. The solution is treated with tartaric acid, made ammoniacal, heated, and any copper and a little iron are precipitated with hydrogen sulphide. The filtrate from the sulphides contains titanium only, which is recovered by means of *o*-hydroxyquinoline (ANALYST, 1930, **55**, 596). W. R. S.

Separation of Caesium from other Alkali Metals. N. A. Tananaeff and E. P. Harmasch. (*Z. anal. Chem.*, 1932, **89**, 256–262.)—Caesium iodobismuthate, $Cs_3Bi_2I_9$ (Wells's salt) forms sparingly-soluble blood-red hexagonal crystals, whilst none of the other alkali metals yields such a compound. The reaction can be used for the separation of caesium from the other alkali metals, with a negative error of one per cent. The saturated solution of the chlorides is treated with a hot solution of iodobismuthic acid (3 times the required amount of bismuth iodide dissolved in a minimum of hydriodic acid), the liquid being carefully stirred so as to prevent the formation of lumps of precipitate. After some minutes' stirring the beaker is placed in cold water for 20 minutes; the precipitate is collected in a porous glass crucible, washed with a saturated solution of the complex caesium salt and then with water cooled below 7° C., and dried to constant weight at 140° to 150° C. If the washing is conducted with cold water only (to disappearance of the bismuth reaction), the results are slightly lower than 99 per cent. Cs factor, 0·2038. W. R. S.

Test for Iodide. C. I. Kruisheer. (*Z. anal. Chem.*, 1932, **89**, 196–197.)—The aqueous solution of an iodide, treated with an equal volume of 25 per cent. hydrochloric acid and strong sodium sulphite or bisulphite solution, gives

a yellow coloration. If the liquid is shaken with amyl alcohol the latter will assume a yellow colour. The reaction detects 0·0005 grm. of iodine in 2 c.c. of the original liquid; bromides and chlorides do not interfere, nor do the common acid ions. Iodate, hypoiodite, and free iodine give the reaction. If the solution contains coloured organic compounds which would colour the alcohol, they must first be removed by repeated extraction with the solvent until it remains colourless.

W. R. S.

Microchemical

Microchemical Detection of Vanillin and Piperonal. M. Wagenaar. (*Mikrochem.*, 1932, **11**, 135–138.)—*Vanillin.*—Vanillin forms colourless monoclinic prisms, slightly soluble in cold water, readily soluble in most organic solvents. It sublimes readily, forming drops which dissolve easily in water containing some acetone, and, on evaporation, good crystals are formed. The smallest amount recognisable is 1 mgrm. A precipitation test consists in diluting the solution of vanillin in water containing acetone, the vanillin then crystallising in needles having an acute angle of 63°. The smallest amount recognisable is 0·5 mgrm. *Crystalline compounds.*—(1) With concentrated nitric acid vanillin is nitrated, the resulting compound forming good crystals about 0·1 mm. long. The smallest amount recognisable is 0·5 mgrm. (2) With ferric chloride and other weak oxidising agents (such as gold chloride, potassium or ammonium dichromate in dilute sulphuric acid) crystalline oxidation products are produced which are similar, but not identical. That obtained with ferric chloride melts at 302–305° C. (3) Griebel's test (*Mikrochem.*, **1931**, **9**, 311), in which a drop of 3 per cent. hydrogen peroxide and a small drop of 25 per cent. hydrochloric acid are used and the mixture is allowed to evaporate, gives hair-like, dark crystals, melting at about 200° C. Perborates or magnesium peroxide may be used instead of hydrogen peroxide, but not hypochlorites, perchlorates or nitrites.

Piperonal.—Piperonal is slightly soluble in water, and very soluble in organic solvents, and crystallises in long prisms with pyramid-shaped ends. *Precipitation.*—Piperonal behaves in the same way as vanillin in sublimation and precipitation tests. *Crystalline compounds.*—(1) Only in concentrated form (sp.gr. 1·5) does nitric acid dissolve piperonal, giving a yellow colour. The nitrated product crystallises on cooling. The smallest amount recognisable is 1 mgrm. (2) With bromine water hair-like crystals of the brominated aldehyde are formed. The smallest amount recognisable is 0·1 mgrm. (3) With iodine in potassium iodide solution good crystals are obtained. A few crystals of piperonal are stirred into a drop of iodine solution, and the precipitate is dissolved in a few drops of acetone. After evaporation of the solvent the periodide crystallises out in black dichroic hair-like crystals. The smallest amount recognisable is 0·1 mgrm. (4) With phenylhydrazine a hydrazone is obtained, which crystallises from alcohol or acetone, but this reaction is not very distinctive.

J. W. B.

Rinnmann's Green Test for Zinc. A. A. Benedetti-Pichler. (*Ind. Eng. Chem., Anal. Ed.*, 1932, **4**, 336–337.)—The reagent paper is prepared by soaking "ash-free" filter paper in a solution of 4 grms. of potassium cobalticyanide

and 1 grm. of potassium chlorate in 100 c.c. of water, and drying. When slightly heated such paper takes fire, leaving a black ash consisting mainly of cobaltous oxide. A drop of the solution to be tested is transferred to the centre of the paper, about 2 cm. square, and, after absorption of the liquid, the paper is held high above a Bunsen flame. A yellow line first appears along the outline of the drop, the centre then turning brown. The paper is then lighted, and the ash is examined on a porcelain plate. If zinc was present, a disc of green ash will be seen where the drop of solution was added, surrounded by a circular zone containing very little ash. For micro-technique the size of the cobalticyanide paper may be diminished to less than a quarter, and the test solution drawn into the paper by means of a micro-pipette with fine bore. The final appearance of the test will depend not only on the absolute amount of zinc present, but also on the concentration and volume of the zinc solution, etc., and with very small amounts a small thread or delicate network of green fibres only may be seen under the microscope. With the macro-technique the limit of concentration is 1 mgrm. of zinc per c.c., and the limit of identification is 0·05 mgrm. of zinc, and with the micro-method, the limit of concentration is 0·4 mgrm. of zinc per c.c., and the limit of identification is 0·0006 mgrm. The limit proportions for the presence of other metals are: Zinc : cadmium, 1 : 5; zinc : manganese, 10 : 1; zinc : cobalt, 2 : 1; zinc : nickel, 1 : 1; zinc : titanium, 1 : 1; zinc : aluminium, 1 : 2. D. G. H.

Micro-vacuum Distillation. R. A. Smith. (*Mikrochem.*, 1932, **11**, 221–226.)—A simple apparatus for fractional micro-vacuum distillation, with thermo-couple temperature reading, is made from a test-tube (1 cm. diameter) or glass tubing of similar bore. The test-tube is drawn out to capillaries of 1 to 2 mm. inner diameter and 25 to 30 cm. long, each capillary being separated from the next by a short bulb of the original tubing. Three or four bulbs can be formed from one test-tube; the number of bulbs depends on the number of fractions required from the liquid. The mouth of the test-tube is left intact, and can be attached to the vacuum-pump. The other end of the apparatus consists of a capillary tube. The apparatus is bent so that the bulb next to the end capillary is vertical, and the others horizontal, each suitable for immersion in a bath. A thermo-couple (Constantin-nichrome, 0·0125 mm. in diameter) is attached to the vertical bulb. The liquid is introduced by means of the suction-pump through the end capillary, which is then drawn out as a fine tip. By suitable arrangement of heating baths each fraction can be obtained in a different bulb. When the distillation is complete the bulbs can be sealed separately. J. W. B.

Physical Methods, Apparatus, etc.

Optical Determination of Sodium Nitrite. K. Weber. (*Chem.-Ztg.*, 1932, **56**, 642–643.)—Unlike all other colourless inorganic salts, sodium nitrite exhibits marked absorption of ultra-violet light of wave-length between 300 and 400$m\mu$, the absorption following the law, $A=100(1-10^{-\epsilon cp})$, A being the percentage of the light absorbed, p the thickness of the layer in cm., and c the concentration of the sodium nitrite in grms. per 100 c.c. A curve is given, showing the variation,

with concentration, of the absorption of a 2-cm. layer of the nitrite solution, for light of 386$m\mu$ wave-length. To determine the content of sodium nitrite in a pickling salt, 10 grms. of the salt, which should contain from 0·5 to 0·6 per cent. of the nitrite, are dissolved in water, and the solution is made up to 100 c.c. The ultra-violet absorption (386$m\mu$) is then measured by means of Plotnikow's fluorometer (*Z. Elektrochem.*, 1929, **35**, 432), and the content of sodium nitrite is read off from the curve. With practice, the absorption can be measured to within 1 per cent., this corresponding with an accuracy of 0·015 per cent. for the dry salt; the measurement occupies about 5 minutes. In laboratories where a quartz lamp, a polarisation colorimeter, a step-photometer, etc., are available, a fluorometer can be easily assembled, a suitable fluorescing substance being a 0·1 per cent. solution of quinine sulphate in *N* sulphuric acid. T. H. P.

Spontaneous Ignition of Beech-Wood Charcoal Dust. E. Möhlau. (*Chem.-Ztg.*, 1932, **56**, 581–582.)—The spontaneous ignition which is proved to occur when finely divided carbonaceous matter is stored in bulk has been studied in the case of beech-wood charcoal. By the method of Dennstedt (*Z. angew. Chem.*, 1912, **28**, 2627; *Chem.-Ztg.*, 1919, 402), as modified by Mildner (*Braunkohlarch.*, 1927, **15**, 42), in which a current of oxygen is made to pass through a column of the powder kept under controlled conditions of temperature, the following results were obtained:

State of division	Commencement of spontaneous rise of temperature °C.	Ignition point °C.	Time of burning	
			at 120° C. Minutes	at 130° C. Minutes
Coarser grains	110–120	180	30	22
Finest grains	95–100	170	30	16

The temperature of spontaneous ignition of beech-wood charcoal dust is stated to be near to that already established for lignite dust. On the basis of the above results the author considers that a rise in temperature to 50° C. within a mass of the material would definitely indicate the onset of auto-oxidation which would ultimately lead to spontaneous ignition, and, therefore, proposes that when large quantities of this material are stored, some form of automatic temperature indicator should be employed, in order to give warning in time for preventive measures (which are not detailed) to be taken. S. G. C.

Reviews

ORGANIC SYNTHESES. Collective Volume I. Editor-in-Chief: HENRY GILMAN. Pp. ix+564. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. Price 37s. 6d. net.

To those who, during the past years, have used the previous nine annual publications of "Satisfactory Methods for the Preparation of Organic Compounds," the issue of the collective volume, with its copious indexes, is indeed welcome. The new work is more than a reprint of the separate books, for the whole subject matter has been recast. In the separate volumes one occasionally finds notes

referring to newly-discovered improvements in previously published methods; in the Collective Volume these are either incorporated in the process to which they belong or are inserted in their proper places. The 260 or so preparations described are arranged in alphabetical order.

As in the original publications, each preparation is set out under the headings: (i) Scheme or equation representing the reactions; (ii) details of procedure; (iii) notes; (iv) summary of methods available; and (v) original references. Since the object of the authors is to provide, after practical test, details for obtaining the maximum yield in the preparations described, no theoretical discussions, such as are a feature of books like Gattermann's *Praxis des organischen Chemikers*, are to be found. In passing, it may be mentioned that some of the formulae used are a little difficult to recognise; thus, $HO_2CCH_2C(OH)(CO_2H)CH_2CO_2H$ for citric acid would be improved by a few full stops. There seems to be no reason why such formulae should not be written structurally.

The range of preparations is extensive, and those engaged in organic research or the manufacture of fine chemicals will do well to consult this book. For the analyst, however, only a few of the preparations are of interest, for, while details are given for the preparation of cupferron, isatin, aurin-tricarboxylic acid and α-nitroso-β-naphthol, no mention is made of 8-hydroxyquinoline, dimethyl-glyoxime, or diethyl-dithio-carbamate. Only one indicator, *viz.* methyl red, is mentioned. Among the substances of general interest which are treated in detail may be mentioned ketene, nitromethane and *p*-nitrobenzoyl chloride.

The book is well bound and clearly printed; the illustrations, though few in number, are excellent; and the five thumb-indexes make it extremely easy to use. The indexes are under the following headings: (i) Type of Reaction; (ii) Type of Compound; (iii) Formula (after the style of Richter's Lexicon); (iv) Illustration; and (v) General.

Collective Volume I is up to date, for, during its compilation, the literature up to December 1, 1931 has been consulted. The authors are to be congratulated on having given to the world a set of thoroughly reliable directions for the preparation of what may be regarded as "key-compounds" in organic chemistry, and it is with some impatience that we realise that Collective Volume II cannot be published before 1942! Nevertheless, in the meantime, all whose work, from whatever point of view, is connected with the preparation of organic compounds should obtain a copy of Volume I. HAROLD TOMS

THE SCIENTIFIC PRINCIPLES OF PETROLEUM TECHNOLOGY. By Dr. LEO GURWITSCH and HAROLD MOORE. Pp. xii+572, lx plates. London: Chapman & Hall, Ltd. 1932.

The translation by Mr. Harold Moore of Professor Gurwitsch's book has become greatly enhanced in value by the new edition, containing the latest results of modern investigations.

The book is divided into three parts: I, Chemistry and Physics; II, Manufacture; and III, Products.

The first part, on chemistry and physics, explains the scientific facts regarding petroleum and its fractions. The action of oxygen and oxidising agents is also

dealt with at length (40 pp.). The chemistry of oxygen, sulphur and nitrogen compounds contained in petroleum oils is dealt with more briefly. Of special interest is a fairly full table showing the sulphur-contents of crude oils from North American sources. The section on natural gas could, perhaps, have been expanded, since the technique used in the recovery of natural gas is now becoming more widely applied.

The section on physics deals with specific gravity and viscosity, surface tension, and the optical, electrical and thermal properties of oils. The section on the power-factor of electrical insulating oils is a new feature which should be of considerable value.

Sections C and D form an interesting and concise statement of the differences which distinguish petroleums obtained from different sources, together with a review of modern ideas on the vexed question of the origin of petroleum.

Part II, dealing with manufacture, includes sections on distillation and refining. The section on distillation and heat treatment (30 pp.) includes steam, vacuum and combined distillation, and distillation in inert gases. The treatment is from a scientific, rather than from a technical point of view. A new section includes modern advances in the production of motor spirit, with particular reference to liquid- and vapour-phase cracking, and to hydrogenation. The refining of oils with sulphuric acid is dealt with at considerable length (40 pp.), and includes all the latest important data collected from researches in this difficult field. Alternative methods of refining are also dealt with fully, and include processes involving the use of sodium plumbite, hypochlorite, selective solubility and sulphur dioxide.

The final part, Part III, deals with the properties of motor spirit, illuminating and lubricating oils, paraffin wax and vaselines. The section on motor spirits includes a *résumé* of modern theories upon the cause of detonation in the internal combustion engine.

The whole book is concerned with the scientific facts and principles underlying petroleum technology rather than with the technical or commercial aspects involving the preparation of petroleum products for the market. It is of particular interest, therefore, to the technical man dealing with petroleum technology, but should also have a definite appeal to the commercial man, as explaining fully the underlying principles in the preparation of petroleum for the market.

Mr. Harold Moore is to be complimented on having brought this work so thoroughly up to date.

The book is well printed and is presented in a clear and well-arranged form.

J. G. KING

AUSGEWÄHLTE METHODEN FÜR SCHIEDSANALYSEN UND KONTRADIKTORISCHEN ARBEITEN BEI DER UNTERSUCHUNG VON ERZEN, METALLEN UND SONSTIGEN HÜTTENPRODUKTEN. MITTEILUNGEN DES CHEMIKERFACHAUSSCHUSSES DER GESELLSCHAFT DEUTSCHER METALLHÜTTEN- UND BERGLEUTE E.V., BERLIN. Pp. 457. 2 Auflage. Berlin: Gesellschaft Deutscher Metallhütten- und Bergleute, e.V. 1931.

In 1920, a chemical committee was formed by the Gesellschaft Deutscher

Metallhütten- und Bergleute (the German society dealing with metalliferous mining and smelting) to get together a collection of methods which could be recommended for the referee analysis of materials coming within the sphere of the Society's interests. The present committee is composed of some fifty members from various metallurgical and manufacturing concerns, railways, etc., and includes a number of well-known University workers. The work of the committee appeared in 1924–6 in the form of two volumes of methods, which, in the present (2nd) edition, are collected together, with certain additions, in one volume.

The book contains 21 chapters, the first (2 pp.) dealing with generalities in connection with referee analysis, and the succeeding ones each devoted to an individual metal (or substance) or group of metals as follows: aluminium, antimony, arsenic, beryllium, lead, cerium, noble metals, cadmium, carborundum, cobalt, corundum, copper, magnesium, nickel, mercury, selenium and tellurium, bismuth, zinc, tin, "alloy-steel" metals (chromium, molybdenum, vanadium, tungsten). Each chapter has been prepared by a member of the committee having special experience of the matter dealt with, and has been approved by a small sub-committee. These chapters give plain, straightforward descriptions of the main methods for the determination of the element in question, its essential separations from other elements, and methods for its determination in the ores, alloys, etc., which are most likely to be met with in practice by the analyst.

The methods appear, on the whole, to be well-chosen and sound, and up-to-date as judged by the references to Continental literature. Recent English work is not quite so entirely neglected as is often the case in a German book; thus, it is gratifying to note that one example of it is recommended, *viz.* the internal electrolysis method for bismuth in lead recently published in the ANALYST (H. J. S. Sand, ANALYST, 1930, **55**, 309; E. M. Collin, *id.*, 312), and a reference is made to T. B. Smith's outstanding book, *Analytical Processes* (1929). It would, however, have been possible to improve the book in some directions by the inclusion of more of the advances made during the last few years in laboratories in this country and America. A case in point is the method given (p. 366) for determining antimony in commercial tin, which is as given in the first edition, although this was criticised, on the grounds of probable slight loss of stibine during the solution of the tin in hydrochloric acid, by the reviewer (ANALYST, 1928, **53**, 374).

Considering the wide field covered, the book is moderate in size, and the authors, by adopting a concise style which makes the text easy to follow, have been successful in describing a very large number of methods without sacrifice of essential details. The hope expressed in the preface, that the book might gain many friends, is certain to be realised. S. G. CLARKE

ANALYTICAL CHEMISTRY. Vol. I. QUALITATIVE ANALYSIS, BASED ON THE GERMAN TEXT OF F. P. TREADWELL. Translated and Revised by WILLIAM T. HALL. Eighth English Edition. Pp. xi+640. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. Price 28s. 6d.

That a new edition of this well-known text-book has been called for only two years after the publication of the seventh edition testifies to its wide popularity among English-speaking teachers and students of advanced qualitative analysis.

The last edition suffered from the presence of a large number of printer's errors, and contained several sections which did not fully represent the state of our knowledge of the particular subject at the time of writing. Practically all the errors have been corrected, and the weak parts of the book have been re-written. In his work the author has had the able co-operation of Dr. W. R. Schoeller, who has, among other things, replaced the old sections on tantalum and niobium with new material representing the latest advances made in the qualitative analysis of these troublesome elements.

The only important corrections omitted are in the periodic table of the elements (p. 72), where a tangle in arranging the rare-earth and platinum groups seems to have occurred. A minor criticism might be made of the middle paragraph on p. 500, which describes the separation of caesium from rubidium and potassium by precipitation as chloroantimonite; as this method has already been given at the top of the page, as well as on p. 498, the paragraph is redundant.

A new feature of this edition is a syllabus of the course of instruction in qualitative analysis, as given at the Massachusetts Institute of Technology; this section, which extends to over 26 pages at the end of the book, includes laboratory directions, lecture topics, and a series of well-selected questions suitable for class exercises at the end of a lecture, and is designed to show the utility of the book as a laboratory manual for home study and for reference purposes.

As it now stands, the book is undoubtedly one of the best English works on qualitative analysis. A. R. POWELL

SULPHUR BACTERIA. By D. ELLIS, D.Sc., F.R.S.E. Pp. ix+261, with 66 illustrations. London: Longmans, Green & Co. 1932. Price 21s.

This volume, with its cover appropriately enclosed in a paper of true sulphur yellow, is a monograph on the sulphur-producing bacteria whose habitats are the malodorous muds containing the sulphides of hydrogen and other elements occurring in ponds, rivers and seas in many parts of the world.

The text comprises an extensive summary of our present knowledge of these interesting, but in many ways puzzling, organisms, and is, perhaps, the first attempt to provide a *résumé* of many diverse and scattered investigations conducted during the past century. The most outstanding characteristic of these bacteria is the property they exhibit of liberating a solid element in the free state from at least one of its compounds, but there are many points in their life history which more or less differentiate them from the commoner and better-known organisms. The contents of the volume include the production of hydrogen sulphide in nature and its assimilation by sulphur bacteria; the metabolism, culture, classification and description of the many species of these organisms; the intimate structure of the cell; irritability, chemiotactic phenomena and phylogeny of the sulphur bacteria, and the colouring matter produced by the various species. In addition to the usual general index, one of authors' names and another dealing with the organisms described are provided, together with a comprehensive bibliography, each of which appears to be complete and of a high order of accuracy. The text is by no means a mere abstract of the results obtained by other workers, for the long experience of the author in investigating these organisms, extending over a period

of at least twenty-one years, has enabled him to interpolate many valuable and erudite criticisms, besides offering suggestions which are likely to prove invaluable to future workers. The thoroughness with which the subject-matter has been treated is indicated by the sections on the physical chemistry of hydrogen sulphide in aqueous solution; the spectrophotometric investigation of the colouring matters produced by the sulphur bacteria, and the mechanics of ciliary movement. In spite of the numerous workers who have examined many of the species of these organisms, but little definite knowledge has been acquired about the larger number of them, and any investigator attracted by the subject would find an almost unlimited field open to him.

The text throughout is lucid and readable, and the author's sound statements and criticisms are not only of value in connection with the organisms forming the subject of the book, but are of wide application and will enable the reader to avoid many of the pitfalls incident to the study of general bacteriology. As in other branches of this subject, the question of classification is a thorny one, and, after commenting on the systems adopted by Winogradsky, Molisch, Jensen, and other investigators, the author proposes a new one, which appears to be a great advance on those described in the earlier literature.

The book, as a whole, is an admirable production, the typescript being legible and almost free from error; the illustrations, both diagrams and photomicrographs, are clear and well selected, and the binding is well done. There is little meriting adverse criticism; the only items deserving such comment are a reference to "ferrolactate," the formula of which is not given, on p. 24; the substitution of "nef" for "not" on p. 87, and the phrase "highly refractive *drops* of calcium carbonate" on p. 133. The author has carried out what must have been a tedious piece of work in a very satisfactory manner, and has produced a volume well worth the attention of all bacteriologists. T. J. WARD

BREEDING AND CARE OF THE ALBINO RAT FOR RESEARCH PURPOSES. By MILTON N. GREENMAN and F. LOUISE DUHRING. Second Edition. Pp. 121, 18 figures, 11 charts. Philadelphia: Wistar Institute of Anatomy and Biology. 1931. $3.00.

Twenty years ago the use of the albino rat in physiology was confined to a relatively small number of academic investigators. The first edition of this book appeared in 1923, and was in some respects a propagandist venture, although Donaldson's monograph, "The Rat," had preceded it by eight years. For Donaldson the rat was an end in itself, and his statistical survey of the animal's anatomy and physiology, revised and enlarged in 1924, remains a classic.

During the last decade or so, however, the whole position has changed entirely. The rat has ceased to be a mere qualitative instrument for specialised physiologists, and has become a biochemical reagent essential to many branches of bio-assay, more particularly in nutrition and vitamin studies, and to a less extent in endocrinology. If there is any one strain of rat that more than another deserves the qualification "A.R." it is surely the Wistar rat, as witness the increasing number of authors who are content—and rightly—to remark, under the head of "Experimental Methods," simply "albino rats of the Wistar strain were used throughout."

Part of the reason for the steady replacement of mixed and nondescript strains by the Philadelphian breed is to be found in this book. The description of the methods taken to rear a healthy (which means a clean) and a cheerful (which means a healthy) stock should interest even the reader who is not himself directly concerned with the problem. The varied daily menus served at the Wistar Institute secure to the animals a reserve of minerals and vitamins so high that their first generation offspring are, as a matter of fact, useless for deficiency experiments! The anti-infective measures practised are such as might give many a hospital governor food for thought. And the musical entertainment advocated might give the frequenters of British sea-side boarding houses legitimate cause for jealousy.

If the conditions at the Wistar Institute itself are so exceptional as to warrant the description "de luxe," there is a very good reason for it, even though few ordinary laboratories can, or indeed should, attempt to copy them. The Institute has established itself as a world centre for the distribution of A.R. rats. Through subsidiary breeding centres, such as the reviewer's laboratory, the offspring of Wistar Institute ancestors have gone to New Zealand, Buenos Aires, Bombay, Paris, Oslo, Berlin, and numerous other places, and to nearly every university in Great Britain, by rail, road, sea, and air. They have become an essential part of the equipment of many academic, state and industrial laboratories. The stock at Philadelphia must not fail; with the methods advocated and practised by Drs. Greenman and Duhring, it cannot fail.

For those who have no need to take such extreme methods for breeding and care of their animals, there yet remain, in this invaluable book, innumerable hints and suggestions capable of application in humbler animal departments. The chapters on breeding and on diseases and the reference tables are enough to make the book indispensable.

It would, however, be a mistake to suppose that it has been detailed attention to environment, including diet, that has by itself given us the A.R. rat. Something more fundamental was needed, and it is, perhaps, for their insistence on the part played by inbreeding in producing a stock asymptotic to "pure"—a stock that tends continuously to become homozygotic for all Mendelian factors, except in so far as mutations may counteract the tendency—that the workers at Philadelphia have greatest claim on our gratitude. The scientific evidence for this view is to be found in the publications of the Wistar Institute workers themselves—particularly in Dr. Helen Dean King's monograph on "Inbreeding," and in the well-known volume by East and Jones. But the matter can be put fairly simply.

In scientific experiments every reduction in the number of independent variables increases control and accuracy. When animals are used as analytical reagents their genetic "make-up" is an uncontrolled variable, unless animals of identical genetic "make-up" can be compared. The nearest approach we can get to this condition is by using animals as closely inbred as possible, for, the larger the number of ancestors two animals have in common, the more closely alike will they be. A colony of animals bred exclusively by brother-sister mating will, other things being equal, tend to uniformity.

The proof of the homozygosis is in the assay. Winton has shown that intensely inbred animals give, in certain toxicological work, results ten times as certain as an equal number of animals from a mixed stock. The Wistar Institute started its "Tyler Strain" from a pair of animals. In the reviewer's laboratory there are to-day animals removed from these ancestors by some 40 generations, and brother–sister matings have been practised for at least 30 of them. The stock to-day is as fertile and viable as it has ever been, for the bad characteristics thrown up by inbreeding were eliminated at the Wistar Institute during the first dozen or so generations. For later workers the task has been made relatively easy by the foresight, skill and care of Drs. Greenman and Duhring and their colleagues.

A. L. BACHARACH

ASSOCIATION THEORY OF SOLUTION AND INADEQUACY OF DISSOCIATION THEORY. JITENDRA NATH RAKSHIT. Pp. 298. Calcutta: S. C. Auddy & Co. 1930.

This book is written by the Opium Chemist to the Government of India. The author has carried out a number of investigations into the contractions which occur on the dissolution of solids, most of the work being described in the German literature, and in this book he discusses the phenomenon of solution in connection with the properties of specific gravity, contraction effects, surface tension, viscosity, osmotic pressure, thermal effects, optical properties and electrical effects. The views of the author are unorthodox, and he finds little use for the concept of ionic dissociation as an explanation of the deviations from the simple law of mixture usually found in the properties of solutions. As an alternative, an association theory of solution is proposed, it being supposed that a solute can form compounds with a solvent in any proportion. "When a solute dissolves in a solvent all the molecules of both combine with each other in proportion to their dilution, and when the solvent molecules are increased or decreased corresponding association of solvent and solute takes place uniformly. . . . The process . . . is reversible at all conditions." As I understand this theory, there are no free solvent molecules in a solution, but each is bound to a solute molecule. Moreover, "the associated molecules of the solute and the solvent need not bear similarity to those of either component in respect of either chemical, electrical, optical, osmotic, etc., properties." A hypothesis as broad and flexible as this will obviously explain the facts of solution, even if it makes it a very difficult task to correlate the properties of a solution with those of its components, but it is a different matter to substantiate the hypothesis. The author pursues his argument with enthusiasm and vigour, but I closed the book without being convinced of the soundness of his case.

It is admitted in the preface that "it seemed suitable to write this book in English. It would have been happy if I had more control over the language. Readers may have an unavoidable additional inconvenience on account of the book being written in a language foreign to me." While this explains adequately why the argument at times becomes very difficult to follow, it is hardly an excuse for the large number of errors which could have been detected by a more rigorous proof-reading.

R. A. ROBINSON