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# THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS.

## A Study of the Determination of Saccharin, Colorimetrically and by the Ammonia Process.

BY A. F. LERRIGO, B.Sc., F.I.C., AND A. L. WILLIAMS, A.I.C.

*(Under the Analytical Investigation Scheme.)*

*(Read at the Meeting, March 2, 1927.)*

### A. POSSIBLE COLORIMETRIC METHODS.

As some of the reactions to be examined required the application of a certain amount of heat, the effect of heat on saccharin was first investigated.

I. THE VOLATILITY OF SACCHARIN.—According to Allen, sublimation of saccharin takes place just above its melting point (220°–224° C.), but Thorpe states that it occurs at 100° C. Testoni (*Z. Unters. Nahr. Genussm.*, 1909, **18**, 577; ANALYST, 1910, **35**, 63) separates benzoic acid from saccharin by heating in a hot-air oven at 110°–115° C., until constant in weight; in his experience the acid is completely volatilised, whilst the saccharin remains unchanged.

Strangely enough, E. Schowalter (*ibid.*, 1919, **38**, 185; ANALYST, 1920, **45**, 266) states that the two substances cannot be separated from each other by sublimation, since they both begin to sublime at about 120° C. (*cf.* ANALYST, 1926, **51**, 405).

Two quantities, each of 2 grms., of pure saccharin, spread in flat dishes, after standing some hours in a dessicator, were heated for 4½ hours at 100° C., without loss of weight. On heating for 21 hours at 115° C., in an air oven, the rate of loss was 0·001 grm. per hour. At 130° and 165° C. the rates of loss were, respectively, 0·003 and 0·024 grm. per hour.

II. BORNSTEIN'S REACTION.—(*Z. anal. Chem.*, **27**, 165). The residue from the ether extraction of the acidified sample is directed to be heated with resorcinol and a few drops of sulphuric acid in a test-tube until swelling occurs, and

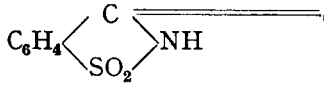
then cooled. After repetition of the heating and cooling processes several times water is added, and the solution neutralised with sodium hydroxide. A red-green fluorescence indicates the presence of saccharin. (This is comparable with the general fluorescein reaction between *m*-dihydric phenols and the inner anhydrides of di-carboxylic acids.)

The reaction is also given, according to Gannter (*Z. anal. Chem.*, **32**, 309) by hop resin, and, according to Durand (*J. Ind. Eng. Chem.*, 1913, 987), by many other organic substances.

Working with quantities of saccharin of the order of about 1 mgrm., we found it difficult to obtain successful qualitative results. A sample of resorcinol by itself gave a well-defined fluorescence under the same conditions.

III. KASTLE'S REACTION.—Kastle (*U.S. Public Health Bull.*, 1905, **26**, 31) directs the saccharin to be heated for five minutes, at a temperature of from 160° to 170° C., with a mixture of 5 c.c. of phenol and 3 c.c. of sulphuric acid. After the product has been dissolved in water, the addition of potassium hydroxide gives a purple colour. Thorpe states that the colour is proportional to the amount of saccharin present, and Parry says that 0·25 mgrm. may be detected in this way.

The condensation of phenols and saccharin, of course, produces bodies analogous to phenolphthalein; in this case the product is




According to Klostermann and Scholta (*Z. Unters. Nahr. Genussm.*, 1916, **31**, 67–78; ANALYST, 1891, **16**, 309) the reaction is given by clove and cinnamon oils, salicylaldehyde and benzaldehyde, gum benzoin, tannin and vanillin.

Attracted by the readiness with which this reaction is given by small quantities of saccharin (considerably less than 0·25 mgrm., in our experience) we spent a considerable time in investigating its possibilities as regards quantitative work. The attempt failed, mainly for two reasons:

(1) *The extreme Susceptibility of the Reaction to the Conditions of Experiment.* (*a*) *Time.*—The reaction is by no means complete in five minutes; on the contrary, when 1 mgrm. is taken, the resulting colour increases very considerably with the time of reaction, up to about 1 hour. (*b*) *Temperature.*—The reaction does not proceed very quickly below 150° C. (not at all at 100° C.), and above 180° C. the rate increases rapidly, with a tendency for charring to occur. Incidentally, saccharin is very appreciably volatile at the temperature used, although it was not actually proved that this was sufficient, under the conditions of the experiment, to affect the result.

(2) *The Effect of the Presence of Impurities.*—In actual practice the final extract of the sample under examination may contain, in addition to saccharin, small quantities of any acid organic matter that may be present. This gives rise to sufficient charring to prevent any accurate comparison of colours, even supposing that other circumstances were favourable.

Very small quantities of tartaric, citric, lactic, and tannic acids all gave brownish colours in Kastle's reaction. Formaldehyde must be added to the list of substances which respond to this test in a similar manner to saccharin.

It is interesting to note that Durand (*loc. cit.*), searching for a reliable qualitative test for small quantities of saccharin, condemned Kastle's test on account of the readiness with which impurities interfere with or prevent the appearance of the characteristic colour. Incidentally, it may be pointed out that the title of his paper refers to the "determination" of saccharin, whilst actually "detection" only is its subject; this error persists in the ANALYST abstract (**1914, 39,** 86).

IV. SCHMIDT'S REACTION.—Its conversion into salicylic acid by fusion with sodium hydroxide has long formed the basis of a qualitative test for saccharin. The reaction appears to be due to C. Schmidt (or Schmitt) (*Rep. anal. Chem.*, **30**; ANALYST, 1887, **12**, 200); he advises evaporation of the ethereal residue (of an acidified wine) with a few c.c. of sodium hydroxide solution, heating when dry for half-an-hour up to 250° C., and then testing the fused mass, after cooling, for sodium salicylate in the usual way. Several other writers give additional details and varying procedure for this qualitative test. H. E. Cox states that the method may be made approximately quantitative.

Proctor (*J. Chem. Soc.*, 1905, T., 242) states as follows:—"The transformation of real saccharin into salicylic acid, appears to be a fairly quantitative reaction under suitable conditions." He is speaking here of commercial samples of saccharin, and says that a pure sample must be treated alongside the sample under examination as a control. "Apparently very slight variations at any stage of the experiment will cause somewhat wide differences in the results, and rigorous attention to every detail is therefore necessary. There is always some difficulty in exactly matching the coloration produced by the iron salt." He says later, however, that, "When applied to commercial saccharin, the salicylic acid method is a comparatively simple process," but no information is given as to the exact conditions or degree of accuracy.

E. Carlinfanti and P. Marzocchi (*Boll. Chim. Farm.*, 1911, 609; ANALYST, 1912, **37**, 22) appear to have been able to convert saccharin into salicylic acid quantitatively, but no details are given. H. Bonis (*Ann. Falsif*, 1917, **10**, 210; ANALYST, 1917, **42**, 303) states that saccharin may be determined by Schmidt's process, but says that a control test with pure saccharin must be made alongside the sample. He does not give the results of any experiments on the reliability of this as a quantitative method.

C. B. Gnadinger (*J. Assoc. Off. Agr. Chem.*, 1917, **5**, 25) prefers the determination of saccharin by the sulphur method to that of Schmidt.

Durand (*loc. cit.*) draws attention to the fact that treatment of saccharin with concentrated sodium hydroxide in the cold for several hours results in the formation of sodium benzoate, and states that this is utilised as a qualitative test in his laboratory (Dept. of Health, N.Y.). The difficulty referred to by Proctor with regard to matching the colour produced by the iron salt, was repeatedly encountered by us during our attempts to convert saccharin into salicylate; the

purple colour was frequently more or less modified by a pronounced turbidity. It seems very probable that this is due to the formation of a certain amount of benzoate, as well as salicylate; and although this was very slight, it was sufficient in some cases to vitiate any attempt at accurate comparison. Most of our experiments were free from this trouble, but no conclusions were arrived at as to the nature of the conditions favouring benzoate formation.

We made from 30 to 40 experiments, following Schmidt's original method in outline, with varying conditions, and made the following observations:

(1) *Temperature.*—Variation of temperature between 200 and 240° C. appears to have little effect on the course of the reaction. Above the higher temperature charring occurs, and even below it there was occasionally noticed a suspicion of a phenolic odour, suggesting loss by decomposition. Conversion of saccharin into salicylate takes place, to some extent, at 150° C., but none was observed at 100° C.

(2) *Time.*—The time of reaction was varied from 15 to 80 minutes without affecting the amount of salicylate formed.

(3) *Sodium Hydroxide.*—Both 15*N* aqueous and 3*N* alcoholic sodium hydroxide were used for evaporation with the saccharin before the final fusion; this was also carried out in many cases directly with solid sodium hydroxide. The results did not indicate decided superiority of any one method over the others.

(4) *Conditions of Heating.*—The actual heating of the mixture of saccharin and caustic soda was conducted

(i.) *In an air-bath* : (*a*) in silver crucible; (*b*) in open porcelain dishes; (*c*) in open glass tubes; (*d*) in sealed glass tubes.

Fusion in open tubes gave better results than in open dish or crucible; the melt was clear in the former case and free from the crust of carbonate that formed under the latter conditions. Sealed tubes gave no better results than open ones.

(ii.) *Over flame in open dishes.*—This method was difficult to control and gave very low results.

(5) *Results.*—In each case the residue, after being heated with sodium hydroxide, was taken up with water, acidified, and extracted with ether. The ethereal solution was usually shaken out with dilute sodium hydroxide solutions, and the salicylate in the neutralised alkali extracts was determined colorimetrically by means of iron alum solution.

In some cases the ethereal solution was allowed to evaporate spontaneously and the residue weighed; invariably the weight was in excess of the amount of salicylic acid present, as found by subsequent colorimetric determination, suggesting the presence of unaltered saccharin.

The amounts of saccharin taken varied from 0·001 to 0·500 grm., but most of the experiments were on quantities below 0·02 grm. The results were erratic in the extreme, the amount of saccharin converted into salicylate varying, for the most part, from 60 up to 90 per cent. Many experiments, however, gave

lower results. Equal quantities of saccharin under apparently identical conditions sometimes gave widely divergent results.

Schmidt's reaction will readily detect less than 0·001 grm. of saccharin, but it does not seem readily adaptable to quantitative work.

V. TARUGI AND LENCI'S REACTION. (*Rend. Soc. Chim. Ital.*, 1911, **7**, 320; abstr. *J. Soc. Chem. Ind.*, 1915, 569.)—A small quantity of saccharin is heated with a few drops of concentrated sulphuric acid until white fumes appear, cooled, diluted with water, neutralised with sodium hydroxide, and added to a little phenol dissolved in sodium hydroxide solution. On the addition of freshly prepared solution of sodium hypochlorite, drop by drop, a blue colour is obtained. It was found that 15 minutes' heating on the steam bath was quite as efficient as heating the sulphuric acid to the fuming stage, and had the advantage of eliminating the possibility of charring the saccharin. With quantities of the order of 1 mgrm. the colour is developed slowly over a period of 30 minutes, and in all cases three to four hours' standing were required to reach the maximum. Its intensity, however, is not proportional to the amount of saccharin present, and it was impossible to make quantitative determinations by this method. As a qualitative test for quantities exceeding 0·5 mgrm. the reaction is recommended.

VI. REACTION OF A. LEYS. (*Ann. Chim. anal.*, 1901, **6**, 201.)—A violet tint is produced when a solution of saccharin is treated with two drops of dilute ferric chloride solution and 2 c.c. of dilute hydrogen peroxide.

In our experience this reaction is without value, even for the detection of moderately small amounts of saccharin.

## B. CONVERSION OF SACCHARIN INTO AMMONIA.

On boiling saccharin with dilute acids, the ammonium salt of sulpho-benzoic acid is formed, and the ammonia may be determined in the usual way by distillation; this forms the chief method of determining the purity of commercial samples of saccharin.

We have endeavoured to adapt this process to the determination of quantities of saccharin of the order of a few mgrms., the method followed consisting in:—(1) Heating the saccharin with dilute hydrochloric acid at 100° C. (2) Direct Nesslerisation of the neutralised product against pure saccharin which had been similarly treated.

Some fifty or more trial experiments were carried out (mostly on quantities of 1 to 5 mgrms.), and the following observations were made:

(*a*) A concentration of acid approximately equivalent to 3*N* hydrochloric acid was most suitable for the hydrolysis. (*b*) With such a concentration two hours' heating on the steam bath gave satisfactory results. (*c*) The colour produced by a solution of hydrolysed saccharin differed in tint from that given by a standard solution of ammonium chloride.

An attempt was made to determine the ammonia by means of the blue colour produced by phenol and hypochlorite (P. Thomas, *Bull. Soc. Chim.*, 1912, **11**, 796), but consistent results were not obtained.

Satisfactory determinations could only be carried out by comparison with pure saccharin hydrolysed under the same conditions.

The following is the method finally adopted:—The ethereal extract of the acidified sample is evaporated in a 100 c.c. flask and weighed. A quantity of pure saccharin rather less than the weight of the ethereal extract is weighed into a 100 c.c. flask, and 25 c.c. of approximately 3*N* hydrochloric acid are added to each. The flasks are covered by watch glasses and placed on the steam bath for two hours. After cooling, the solutions are made alkaline to litmus paper by the addition of approximately 3*N* sodium hydroxide solution and made up to 100 c.c. Aliquot parts of these two solutions are then Nesslerised. A quantity equivalent to between 0·5 and 1 mgrm. of saccharin was found to be most convenient.

The following table gives the results of some determinations made by this method on quantities of saccharin unknown to the workers.

| | Present. Per Cent. | Found. Per Cent. |
|---|---|---|
| Solution in water .. .. .. | 0·033 | 0·030 |
| ,, ,, ,, .. .. .. | 0·033 | 0·030 |
| ,, ,, ,, .. .. .. | 0·037 | 0·042 |
| ,, ,, ,, .. .. .. | 0·037 | 0·038 |
| ,, ,, ,, .. .. .. | 0·037 | 0·040 |
| Ginger wine .. .. .. | 0·023 | 0·020 |
| ,, ,, .. .. .. | 0·023 | 0·022 |
| Custard powder .. .. .. | 0·018 | 0·020 |
| Plum jam .. .. .. .. | 0·050 | 0·050 |

In each of the above cases a control experiment was made, in which the same materials without the saccharin were used. In none was any ammonia-yielding residue extracted.

It will be seen that the method just described is fairly consistent and capable of giving results with an accuracy approaching 10 per cent. The advisability of distilling off the ammonia, and then Nesslerising against standard ammonium chloride was considered. This was not adopted, for two reasons: (1) A 10 per cent. accuracy is not far from the limit attained when Nessler solution is used, and one was able to approach this accuracy without the introduction of an extra process. (2) When dealing with such small quantities of ammonia the use of a control experiment as a standard gives a feeling of security.

Distribution Coefficients.—Attention may be called here to a paper by J. W. Marden (*J. Ind. Eng. Chem.*, 1914, **6**, 315) on "Methods of Extraction by means of Immiscible Solvents. . . ." He emphasises the importance, in extracting saccharin with ether from aqueous solution, of making the solution strongly acid.

The following determinations of Distribution Coefficients, made by us, fairly illustrate this point.

Distribution Coefficient of Saccharin between:

| | | |
|---|---|---|
| 1. | Water and methylated ether at 15° C. .. .. | 0·42 |
| 2. | 0·034 *N* hydrochloric acid and methylated ether at 15° C. | 0·12 |
| 3. | 0·34 *N* hydrochloric acid and methylated ether at 15° C. | 0·036 |
| 4. | 0·67 *N* hydrochloric acid and methylated ether at 15° C. | 0·032 |

SUMMARY.—The principal colour reactions of saccharin have been examined and are considered unsuitable for quantitative work. The well-known conversion into ammonia process has proved to be readily adaptable to the determination of small quantities.

Our thanks are due to the Birmingham Public Health Committee for facilities for carrying out this work.

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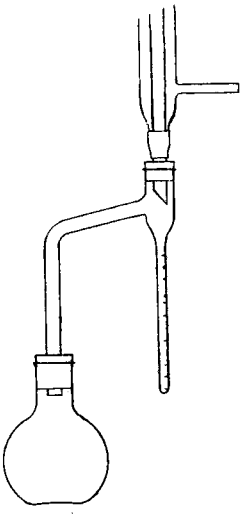
# The Determination of Moisture by the Volatile Solvent Method.

BY J. M. JONES AND T. McLACHLAN

(*Read at the Meeting, May* 4, 1927.)

IN view of the increasing popularity of the method of determining moisture by the volatile solvent method, first recommended by Marcusson in 1905,[1] later by numerous other workers, notably Dean and Stark,[2] and since adopted tentatively by the Association of Official Agricultural Chemists[3,4], it was decided to compare it with other methods commonly in use.



The method consists essentially in placing thes ample, together with a little sand, in a flask with a suitable liquid, which must be immiscible with, and lighter than, water. The liquid is distilled, and the mixed vapours are condensed in a reflux condenser, the condensate falling into a graduated tube. The water settles out at the bottom of the tube and is measured, while the excess of immiscible liquid overflows back into the flask.

The apparatus is shown in the figure. It has been altered slightly from that used by the A.O.A.C., so as to occupy less space. The flask has always been heated in a paraffin bath, owing to the difficulty encountered with substances such as malt extract, due to charring with direct heat.

Different workers have suggested various volatile solvents, such as xylene[6,7], kerosene,[8] a mixture of xylene and benzene,[2] petroleum naphtha,[2] toluene,[5,9] and benzene.[10]

Early experiments showed that for general use xylene is unsuitable, as it causes decomposition and charring of many products, especially those containing sugars. It was therefore obvious that kerosene was unsuitable for general use, as this boils at a higher temperature.

It is difficult to understand the necessity for using a mixture of benzene and xylene or a petroleum naphtha of the specification laid down by Dean and Stark; it appears much simpler to employ a chemical as nearly as possible pure.

Theoretically, all water other than water of hydration should be removed in a reasonable time by any solvent boiling about 100° C., and with this end in view work was begun with benzene, before a reference to Norman's[10] paper was found. Another solvent which appeared very suitable was a sample of petrol boiling fairly constantly at about 104° C.

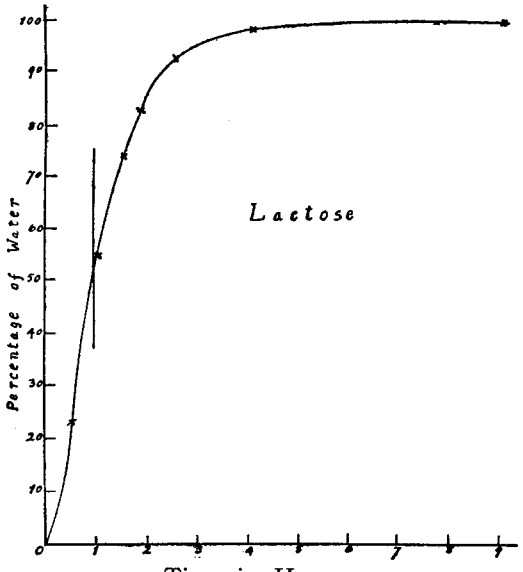
Dean and Stark, and Bidwell and Sterling[5] state that the distillation is, to all intents and purposes, completed in 1 hour. This was not found to be the case in early experiments with toluene and xylene, and it was decided that the safest method was to continue the distillation till no further water came from the samples under examination. In most cases several hours were needed; and for lactose, the distillation with benzene required 16 hours to reach a constant value.

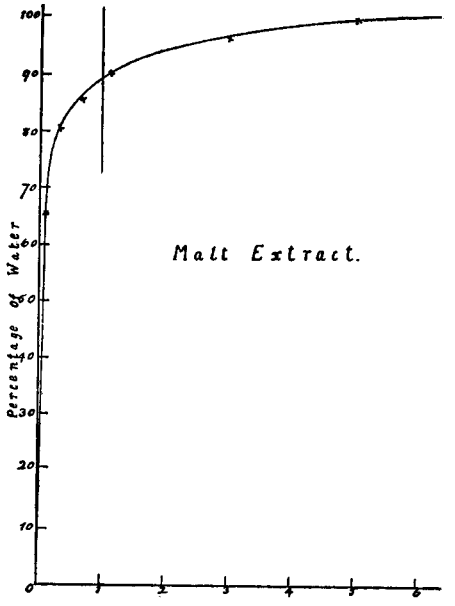
Two other slight modifications have been made. A long thin copper spiral has been introduced capable of fitting inside the graduated tube and the condenser, so that periodically during the distillation this wire can be moved up and down the condenser and, if necessary, the graduated tube, causing the small drops of water to coalesce and fall to the bottom of the graduated tube. In the case of sticky substances pieces of porcelain have been placed on top of the layer of sand in the distillation flask, and the substance under examination placed on top of this. When the flask is heated bubbles of air leaving the porcelain tile are caught in the substance being examined and carry it to the top of the solvent, so that water is more easily removed.

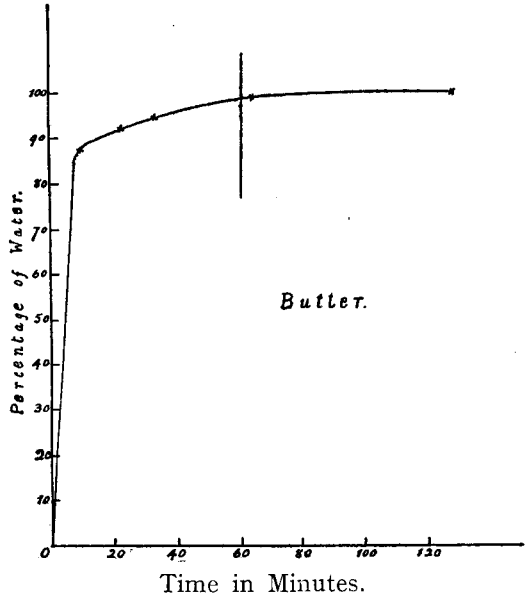
It was thought advisable to construct distillation curves for a few substances, to find out the optimum time required for distillation, and curves have been made for the distillation of lactose, citric acid, malt extract, and butter, toluene being used as the immiscible solvent.

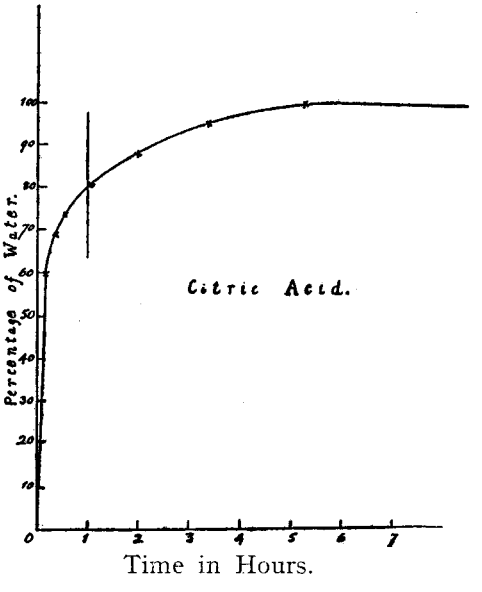
In the case of lactose a little more than half the water is removed in the first hour; with citric acid four-fifths; with malt extract 90 per cent.; and with butter nearly all the water is distilled in 1 hour. It has been found that water due to moisture and water of hydration are always removed simultaneously, and the distillation must be carried on until all water of hydration has been removed. Calculations must be made on the assumption that the substance left is anhydrous. Lactose does not lose its water of hydration below 130° C., when heated in air, but benzene and petrol cause its dehydration, although they require longer than toluene to do so.

It is obvious, therefore, that the only safe method of procedure is to distil until no more water is given off from the substance being treated.


Lactose
Percentage of Water
Time in Hours.


Malt Extract.
Percentage of Water
Time in Hours.


Butter.
Percentage of Water.
Time in Minutes.


Citric Acid.
Percentage of Water.
Time in Hours.

Several commercial products were examined by this method, and the results compared with those obtained in the water oven, air oven and a steam pressure vacuum oven. A constant-temperature air oven is readily obtained by using a small water oven fitted with a condenser, and filling it with toluene instead of water; a constant temperature of 108° C. is maintained. The results obtained are shown in the table.

PERCENTAGES OF WATER BY DIFFERENT METHODS.

| | Benzene. | Petrol. | Toluene. | Water Oven. | Air Oven. | Vacuum at 135° C. | Specific Gravity Method. |
|---|---|---|---|---|---|---|---|
| Jam (1) | 22·56 | 24·62 | 26·92 | 16·05 | 20·78 | 28·86 | — |
| Jam (1) | 18·47 | 23·32 | 25·55 | 17·58 | 19·55 | 26·5 | — |
| Honey | 10·13 | 15·12 | 16·57 | 11·62 | 13·85 | 18·18 | — |
| Wheat Extract (1) | 25·56 | 27·8 | 28·2 | 18·37 | 25·46 | 28·18 | 27·00 |
| Wheat Extract (2) | 21·8 | 25·5 | 25·5 | 16·31 | 24·12 | — | 26·5 |
| Malt Extract (1) | 20·44 | 22·8 | 24·11 | 17·53 | 19·71 | — | 22·25 |
| Malt Extract (2) | 18·95 | 21·7 | 22·2 | 14·71 | 17·30 | — | 21·75 |
| Malt Extract (3) | — | — | 28·8 | 18·8 | 26·0 | — | 27·25 |
| Lactose | 5·0 | 4·96 | 5·27 | 0·06 | 0·08 | — | — |
| Citric Acid | — | — | 8·57 | 8·45 | 8·53 | (Theoretical, 8·55) | |
| Infants' Food (1) | 2·64 | 3·76 | 4·37 | 2·58 | 2·76 | 5·59 | — |
| Infants' Food (2) | 7·39 | 7·45 | 7·47 | 5·81 | 5·76 | 5·76 | — |
| Malted Cocoa (1) | 4·9 | 5·33 | 5·69 | 3·89 | 4·8 | 6·89 | — |
| Malted Cocoa (2) | 4·37 | 4·5 | 4·85 | 3·68 | 4·1 | 6·17 | — |
| Starch | 12·6 | 13·8 | 15·6 | 12.79 | 12·90 | 12·44 | — |
| Fresh Butter | 12·6 | 12·7 | 12·7 | 12·7 | 12·83 | — | — |
| Salt Butter | 12·1 | 12·1 | 12·2 | 12·2 | 12·38 | — | — |
| Margarine | 14·15 | 14·2 | 14·37 | 14·5 | 14·5 | — | — |
| Soft Soap | 35·5 | 35·3 | 36·8 | 31·76 | 33·49 | 38·6 | — |
| Hard Soap | 25·9 | 28·4 | 30·1 | 28·47 | 25·8 | 26·5 | — |

The specific gravity method of determining total solids in malt extracts is merely an approximate one used by the trade. 1·000 is subtracted from the gravity of a 10 per cent. solution of the extract, determined at 15° C. The residue is multiplied by 10,000 and divided by 4, to give the total solids.

Toluene always causes very slight decomposition of malt products, but nothing appreciable. With the Infants' Food No. 1 the distillations with benzene and petrol were not carried far enough; these determinations were made early in the series.

When soap was distilled rosin was added in the distilling flask, as recommended by the A.O.A.C.,[3] and not potassium acid sulphate as recommended by Griffin,[7] or red oil or oleic acid as recommended by Hart.[6]

A sample of oil "foots" was examined for water by distillation with toluene and by drying on the water bath; the results were 24·7 and 24·9 per cent., respectively, but the times taken were about 1 hour and two days.

A sample of paraffin kaolin emulsion was distilled with toluene and found to contain 33·1 per cent. of water. It would have been very difficult to obtain an accurate determination by the water oven method, since only 10 per cent. loss in weight was found after four hours.

SUMMARY.—(1) The method of determining moisture by the immiscible volatile solvent method has been critically examined.

(2) The distillation must be continued till no further water is distilled from the product.

(3) Toluene is recommended as being usually the most satisfactory solvent, as less time is required than with benzene or petrol.

(4) All water of hydration is removed, even with benzene, and this must be considered in calculating results; the water oven and air oven may, or may not, remove water of hydration, according to the substance under examination.

(5) The method is very satisfactory for emulsions, including butter and margarine, and oils, and is far more rapid than ordinary water oven or air oven methods.

(6) This method appears to give more consistent results than any other known at present for substances such as jam, honey and malt extract, but it cannot be said whether they are accurate or not.

(7) For powders and substances which do not cake together, it is quicker and preferable to keep to water oven or air oven methods.

In conclusion, we have to thank Mr. Norman Evers for his continued interest, and Messrs. Allen & Hanburys, Ltd., in whose laboratories this work was carried out.

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# The Relations of the Manley and Reichert Figures for Butter Analysis.

By HERBERT S. SHREWSBURY, F.I.C.

In the February Analyst (1927, p. 67) Manley published an interesting rapid method for sorting butters and margarines, which depends on the determination of a certain proportion of the soluble fatty acids in 5 grms. of fat, expressed in c.c. of decinormal sodium hydroxide solution.

The Manley figures are lower than the corresponding Reichert-Meissl figures, thirteen Reichert values showing a mean of 28·1, as against 23·7, the mean of the thirteen corresponding Manley figures; or (calculated on the Reichert values) a shortage of 15·7 per cent.

Manley suggests that this is due to "preferential solubility of the soluble acids (other than butyric) in the liquid layer of the higher fatty acids (partition co-efficient)."

This did not appear to be a satisfactory explanation, in view of the fact that after thorough washing of the insoluble fatty acids with hot water there was still a shortage, a Reichert–Meissl value of 29 corresponding to a Manley figure of 24·2 after adding the additional acids washed out by the hot water.

This shortage, calculated on the Reichert figure, amounts to 16·6, against the shortage of 15·7 obtained from the mean figures.

It seemed more probable that the difference was largely due to the reactions of the soluble fatty acids to methyl orange, and it is suggested that the following experiments prove this to be the case.

The first six homologues of the fatty acid series were all found to give an acid reaction to methyl orange, but, on titration, the neutral tint of methyl orange was obtained when only a small percentage of each fatty acid had been neutralised. In the case of formic acid as much as 56 per cent. was neutralised.

Titrations were made of 0·1 *N* solutions of the first six homologues with 0·1 *N* sodium hydroxide solution in the presence of methyl orange and phenolphthalein, under the following conditions:

(*a*) Ten c.c. of each acid. With caproic acid 20 c.c. of 0·05 *N* strength. At Trinidad air temperatures (about 30° C.; 86° F.), it is possible to make 0·05 *N* solutions, but the acid is not soluble enough to yield 0·1 *N* solutions.

(*b*) Ten c.c. of each acid mixed with 2 c.c. of glycerol. Twenty c.c. of 0·05 *N* strength in the case of caproic, with 4 c.c. of glycerol.

(*c*) Twenty-eight c.c. of each acid (56 of 0·05 *N* caproic) under the conditions of the Manley determination, *i.e.* 20 c.c. of glycerol sodium hydroxide (made after Manley's formula) were mixed with 100 c.c. of water, made just acid to

methyl orange with $25^{v}/_{v}$ sulphuric acid (about 2·5 c.c.), neutralised with 0·1 *N* sodium hydroxide (0·8 c.c.); blank determined by adding phenolphthalein and further titrating with 0·1 *N* sodium hydroxide solution; this amounted to 1·2 c.c. At the point of neutralisation to methyl orange the 28 c.c. of fatty acid were added (28·1 is the mean of the thirteen Reichert–Meissl values), the liquid titrated with 0·1 *N* sodium hydroxide to the point of neutrality (with methyl orange), and the titration completed after addition of phenolphthalein.

(*d*) The conditions of the Manley determination were more strictly observed, *i.e.* 20 c.c. of the glycerol sodium hydroxide solution were mixed with 100 c.c. of water, 28 c.c. of a 0·1 *N* solution of the acid, and methyl orange as indicator. The mixture was treated with $25^{v}/_{v}$ sulphuric acid until one drop caused an acid reaction: thereafter with 0·1 *N* sodium hydroxide solution until neutral to methyl orange. Phenolphthalein was now added, and the titration made with 0·1 *N* sodium hydroxide solution. This figure, less the blank (1·2 c.c.), which was probably due entirely to carbonic acid, gave a figure corresponding to a Manley figure, but obtained on a single fatty acid. By subtracting it from 28 and multiplying by 100/28, a percentage shortage is obtained, corresponding to the figures given under (*a*), (*b*) and (*c*).

The distilled water used was neutral to phenolphthalein. The following table shows the percentages of each acid which were neutralised (under conditions *a*, *b*, *c*, and *d*) when the methyl orange showed the neutral tint, calculated on the full strength, as shown by the completion of the titration with phenolphthalein.

| | Neutralised to Methyl orange. | | | |
|---|---|---|---|---|
| Acid. | *a* | *b* | *c* | *d* |
| | Per Cent. | Per Cent. | Per Cent. | Per Cent. |
| Formic .. | 56 | 56 | 58·3 | 62·6 |
| Acetic .. | 10 | 10 | 15·0 | — |
| Propionic .. | 8 | 8 | 9·6 | 9·3 |
| Butyric .. | 12 | 12 | 10·9 | — |
| Valeric .. | 10 | 10 | 13·0 | — |
| Caproic .. | 14 | 14 | 12·9 | — |

These figures represent the shortage from the Reichert–Meissl figure (compared with the Manley figure), which would be caused by the varying strengths of ionisation of each acid.

If the composition of butter fat recorded by Lewkowitsch (1922, Vol. II, p. 820) is correct, the acids concerned will be: Butyric, 3·1; caproic, 1·3.

Higher homologues than caproic acid (excluding oenanthic acid), though slightly soluble in water, are neutral to methyl orange. The solubility of caprylic acid is stated to be $0{\cdot}25^{100}$ (Van Nostrand); pelargonic is said to be slightly, and capric acid to be very slightly soluble. Aqueous solutions of these three acids are neutral to methyl orange. Undecylic and lauric acids are said to be insoluble; filtrates of their mixtures with water are neutral to methyl orange.

The shortage, calculated on this basis, for a Reichert–Meissl value of 28 would be 11·5 per cent., whilst the shortages between the thirteen Reichert's and fourteen

Manley's are as follows:—5·7; 8·4; 9·0; 12·0; 13·3; 15·8; 16·6; 17·3; 18·4; 19·6; 20·0; 20·3; 22·4; and 24·2. These figures include the Manley figure increased by hot water washing (shortage of 16·6); the mean shortage is 15·9 per cent.

If it may be assumed that 11·5 per cent. of the shortage is caused in the way suggested, there remains 4·4 per cent. shortage to explain. In the case in which the insoluble acids were further treated with hot water, these figures were obtained by Manley:—Reichert value, 29; Manley figure, 23·1; Manley figure washing, 24.2. On a Reichert–Meissl value of 29 the shortage of $24{\cdot}2-23{\cdot}1=1{\cdot}1=3{\cdot}8$ per cent. may, perhaps, be explained by retention of slightly soluble acids in unwashed insoluble acids. This is not fair, because a single figure is contrasted with the mean of fourteen figures; but may it not reasonably be suggested that the difference between the Manley and Reichert–Meissl figures is mainly due to: (1) In a major degree the reactions of soluble fatty acids to methyl orange; (2) in a minor degree to the retention of a small amount of slightly soluble acids by insoluble acids?

Small quantities of the slightly soluble caprylic, pelargonic and capric acids (neutral to methyl orange, acid to phenolphthalein) would increase the Manley figure; a small amount of such acids is probably retained by the insoluble acids.

There still remains a shortage of 0·6 per cent. on the average, and much larger amounts in individual cases; *e.g.* the largest shortage (24·2 per cent.) occurs with a Reichert–Meissl value of 28·5 and a Manley figure of 21·6. Allowing

$\frac{28{\cdot}5\times11{\cdot}5}{28}=11{\cdot}7$ for acid reactions to methyl orange, and $\frac{28{\cdot}5\times3{\cdot}8}{29}=3{\cdot}7$

for retention of slightly soluble acids, we obtain a shortage of 15·4 per cent., and have not accounted for $24{\cdot}2-15{\cdot}4=9{\cdot}8$ per cent.

These figures, when taken in conjunction with the titration figures for the individual soluble fatty acids, may, perhaps, indicate the possibility of butter fat containing acetic and (particularly) formic acids in the form of glycerides, and this has been stated to be the fact by some workers.

The Manley figure gives an approximation to the Reichert–Meissl value by application of the equation: $R=\frac{100M}{84{\cdot}16}$, where R represents the Reichert–Meissl value and M the Manley figure.

It is, perhaps, worth mentioning that the neutralisation of the lower acids to methyl orange is a very difficult proceeding and will probably be attended by large errors, due to the personal equation.

It is suggested that the difficulties of this neutralisation are reflected in the range of the Manley figures—21·6 to 26·4 or 20·2 per cent. of the mean of thirteen, as against the range of the corresponding Reichert–Meissl values—27 to 29·4 or 8·5 per cent. of the mean; or, expressed differently, a range of 4·8 for the Manley figures against 2·4 (or half the range) for the Reichert–Meissl values.

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# The Presence of Formaldehyde in Wood Smoke and in Smoked Foodstuffs.

By ERNEST HAROLD CALLOW, Ph.D., B.Sc., A.I.C.

Wood smoke has long been known to possess preservative properties and is widely used to preserve many foodstuffs. In addition to its preservative action, wood-smoke also imparts an agreeable flavour to the food. The general ban on the use of preservatives in this and other countries, has never been extended to wood-smoke. On page 11 of the Ministry of Health's "Final Report of the Departmental Committee on the Use of Preservatives and Colouring Matters in Food" (London, 1924) it is expressly stated that "it will be understood that in the term 'preservative' we do not include salt, salt-petre, sugar, etc. . . . or the minute quantities of preservative agents introduced by the process of curing known as 'smoking' . . ."

What are the preservative agents introduced by 'smoking'? A large firm of bacon curers recently raised this question by drawing the attention of the Food Investigation Board to the fact that smoked bacon and ham gave a strong reaction for formaldehyde when tested by Schryver's method.[1] As unsmoked bacon or ham only gave a faint or negative reaction, the smoke itself was naturally suspected as the source of the formaldehyde. A search of the literature on wood distillation failed to reveal any record of formaldehyde having been detected in wood distillates. This is not surprising when it is remembered that the distillation of wood is carried out in the absence of air, whilst for the production of smoke air is essential. In wood distillation methyl alcohol is produced; and this, on oxidation, gives rise to formaldehyde. This substance, therefore, is likely to be a component of wood-smoke. Experiments were therefore carried out to test for the presence of formaldehyde in wood-smoke and in smoked foodstuffs.

Application of Schryver's Method.—Schryver's method[1] for the determination of formaldehyde is the most convenient one which can be employed. For general purposes, the method consists in adding 10 c.c. of the solution containing the aldehyde to 2 c.c. of a freshly made and filtered 1 per cent. solution of phenylhydrazine hydrochloride. To this is added 1 c.c. of fresh 5 per cent. potassium ferricyanide solution, followed by 4 c.c. of concentrated hydrochloric acid. In the presence of formaldehyde, a brilliant magenta-like colour, is developed which reaches its full intensity in a few minutes and keeps without marked deterioration for several hours. The method, as applied to meat, is as follows:—Ten grms. of the suspected meat are cut up finely, placed in a boiling-tube containing 10 c.c. of distilled water and 2 c.c. of the phenylhydrazine hydrochloride solution, and the mixture is heated in a bath of boiling water for 5 minutes and filtered. The filtrate, after cooling, is treated as in the normal procedure. To make the method quantitative, solutions containing known amounts of formaldehyde are made up

and treated with the above-mentioned reagents. A standard series of colours can thus be obtained, and all unknown solutions can be compared with the standards.

In view of the fact that this method has been used in experiments to detect the presence of formaldehyde in wood-smoke and smoked foodstuffs, it appeared advisable to carry out tests designed to prove its specificity for formaldehyde. Accordingly dilute solutions of a number of substances were tested, with the following results:—

TABLE I.

| *Substance.* | *Remarks.* |
|---|---|
| Acetaldehyde | Brown colour, rapidly fading. |
| Acetic acid | Completely negative. |
| Acetone | ,, ,, |
| Acetophenone | ,, ,, |
| Acrolëin | Freshly prepared acrolëin was diluted to 1 part in $10^3$. This solution gave a colour of the same intensity as 1 part in $10^6$ formaldehyde in 5 minutes. In an hour the colour had increased and was equal to 3 parts in $10^6$ formaldehyde. |
| Allyl alcohol | Completely negative. |
| Dimethyl acetal | ,, ,, |
| Formic acid | ,, ,, |
| Formaldehyde | Rapidly forming magenta colour; even a dilution of one in $10^7$ gave a perceptible colour. |
| Furfuraldehyde | Greenish colour, gradually changing to a brown colour. |
| Methyl alcohol | Completely negative. |
| ,, acetate | ,, ,, |
| Methylamine hydrochloride | ,, ,, |
| Pyridine | ,, ,, |
| Sodium nitrate | ,, ,, |
| Sodium nitrite | 1 part in 1000 gave a dull brown colour.<br>1 part in 10,000 gave a complete negative. |
| Wood-smoke distillates | Strong positive reaction. |

In practice, it is unlikely that acrolein would be present in such large quantities as 1 part in 1,000 (for example, in smoked bacon), but, even if it were, it could not make a very large difference to many of the results obtained (see Table II). The other substances chosen in the above list represent classes of compounds or the compounds themselves that might be expected to occur in wood distillates. There is a great probability, therefore, that the colour produced by formaldehyde in the Schryver test is not given by any other substance present in smoke or smoked meat and fish.

IDENTIFICATION OF FORMALDEHYDE IN WOOD-SMOKE.—For the purposes of the present investigation, wood smoke distillates were obtained in the following manner. Oak sawdust, which is the principal type of sawdust used in the smoked food industries, was heated in a glass distillation flask through which a current of air was drawn. The smoke thus produced was passed through a condenser, and the distillate was collected in a Buchner flask. The cooled smoke was then bubbled through water. Both the distillate and the water through which the

smoke had been bubbled gave a strong reaction for formaldehyde when tested by Schryver's method.* Similar results were obtained with deal sawdust and sawdust supplied by a local bacon-smoking firm.

Since the Schryver method had been shewn to be specific, the evidence of a positive test was strong indication of the presence of formaldehyde in wood-smoke.

In order to obtain confirmatory evidence it was decided that an attempt to prepare a crystalline derivative of formaldehyde should be made. The compound chosen was the di-$\beta$-naphthol ether of formaldehyde, which crystallises in long needles and melts at 190° C.

As a control this derivative was prepared from commercial formalin by the following method. Three drops of formalin were added to 2 c.c. of distilled water and 1 c.c. of ethyl alcohol. Abour 0·05 grm. of $\beta$-naphthol was added and then 3 to 5 drops of concentrated hydrochloric acid. The mixture was warmed on a boiling water bath until a cloudiness appeared. The di-$\beta$-naphthol ether crystallised out on cooling and was filtered off, and washed with 33 per cent. alcohol in order to remove excess $\beta$-naphthol. Alcohol was used for recrystallisation and the crystals dried on a porous plate.

In order to prepare the $\beta$-naphthol derivative from the wood smoke distillate it was necessary to remove the tarry matters. This was done by filtration and several extractions with small quantities of ether. Finally the volume of the concentrated, ether-extracted distillate was determined, and half this volume of ethyl alcohol was added. An appropriate amount of $\beta$-naphthol and concentrated hydrochloric acid were added, and the mixture heated on a boiling water bath. Even with all the precautions adopted the product, which crystallised in long needles, had a brown colour due to tarry matter. A long series of recrystallisations was carried out. The crystals obtained were washed with alcohol, and the mother liquors boiled with charcoal before being used to produce fresh crops of crystals. The final product after all these recrystallisations had a melting point of 190° C., which is the melting point of the pure substance. Thus it may be concluded that wood smoke contains formaldehyde.

THE PRESENCE OF FORMALDEHYDE IN VARIOUS SMOKED FOODSTUFFS.—In order to show that wood smoke can cause meat to become impregnated with formaldehyde, the following experiment was carried out. Some unsmoked bacon was purchased, and the lean tested for formaldehyde. The result by Schryver's method was negative (*i.e.* less than 1 part in $10^7$). A rasher which had been smoked for two hours gave a strong reaction (2 parts in $10^6$). The smoking was carried out in a fume cupboard, and the sawdust used was freshly prepared in the laboratory workshop. This was done in order to avoid the criticism that sawdust used in the food-smoking industry might purposely be adulterated with formaldehyde or paraformaldehyde.

An examination of various foodstuffs purchased from Cambridge shops has been made. Whenever more than one sample was used the material was

* The actual colour obtained could be imitated by a mixture of formaldehyde and furfuraldehyde.

purchased from two shops. The results, expressed in parts per million of the material, are given below.

TABLE II.

| Foodstuff. | Concentration of formaldehyde necessary to match intensity of colours obtained. Parts. | Samples. |
|---|---|---|
| Herring .. .. .. | 0·5 to 3·0 | 3 |
| Bloater .. .. .. | 50 to 100 | 2 |
| Kipper .. .. .. | 250 to 1,000 | 2 |
| Ham, smoked, raw .. | 4·0 to 15 | 2 |
| Ham, smoked, cooked .. | 0·5 | 1 |
| Bacon, smoked .. .. | 30·0 to 50·0 | 2 |
| Bacon, unsmoked .. .. | Negative | 2 |

The occurrence of a substance giving a positive reaction with herrings when tested by Schryver's method is well known. It has been suggested that it is trimethylamine which is transformed into formaldehyde during the reaction.[2] The ice in which the herrings had been packed was tested for formaldehyde, but with negative results.

It is worthy of note that Schryver[1] only found 285 parts of formaldehyde per million in meat which had been exposed to formaldehyde vapour. The results obtained for kippers are very interesting and should repay further investigation. In this connection a reference may be made to the work of Dill and Clark.[3] These workers investigated the occurrence of formaldehyde in canned fish and found as much as 33 parts per million. In the case of canned fish they suggest that trimethylamine is not the precursor of the formaldehyde, as is the case with fresh fish.[4]

APPENDIX.—In Germany the use of formaldehyde was forbidden in 1908. Regulations regarding the meat imported into the German Empire were published on February 22nd, 1908.[4] They give explicit directions for the detection of formaldehyde in meat. It will be noted that the procedure is different for smoked meats as compared with unsmoked meats. "Formaldehyde is sought for by taking 30 grms. of the meat cut into small pieces, adding 200 c.c. of cold water, allowing the mixture to stand half-an-hour, acidifying with phosphoric acid and distilling 50 c.c. in a current of steam. In the case of unsmoked meats 5 c.c. of the distillate are mixed with 2 c.c. of fresh milk (free from formaldehyde) and 7 c.c. of hydrochloric acid (sp. gr. 1·12) containing 0·2 c.c. of 10 per cent. ferric chloride in 100 c.c. In the case of smoked meats, a portion of the distillate is diluted with four times its volume of water, and 5 c.c. of the dilution is used. If a violet colour is produced, the remainder of the distillate is evaporated after the addition of ammonia. The crystals of hexamethylene tetramine are looked for and tested with mercuric chloride and potassio-mercuric iodide. The presence of formaldehyde is taken as proved when, in the case of unsmoked meats, the

evaporated *residue* gives a positive results with mercuric chloride, but in the case of smoked meats only if it yields a positive test with both reagents." Japanese workers have actually detected the presence of formaldehyde in smoked meats.[6,7]

FOOD INVESTIGATION BOARD OF THE DEPARTMENT OF
SCIENTIFIC AND INDUSTRIAL RESEARCH AND THE LOW
TEMPERATURE RESEARCH STATION, CAMBRIDGE.

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# Examination for Halophilic Micro-Organisms.

BY WILLIAM CLAYTON, D.Sc., F.I.C., AND WILLIAM EDWARD GIBBS, D.Sc., A.I.C.

"PINK" on salted cod (ANALYST, 1923, **48**, 20; 1924, **49**, 86) and "salt stains" on hides may be produced by salting or "curing" with solar salts. Solar brines and salts almost invariably contain micro-organisms (cocci, bacteria and spirilla), which only exist and develop in the presence of abundance of common salt. A new class of micro-organisms must be admitted, and, as yet, singularly little work has been reported on these so-called halophilic strains. Chromogenic halophilic growths have been described by Harrison and Miss Kennedy (*Trans. Roy. Soc. Canada*, 1922, **16**, 101–152), and Cloake (*Dept. of Sci. and Ind. Res. Food Investigation Board, Special Rept.*, No. 18, 1923, pp. 23), and numerous non-chromogenic cultures were obtained from ham-curing pickles by Sturges and Heideman (*Abst. Bact.*, 1923, **7**, 11; 1924, **8**, 14), and from fermenting cucumber pickles by LeFavre and Round (*J. Bact.*, 1919, **4**, 177–182).

Halophilic cultures cannot be obtained on the ordinary bacteriological nutrient media; at least 15 per cent. of sodium chloride must be present. In the course of extensive investigations in this laboratory a complete examination has been made of the media suggested for the culture of halophilic micro-organisms, and special media have been devised for obtaining rapid growths. The following scheme is followed in routine work.

(1) MICROSCOPICAL EXAMINATION OF BRINE.—A loopful of the brine is placed on a clean, sterile slide, or is mixed on the slide with sterile, 16 per cent. sodium chloride solution. (Water cannot be used, as plasmoptysis occurs and a slimy mass results.) The slide is dried by gentle warming, and then flooded for two

minutes with methyl alcohol. Staining follows with Giemsa's stain for thirty minutes. After being rinsed in water the slide is dried, and a cover-glass affixed with Canada balsam.* Examination follows with the $\frac{1}{12}$ in. oil-immersion lens.

(2) EXAMINATION OF SALT.—The salt (usually solar salt) is dissolved in sterile, 16 per cent. sodium chloride solution in the incubator at 37° C. This concentration of salt prevents plasmoptysis and yet permits fairly rapid solution. The resulting brine may then be examined microscopically, as above.

(3) PREPARATION OF CULTURES.—(*a*) *Chromogenic Strains.* Excellent growths (most frequently as red colonies) develop in from seven to fourteen days on the following medium:—Ten grms. of rice grains in an Erlenmeyer flask are covered with 25 c.c. of fish broth and sterilised in an autoclave. (The fish broth is prepared by digesting 1 lb. of minced fresh cod with water. After the liquid has been filtered and made up to 1 litre, 0·1 per cent. of peptone is added and 20 per cent. of sodium chloride (pure or solar). The $P_H$ is adjusted to 8·2, and the broth again heated in the autoclave, and filtered.) The sterilised medium consists of swollen rice grains containing no free liquid, and presents a clean, white surface. Infection is made with the brine under test and the wool plug sealed with paraffin wax to prevent evaporation. Incubation follows at 37° C. or at 42° C. (optimum growth).

For direct culturing from salts, the salt crystals are added directly to the rice medium. Solution occurs on incubation, and colonies later develop where the salt has been in contact with the medium. This method rapidly decides on the presence or absence of chromogenic micro-organisms in a salt sample.

When sub-culturing chromogenic strains excellent growths are obtained on potato. The usual potato cylinders are soaked overnight in saturated brine. After draining, they are soaked in the fish broth described above. The cylinders are then placed in Roux tubes over fish broth, and sterilised in the autoclave.

(*b*) *Non-chromogenic Strains.*—After it had been found that rice favoured rapid development of halophilic cultures, the following modification of agar jelly was finally adopted. Two per cent. of agar powder and 0·1 per cent. of peptone are dissolved in a mixture of fish water (1 lb. minced cod to 1 litre water) and rice water (25 grms. of rice digested with 1 litre of water) in equal parts by volume. Salt is added to 20 per cent. concentration, the reaction adjusted to $P_H$ 8·2, and the mixture heated in the autoclave and filtered. A clear, almost transparent, jelly is thus obtained, which gives consistently good results. It is essential to prepare agar slopes, as stab inoculations are ineffective. The surface is rubbed with a loopful of the brine under examination, and the plugged tube sealed with paraffin wax, and incubated at 37° C. or 42° C. For the isolation of different species, the usual method of plating out is followed, dilutions being made in sterile, 20 per cent. brine instead of water.

(4) THE EXAMINATION OF SEA WATER.—In our routine examination of sea water samples from the chief ocean routes, the method adopted is to add the sea

* For an alternative method, *vide* Browne, *Abst. Bact.*, 1921, **6**, 25.

water (diluted in sterile, 3·5 per cent. solar-salt solution, when necessary) to the rice medium described above. The medium in this case contains only 3·5 per cent. of sodium chloride, and solar salt is used in preference to pure salt, when making up the fish broth.

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# The Occurrence of Sulphur Dioxide in Malt Vinegar.

BY H. E. COX, M.Sc., Ph.D., F.I.C.

IN addition to their value from the legal point of view, the Preservatives Regulations have proved to be of some heuristic value, and have led to investigations into unforeseen, but forensically important, points connected with the natural or unavoidable presence of substances classed as preservatives in various articles of food. Malt vinegar provides yet another instance of this. As is well known, the first stages of the brewing of vinegar are essentially similar to those of the manufacture of beer; the presence of sulphur dioxide in beer was recognised by the Minister of Health in the allowance, according to the Regulations, of up to 70 parts per million, and it appears from the Report of the Departmental Committee (§ 138) that it was realised that part, at least, of this quantity is unavoidable, by reason of the small amounts of sulphurous acid derived from the materials employed in the brewing operation. "Thus, malt (hops) and finings are frequently a source of sulphurous acid; and after cleansing the casks with sulphite solutions a little sulphite often remains in the casks. The total amount of sulphurous acid from all these sources is not great, but must be taken into account. . . ." In spite of these facts, no mention of vinegar appears in the Schedule of the Regulations. There is, however, the general reservation "that an article may contain any preservative necessarily introduced by the use in its preparation of an article specified in the Schedule."

Of many different vinegars recently examined, not one has been found to be quite free from sulphur dioxide, even samples which have not been in contact with sulphured casks. This led to an enquiry as to what quantity of sulphur dioxide might reasonably be derived from the ingredients themselves; whether the sulphur dioxide is removed during acetification, and what quantity would be introduced by the use of sulphured casks.

Malt doubtless derives its sulphite from the same source as in the past it has obtained its supplies of arsenic, namely, the kilning process. It is remarkable that, although sulphur dioxide is so readily oxidised, small amounts of it remain in contact or in combination with carbohydrate materials for a very long time. An average sample of malt was found to contain 12 parts per million of sulphur dioxide.

The worts prepared from other malts were found to contain 9 and 10 parts per million. In acetified gyles, prepared from malt only, I found 10 and 12 parts

per million, even though there had been no addition of glucose or contact with sulphured vessels. Glucose is sometimes used, in addition to malt, in the manufacture of certain vinegars, and this is permitted to contain, according to the Regulations, 70 parts of sulphur dioxide, if solid, or 450 parts if liquid. Another operation to be considered is the fining. Commercial finings may contain very large quantities of sulphur dioxide; two samples recently examined showed 750 and 7130 parts per million respectively. The second sample was a newly prepared batch and is unusually strong. The addition of even small quantities of such finings materially affects the vinegar. Caramel, also, is a potential, if small, additional source.

The foregoing facts together may account for the following findings in malt vinegar which had been stored for a long time in vats:

| | | | |
|---|---|---|---|
| 1. | 10 parts per million. | 4. | 15 parts per million. |
| 2. | 12 ,, ,, ,, | 5. | 10 ,, ,, ,, |
| 3. | 10 ,, ,, ,, | 6. | 12 ,, ,, ,, |

These quantities, and in fresh vinegars, even larger amounts, appear to be covered by par. (ii) of Section 4 of the Regulations.

There remains to be considered the amount introduced by sulphuring the casks. This operation consists generally in pouring a quantity of bisulphite solution, of about 5 per cent. strength, into the casks. This is swirled round and the casks are drained. The amount remaining depends upon the size of the cask, and is relatively greater in the smaller casks. Experimenting on dry casks I found that the amounts retained were:

| | | | | | |
|---|---|---|---|---|---|
| 6-gallon | cask | $2\frac{1}{2}$ | ozs. | solution. | |
| $12\frac{1}{2}$ | ,, | ,, | $4\frac{1}{2}$ | ,, | ,, |
| 25 | ,, | ,, | $8\frac{1}{2}$ | ,, | ,, |

If the liquor contained 5 per cent. of sulphur dioxide, and the casks were subsequently filled with vinegar, it would impart to the vinegar 130, 112 and 80 parts per million respectively, in addition to the amounts already present from the other causes. In practice, three factors tend to reduce these quantities; one is that the cask may be wet before it is rinsed with bisulphite, in which case the actual amount of sulphur dioxide retained would be less; the second is that the cask may be steamed or rinsed after sulphuring; the third is the continuous oxidation of the sulphur dioxide, which reduces it to a smaller, though by no means negligible, figure.

Samples examined from sulphited casks have been found to contain from 20 to 60 parts per million, some considerable time after being filled.

It is concluded, therefore, that, without any intentional addition of preservative, malt or other vinegar may contain sulphur dioxide, from 10 to 30 parts (or more) per million, derived from the malt, glucose (if used) caramel and finings, and a further and larger quantity from the sulphuring of the casks.

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# Notes.

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

## THE IDENTIFICATION OF SEMINAL STAINS.

In *Lyon's Medical Jurisprudence for India*, by L. A. Waddell (7th ed., p. 744), it is mentioned that, in a great majority of cases, the examination of seminal stains fails to show spermatozoa. Many reasons for the failure are given, including the following:—(1) Spermatozoa are not likely to be found in preparations in which no epithelial cells are seen; (2) spermatozoa are not recognisable if very many bacteria are present; (3) in India spermatozoa are not recognisable, owing to the heat causing decomposition; (4) decomposition for even 24 hours is considered sufficient to render spermatozoa unrecognisable; (5) spermatozoa can rarely be detected on garments which have been wrapped up before drying.

In my opinion these reasons are inadequate, because, when spermatozoa are properly stained, they ought to be visible whether the preparation contains epithelial cells or not, or whether the specimen contains numerous bacteria or few. Nor is there any reason why they should not be recognised in stains on garments which have been wrapped up before the stains are dry.

I think that the chief cause for the failure to detect spermatozoa is that the proper stain has not been known. The stain commonly used in India is that recommended by Hankin, which takes several hours to stain, and in most cases gives unsatisfactory results.

In my medico-legal practice I have obtained positive results in 85 to 90 per cent. of the cases examined, by the use of the following simple method. Most of the exhibits submitted are two or three weeks old, and in some cases positive results have been obtained, even after four months. A piece of cloth is cut off from the garments showing suspicious stains. This is placed in a sterile Petri dish containing just sufficient solution to soak the piece of cloth. After a few minutes the film is gently scraped off the cloth on to a slide by means of a clean knife, spread out, allowed to dry in air, and then fixed with the flame of a spirit lamp. It is now covered with a few drops of carbol thionin, and, after few minutes, is washed with distilled water, and the slide is then placed in a slanting position so that the water drains off. When dry, the slide is examined with a 1/12 oil-immersion lens, after addition of cedar wood oil.

The portion of the spermatozoa which lies between the head and the tail takes the stain more deeply, and this portion is generally semi-lunar in shape. The horns of this semi-lunar body have a tendency to unite in front, and, as a rule, are only very faintly stained. This peculiar staining gives a characteristic appearance to the spermatozoa, and enables them to be readily distinguished from epithelial cells and bacteria. The staining is so selective that the intensity of colour is more pronounced towards the tail, and less so towards the front part of the head. In fresh preparations, however, the tail also becomes stained, but in actual practice, if the stains are of some age, the tails do not absorb the stain. I have used this method here for many years, and have always found it very successful as well as rapid, for an examination can be completed within half-an-hour.

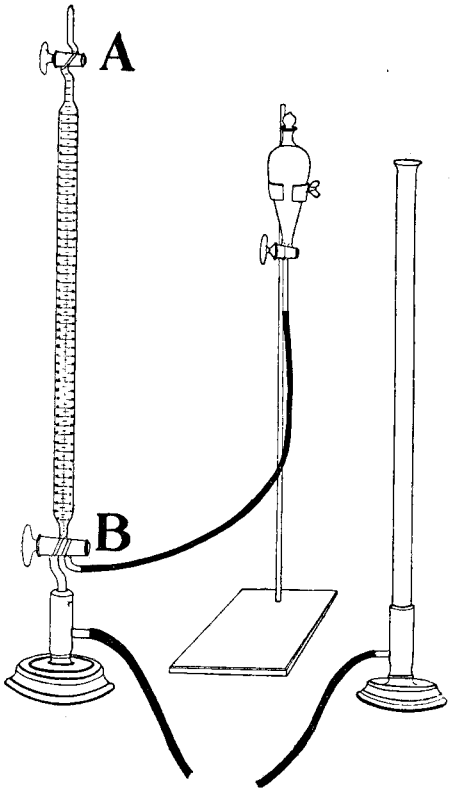
S. Mallannah.

Hyderabad-Deccan, India.

## HEMPEL GAS ANALYSIS APPARATUS WITHOUT ABSORPTION BULBS AND ITS USE IN THE EXAMINATION OF COMMERCIAL OXYGEN.

THE following method of using the Hempel gas analysis apparatus, which avoids the use of absorption bulbs, has obvious advantages when the nature of the gas mixture permits of this modification.

The measuring burette required is of the type with a simple stopcock at the top (A) and a three-way stopcock (B) at the bottom. This burette is connected, by means of a length of rubber tubing, with a levelling burette in the usual manner. The additional tube from B is connected with tubing, as shown in the figure, to a small separating funnel which can be raised to about the height of A.




The details of the analytical process necessary will vary according to the nature of the sample under examination; for illustrative purposes that employed in the analysis of commercial oxygen will be outlined.

All stopcocks are closed, and a solution of 10 grms. of pyrogallol in 30 c.c. of water is placed in the separating funnel. The topcocks are opened to enable the solutions to run down, but only sufficiently to cause the tube connecting with B, and the channel of the stopcock to be completely filled; this stopcock is then turned to establish communication between the burettes. The two burettes are now *very thoroughly* washed out to remove any excess of pyrogallol from the previous operation. Saturated potash solution is added through the open end of the levelling burette, and about 98 c.c. of the sample are collected over this in the usual manner.

The sample having been measured, the levelling burette is lowered to create a slight negative pressure in the measuring burette, and to such an extent as to allow about 1·0 c.c. of potash solution to remain in the measuring burette. The stopcock B is turned to close communication in all directions, and the levelling burette is restored to the bench. The stopcock of the separating funnel has been left open and the stopper removed, so that, with the negative pressure in the measuring burette, pyrogallol solution (about 0·5 c.c.) runs in when B is turned in the appropriate direction. This stopcock is then closed in all directions. The measuring burette is slightly shaken; the two solutions form alkaline pyrogallol and absorb oxygen. With the partial vacuum thus created, the solutions run in freely by simply turning B as required; quantities of pyrogallol and potash solutions, approximately in the ratio 1 to 4, are added alternately until no further absorption occurs after shaking and inverting the measuring burette. After the absorption, which is very rapid, the amount is measured in the usual manner. In calculating the result a correction is required for carbon dioxide, which is

determined separately and conveniently in a Haldane apparatus. Usually the amount may be neglected without introducing serious error.

The method can be simplified still further by placing the complete absorbent reagent in the separating funnel and collecting the sample over water, but in the particular examination under discussion the method of forming alkaline pyrogallol *in situ* prevents loss of absorption power due to contact with atmospheric oxygen.

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ROYAL NAVAL COLLEGE,
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## BORIC ACID IN COFFEE.

EVERY sample of coffee which I have examined during the last two years has been found to contain boric acid. There were 31 in all of these samples, and they were obtained from six different counties. In each case the turmeric test has been applied to the ash from ten grms. of coffee, taken up in water and acidified with hydrochloric acid. The ash of coffee is exceptionally high in alkali content and requires more hydrochloric acid to produce an acid reaction than do most ashes of food products. The amount of boric acid is sufficiently small to escape detection unless the liquid is warmed for a few minutes after the immersion of the turmeric paper.

The boric acid of coffee dissolves when an infusion with hot water is prepared, and can be detected on evaporating the infusion to dryness, igniting and testing with turmeric paper, after taking up in water and acidulating.

A determination of the boric acid in the mixed ashes of seven specimens of coffee, by Thomson's method, showed the average amount in these to be 0·011 per cent.

Neither in English nor French literature have I found any record of the use of boric acid in coffee culture or coffee preparation, nor have any of the published records of analyses of soil from coffee-growing districts mentioned the presence of the substance.

Though only two references in literature have been found, each is of importance in different ways. J. B. André, in a paper entitled "Avant-projet de Code international des Méthodes d'Analyse des Denrées alimentaires." read before the Bromatology Section of the Seventh International Congress of Applied Chemistry, London, 1909, mentioned, as one of thirty-eight objections to disqualify coffee, the presence of borax.

The second reference occurs in a paper entitled "Note sur l'Acide Borique dans les Produits naturels," by Em. Deltour, in *Revue Internationale des Falsifications*, 1893, **6**, 157. This author used a turmeric paper test on the ash, and in a column headed "N'ont pas donné la Réaction," includes two commercial samples of coffee and four museum specimens of the following descriptions: "Java vert," "Preanger jaune," "Moka," and "Saint Dominique," respectively.

Though boric acid also occurred in two samples of coffee and chicory mixtures, Deltour (*loc. cit.*) placed chicory in a column headed "Ont donné la Réaction." He also mentioned that most of the articles he dealt with came from the environs of Liège, and collated with this the then recent discovery of the presence of boric acid in the soil of Belgium.

WILLIAM PARTRIDGE.

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

## MILK PRODUCTS. REPORT No. 1.

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

*Nominated by the Government Chemist:* A. More.

*Nominated by the London Chamber of Commerce:* E. R. Bolton, H. Jephcott, W. J. P. Pelle, J. Tavroges and T. J. Underhill.*

*Nominated by the Manufacturing Confectioners' Alliance:* T. Macara.

*Nominated by the Society of Public Analysts:* G. D. Elsdon, E. Hinks (*Chairman*), E. B. Hughes (*Hon. Sec.*), A. E. Parkes and J. D. Roberts.

In accordance with the terms of reference and in view of the Public Health (Condensed Milk and Dried Milk) Regulations, this Committee has, in the first instance, directed its attention to the analysis of condensed milk.

Particulars of a large number of methods and modifications of recognised methods have been received and considered, and tests have been carried out on many of these. In connection with the work of the Committee, so far, 26 meetings have been held and a total of some 4000 quantitative laboratory determinations have been made.

This Report deals with the work of the Committee and its recommendations on the determination of total solids and fat in sweetened and unsweetened condensed milks. Meanwhile a considerable amount of work on the determination of sugar in sweetened condensed milk has been carried out, and this will form the subject of a subsequent report.

The Committee recognises that, although not within the terms of reference, the sampling of such a product as condensed milk is a matter of considerable importance. It could not deal adequately with this subject until satisfactory methods of analysis had been selected, but with reference to the preparation of a sample submitted for laboratory examination it has the following observation to make:—The most efficient mixing was obtained by hand, using a spoon with an up and down rotatory movement, in such a way that the top layers and the contents of the lower corners of the containing vessel were moved and mixed, care being taken that any separated crystals in the original sample should first be ground and incorporated in the bulk. It is important that frothing or the formation of air bubbles should be avoided.

Because of the difficulty of accurately measuring volumes of the diluted condensed milk and the danger of the separation of fat from the liquid, the principle of taking weighed quantities of the sample sufficient for each determination was adopted in preference to taking aliquot parts by volume of a liquid prepared by diluting a larger quantity of the sample with water. The relatively small weighed quantities that must be taken for analysis emphasise the importance of complete mixing of the sample.

* Resigned before the completion of this report.

# PART I.

## DETERMINATION OF TOTAL SOLIDS OF CONDENSED MILK (SWEETENED AND UNSWEETENED).

DEFINITION OF TOTAL SOLIDS.—The Committee defines the *total solids* as the residue obtained when the milk is dried, under the conditions specified, until the loss of weight between the successive weighings does not exceed 0·0005 grm.

It has been established that the figure for the total milk solids in a mixture of fresh milk and sucrose obtained by subtraction of added sucrose from the total solids thus determined accords with the figure for total milk solids obtained by drying 5 grms. of the same fresh milk for 3 to 4 hours at 98° to 100°C. The percentage of total milk solids in a sweetened condensed milk obtained by deducting added sugar from the total solids determined by the prescribed method can, therefore, be exactly related to the percentages specified for fresh milk in the Public Health (Condensed Milk) Regulations, 1923. It may, however, be remarked in this connection that no authoritative method for the determination of total milk solids in fresh milk has been definitely laid down.

METHOD OF OBTAINING THE DRY RESIDUE.—Extended trials showed that, whilst there is little difficulty in drying an unsweetened condensed milk, the drying of a sweetened one presents considerable difficulty. Simple evaporation and drying of sweetened condensed milk diluted with water gave irregular results and the amount of residue thus obtained was always greater (up to 0·5 per cent.) than when, in order to expose a large area during drying, the diluted milk was dried upon a support. Moreover, drying was more rapid when a support was employed, and this procedure has been adopted.

The effect of adding alcohol or acetone was studied; such procedure was found not to be advantageous.

The indirect determination of total solids from the specific gravity of a mixture of the milk and water was also studied. This method may be useful for routine purposes, but there are difficulties in obtaining the specific gravity exactly, and the method has the disadvantage of not being an absolute one.

THE SUPPORT.—Various supports were tried. Talc has certain advantages, particularly in regard to the small quantity required, but, on the whole, sand was found to be the most satisfactory. The fineness and quality of the sand are of importance, as is also the quantity of sand relative to the quantity of milk and water added. The sand must be extracted with acid, thoroughly washed, and subsequently ignited at a dull red heat; it must afterwards be tested to ascertain that no increase in weight occurs after moistening with distilled water and drying under the same conditions as when the milk is dried on it. A relatively large quantity of sand was found to be necessary in order to prevent the formation of a non-porous skin; the quantity must also be sufficient to incorporate all the diluted milk present without any indication of flooding.

The addition of water to the milk before incorporation with the sand is recommended. In this way any separated lactose which may be present in the sample in a doubtful state of hydration will be dissolved, and, on drying, will be left in the same condition as the lactose in the case of fresh milk. Various methods of incorporating the liquid with the sand were tried, and that prescribed was found to be the most satisfactory.

DISHES.—Owing to the hygroscopic nature of the dried milk solids, dispersed as they are over a large surface, the use of covered dishes is essential. Metal, by reason of its superior conductivity, is preferable to porcelain. Aluminium dishes are convenient and light in weight; these must be tested in order to ascertain that they do not alter in weight under the conditions of the experiment, as some dishes have been found to be considerably affected by steam; they should not be allowed to come into contact with the metal of the water bath.

EVAPORATION OF THE DILUTED MILK.—Much uncertainty attaches both to the degree of hydration of lactose separating from solution at various temperatures, especially in the presence of other substances, and to its final condition after drying. It is probable that much of the difficulty experienced in obtaining concordant results for the total solids is due to the lactose.

Evaporation at various temperatures and initial drying at various temperatures, with final drying at 98°—100°C., were tried. Evaporation with initial drying at temperatures considerably below 100°C., followed by final drying at 98°–100°C. gave, in the end, almost the same figures as when temperatures as close to 100°C. as possible were maintained throughout, but the operation of drying was thereby much prolonged. Evaporation on a rapidly boiling water bath and subsequent drying at 98°–100°C. is the most satisfactory, consistent and expeditious method. As has been stated above, this procedure correlates the total milk solids of condensed milk and of fresh milk.

The object of the support is to distribute the milk in a thin film over a large surface. To ensure and maintain this condition, stirring of the sand mixture during the evaporation stage is helpful. This must be performed at the right moment and before the mixture has become too dry; it is not efficacious if the mixture is too wet and may entail loss if it is too dry. Precise directions cannot be laid down, but attention to the stirring during the few minutes available is of importance; otherwise, undesirable caking may occur.

THE DRYING OVEN.—As has been found in the drying of many other food products, attention to the conditions of drying is of prime importance.

The milk should be dried at a temperature of from 98°–100°C. The temperature of drying, which is taken to be that recorded by a thermometer with the bulb immediately over the drying solids, should be maintained within these limits.

Water ovens and electric air ovens have been used by members of the Committee. Both types of oven have given satisfactory results, but, on the other hand, unless the temperature is carefully controlled and adequate ventilation ensured, results given by either may be highly unsatisfactory. A common fault of water ovens is poor ventilation, but electric ovens seem to be generally satisfactory in this respect. Ventilation sufficient to change the air of the oven in 8 to 10 minutes has been found to be adequate. Electric ovens are liable to varying local heating, and for this reason it is important that the dishes in the oven should be insulated from the shelf (for example, placed on a silica triangle), and should not be placed near the walls of the oven; the shelf used should be at about the middle of the oven. The essentials for both types of oven are adequate ventilation and the maintenance of an internal oven temperature of 98° to 100°C.

THE DESICCATOR.—The dish should be allowed to remain in the desiccator for 45 minutes before weighing, as, owing to the nature and bulk of the contents, the rate of cooling is slow.

The desiccator used should be really efficient, and of size suitable for use with one dish only.

The following results were obtained by members of the Committee, using the process prescribed:—

TABLE I.

*Percentage of Total Solids in Unsweetened Condensed Milk.*

| Sample I. (Divided between 4 members). | Sample II. (Divided between 2 members). | Sample III. (Divided between 2 members). | Sample IV. (Divided between 3 members). |
|---|---|---|---|
| 31·80 | 31·81 | 31·50 | 31·76 |
| 31·87 | 31·82 | 31·58 | 31·82 |
| 31·95 | 31·84 | 31·53 | 31·81 |
| 31·79 | 31·86 | 31·53 | 31·81 |
| | 31·75 | 31·54 | 31·79 |
| 31·78 | 31·76 | 31·55 | |
| 31·79 | | | 31·79 |
| | | | 31·81 |
| 31·87 | | | |
| 31·91 | | | |

TABLE II.

*Percentage of Total Solids in Sweetened Condensed Milk.*

| Sample I. (Divided between 3 members). | Sample II. (Divided between 3 members). | Sample III. Comparative results in 2 types of oven by same analysts. (Divided between 3 members). | |
|---|---|---|---|
| ELECTRIC OVEN. | | ELECTRIC OVEN. | WATER OVEN. |
| 74·88 | 74·70 | 74·56 | 74·64 |
| 74·91 | 74·65 | 74·49 | 74·55 |
| 74·79 | 74·57 | 74·64 | 74·63 |
| | 74·50 | 74·63 | 74·65 |
| 74·74 | 74·56 | | |
| | 74·50 | 74·62 | 74·69 |
| | | 74·65 | 74·67 |
| | 74·58 | | |

## METHOD OF DETERMINATION.

PREPARATION OF THE SUPPORT.—Select for use sand which passes a 30-mesh and is retained by a 90 mesh sieve. Heat a convenient quantity of this sand with strong hydrochloric acid to remove oxide of iron, etc.; decant; repeat the digestion till the acid liquor is nearly colourless; wash, once with dilute hydrochloric acid, and then thoroughly with distilled water; dry, and ignite.

The sand thus prepared should be tested for suitability as follows: Dry a portion at 98°–100°C. and weigh; moisten with distilled water and subsequently dry again at 98°–100°C. There should be no difference between the two weights.

DISHES.—These should be of metal (aluminium or nickel is suitable), with readily removable but close fitting lids; a suitable size is of diameter about 3 inches and depth about 1 inch.

PROCEDURE :—

(1) SWEETENED CONDENSED MILK.—Place about 25 grms. of the prepared sand and a short glass stirring rod in the dish and dry to constant weight in an

oven at 98°–100°C., the lid being removed whilst drying and replaced before removing the dish from the oven. Allow the dish to remain for 45 minutes in the desiccator before weighing.

Tilt the sand to one side of the dish; place on the clear space about 1·5 grms. of the well-mixed sample and weigh rapidly. Add 5 ml. of water to the milk and mix these; then mix the diluted milk thoroughly with the sand by means of the rod.

Place the dish on a rapidly boiling water bath for 20 minutes, carefully stirring during the earlier period. Transfer the dish, with rod and cover, to a well-ventilated oven at 98°–100° C., as recorded by a thermometer in the air immediately above the dish. After 1½ hours, cover the dish and place in the desiccator for 45 minutes; weigh; return the dish to the oven, and heat for one hour with lid removed; remove and weigh as before; repeat this process until the loss of weight between successive weighings does not exceed 0·0005 grm.

(In a satisfactory determination it is generally found that the loss between the second and third weighings does not exceed 0·0005 grm.)

(2) UNSWEETENED CONDENSED MILK.—Weigh out 3 grms. of condensed milk and use 3 ml. of water; otherwise proceed as in (1).

## PART II.

## DETERMINATION OF FAT IN CONDENSED MILK (SWEETENED AND UNSWEETENED).

There is but a limited choice of methods for this determination. Rapid methods, such as the Gerber, the Babcock, and the Leffman-Beam, are capable of giving approximately correct results, but are not suited for determination of fat, with the desired degree of accuracy. The Werner-Schmid process gives good results with unsweetened milks, but is not desirable for sweetened milks. The ultimate choice of a method applicable to both sweetened and unsweetened milks lay between a coagulation method and the Röse-Gottlieb method. Coagulation by copper sulphate and by dialysed iron were investigated; their advantage lies in the fact that relatively large quantities of the sample can be taken for analysis: their disadvantage in the necessity for the adoption of some such treatment of the coagulum as that of Werner-Schmid. These methods are capable of giving accurate results, but the Committee considers that the Röse-Gottlieb process, with the modifications detailed hereafter, is preferable, and accordingly recommends its use.

In the process recommended it has been found that very strict adherence to details is essential.

QUANTITY OF SAMPLE TAKEN.—In the procedure recommended, from 2 to 2·5 grms. of condensed milk are taken. Tests were made, taking larger quantities, up to 4 and 5 grms., without increasing the amount of solvents, but with the extraction apparatus usually available, the employment of the larger quantities did not result in greater accuracy and was often found to lead to a less perfect separation of the extracting liquids.

EXTRACTION OF THE FAT.—Thorough mixing should follow the successive additions of water, ammonia and alcohol. If clots or lumps appear at these stages, subsequent treatment with ether will not completely extract the fat, and the determination should be discarded. Thorough agitation of the ether with the milky emulsion is necessary, and vigorous shaking for one minute is prescribed; after the addition of the petroleum spirit the agitation need not be so extended. Washing with ether after the first ether and petroleum spirit extraction was

found to be of advantage, in that it removes more completely the first extract and prevents the re-incorporation of "strong" extract with the aqueous layer during the next extraction.

The maintenance of an adequate proportion of alcohol in the aqueous layer is of importance, and accordingly the addition of 0·5 ml. of alcohol before the second and third extractions is prescribed, in order to counteract the removal of alcohol by the ethereal layer.

Analysts should bear in mind the fact that the first ether and petroleum spirit extract contains most of the fat, and the greatest care should be taken to avoid loss of any of this by spurting or absorption by the cork. All corks used should be sound and proved to yield nothing to the solvents employed; moistening the corks with water before insertion into the extraction vessel is advisable.

Too much stress cannot be laid upon the necessity for complete separation of the emulsions into ethereal and aqueous layers. Sedimentation until the ethereal layer is quite clear is prescribed; centrifuging, where practicable, is of service. Incomplete separation results in the presence of non-fatty substances suspended in the ethereal solutions and the retention of ethereal droplets containing fat in the milky layer. In a well-conducted operation the non-fatty residue finally obtained is usually negligible and does not exceed a fraction of a milligram in weight.

RE-SOLUTION OF THE FAT.—As mentioned above, the non-fatty residue obtained from the ethereal solutions should be very small. Difficulty lies in preventing the flotation of this residue in the petroleum spirit in the final operations. It has been found that the addition of a few drops of water to the ethereal liquid before distilling off the solvents is of use in concentrating this non-fatty substance, such as it is, into a small compass and inducing it to adhere to the flask.

WEIGHING THE FAT AND FLASK.—Care is necessary to ensure that the conditions of weighing the flask, when containing the fat and the final non-fat residue, are strictly comparable. The use of a counterpoise flask has been investigated, but has not been found to confer any advantage. Analysts are advised to adopt such methods of weighing as they find to give the best results in the conditions of their laboratories, and to bear in mind that an error of 0·0005 grm. in weighing means an error of from 0·02 to 0·025 in the percentage of fat.

The following results were obtained by members of the Committee, using the process prescribed:—

TABLE I.

*Percentage of Fat in Unsweetened Condensed Milk.*

| Sample I. (Divided between 3 members). | Sample II. (Divided between 2 members). | Sample III. (Divided between 2 members). |
|---|---|---|
| 9·95 | 9·92 | 9·96 |
| 9·93 | 9·92 | 9·90 |
| | 9·89 | |
| 9·86 | | 9·86 |
| 9·87 | 9·91 | 9·86 |
| 9·88 | 9·92 | 9·87 |
| 9·91 | 9·95 | 9·82 |
| | 9·95 | |
| 9·94 | | |
| 9·95 | | |
| 9·98 | | |
| 9·95 | | |

TABLE II.

*Percentage Fat in Sweetened Condensed Milk.*

(Samples from the same bulk examined by 9 members.)

| | | |
|---|---|---|
| 9·24 | 9·20 | 9·22 |
| 9·28 | 9·25 | 9·24 |
| 9·21 | 9·21 | 9·23 |
| 9·27 | 9·24 | |
| | 9·20 | 9·19 |
| 9·19 | | 9·19 |
| 9·20 | 9·19 | |
| | 9·20 | |

## METHOD OF DETERMINATION.

Determine the fat according to the following modification of the Röse-Gottlieb method:—

REAGENTS.—Concentrated ammonia solution, nominal 0·880.
Alcohol or industrial methylated spirit, 95 per cent. by volume.
Ether (methylated), sp. gr., 0·720.
Petroleum spirit, boiling between 40°C. and 60°C.
These reagents should leave no appreciable residue on evaporation.

PROCEDURE:—

Transfer to a suitable apparatus from 2 to 2·5 grms., accurately weighed, of the well-mixed sample; add 8 ml. of warm water and mix well; cool; add 1 ml. of concentrated ammonia solution, mix, add 10 ml. of alcohol and again mix. Add 25 ml. of ether and shake vigorously for 1 minute; add 25 ml. of petroleum spirit and again shake vigorously for 30 seconds. Allow the liquids to stand for not less than half an hour, until the ethereal layer is perfectly clear, or centrifuge at a low speed. Transfer the ethereal layer to a suitable flask. To the milk residue add 5 ml. of ether, and transfer without further shaking; repeat this operation in the same manner with a further 5 ml. of ether. Add 0·5 ml. of alcohol, and repeat the extraction with 25 ml. of ether and 25 ml. of petroleum spirit, as before, shaking vigorously for one minute after the addition of the ether and for 30 seconds after the addition of the petroleum spirit. As before, allow the ethereal layer to separate completely and transfer to the flask. Repeat the extraction once more with alcohol, ether and petroleum spirit in the same manner.

Cautiously distil the solvents from the flask and dry the residual fat at 98°–100°C. to constant weight, taking the ordinary precautions to remove all traces of volatile solvent.

Completely extract the fat from the flask by repeated washings with petroleum spirit, allowing any sediment to settle before each decantation. Finally dry the flask at 98°–100°C. The difference in weights before and after the petroleum spirit extractions is the weight of fat contained in the quantity of condensed milk taken.

Make a blank determination, using the specified quantities of reagents, and distilled water in place of the milk, and deduct the figure found, if any, from the weight of fat obtained.

*For and on behalf of the Sub-Committee,*

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

# 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.*

## COUNTY OF LANCASTER.

### ANNUAL REPORT OF THE COUNTY ANALYST FOR 1926.

DURING the year the total number of samples submitted was 5196, of which 4841 were examined in connection with the Sale of Food and Drugs Act. Of these, 2786 were formal and 1961 were informal samples, and 120 were returned as adulterated. It may be mentioned that informal samples require as much time and attention for examination as formal samples.

MILK.—Ninety-nine of the 2714 samples of milk were adulterated.

*Dirt in Milk.*—Since the inauguration, in 1916, of systematic testing for dirt in milk a very great improvement has taken place. So much so, in fact, that during the year 1926 not one sample of milk has been returned as adulterated on account of the presence of "dirt."

It must not be assumed, however, on these grounds, that the milk supply is satisfactory. Whilst the presence of "dirt," which frequently consists of cow-dung and other objectionable substances, in milk undoubtedly proves that the methods used during milking are highly unsatisfactory, its absence by no means implies the contrary. When "dirty" milk is passed through an efficient sieve or centrifugal cleanser the bulk of the sediment is removed, but a milk from which the sediment has thus been removed is little or no better than one still containing it, the only difference being that in the absence of the sediment the consumer is not aware of the unsatisfactory nature of the product, or, in the words of a colleague of the writer's, "the label has been taken off."

Milk that is "clean" merely because it has been apparently "cleaned" is highly unsatisfactory, because the consumer may easily imagine that he is getting a clean, pure article, whereas the soluble material and the serious bacterial contamination which may be present entirely escape notice.

In order that the general standard of cleanliness may be improved, it appears to the writer that there would be many advantages accruing from the total prohibition of all sieves and other apparatus for apparently cleansing a contaminated milk. In practice such methods can be of little, if any, value, and there is little encouragement for the adoption of "clean-milking" principles, which require time and trouble, when their results can be apparently simulated in the eyes of the purchaser by merely passing the product through an efficient sieve. Abolish the sieve, prohibit the removal of dirt from milk, and an immediate improvement would result.

JAM.—Two samples of raspberry jam, which were informal samples, contained a small proportion of apple. They were described as "—— Brand Superior Preserves—Raspberry," and a further label stated that "We guarantee this jar to contain fresh fruit and pure sugar only." The labels were very similar to those of a well-known maker, whose jams are prepared from pure cane sugar, and that fruit only which is mentioned in the description of the particular jam. It is, therefore, obviously undesirable that similar labels should be used for jams which contain a proportion of foreign fruit. It transpired that the firm marketing

these adulterated jams have them made for them. The actual manufacturers, when using their own labels, always state that an addition has been made, but in these cases, where other labels are used, the notification had, by inadvertence, been omitted. Both manufacturers and dealers have given an undertaking that correct labels shall be used in future.

Another sample was described as "Damson Jam," "Prepared from selected fresh fruit and refined sugar only." It contained about 5 per cent. of glucose syrup. The firm preparing this article had their attention called to the matter.

Sample of Raspberry Jam, 605 S.L.D., was a typical example of misdescription. It was labelled "Raspberry" with a small label, whilst another small label contained the words "Home made," printed in script in such a way that the casual observer might think it hand-written in ink. The sample was obviously intended to counterfeit in appearance those jams which are prepared by shopkeepers for sale over their own counters by retail to their own customers, whereas in actual fact it is prepared on the large scale and cannot be described as in any sense a "home-made" article. To make matters very much worse, the sample contained not more than 50 per cent. of raspberry jam, the remainder consisting of apple juice, whilst about 5 per cent. of glucose syrup was present.

It would appear highly advisable for the law to be strengthened, if necessary, so that poor material of this nature not meriting description as "jam" cannot be passed off as "home-made raspberry jam."

A sample of raspberry and gooseberry jam was labelled "Home-made Raspberry and Goose." It was of inferior character, containing, as it did, a trace of salicylic acid (about one quarter of one grain per pound) a quantity of apple juice, and some glucose syrup. There may be objections to food standards, but they would at least prevent material such as this masquerading as home-made jam.

A sample described as "Blackberry and Apple Preserve" was found to contain a considerable proportion of glucose syrup. The fruit consisted almost entirely of apple, only a very small proportion of blackberry being present, the absence of the latter being more or less masked by the addition of artificial colouring. There is at present no standard for jam in this country, and a prosecution for a faked article of this type, which is undoubtedly sold to the prejudice of the purchaser, would at the best entail much trouble and expense.

In my opinion the existence of inferior products of this nature is strong evidence in support of the desirability of the standarisation of food products.

TABLE CREAM.—A sample, described as "Table Cream," consisted of maize starch and sugar flavoured with cocoa, and was, in fact, merely sweetened blanc mange powder. It would appear undesirable that articles of this nature should be described as "Table Cream." The makers of this article, on the possibility of misinterpretation being pointed out to them, immediately agreed to alter the printing on their cartons, and they have added a definite statement that the substance contains neither milk nor cream.

ARROWROOT.—A single sample of arrowroot, returned as adulterated, was found to contain 20 per cent. of the starch of sweet potato. In my opinion this constitutes adulteration—an opinion which is supported by a mass of trade evidence which has recently been obtained. The sample was an informal one, and the vendor was cautioned by the Clerk to the County Council.

CREAM OF TARTAR.—A sample consisted of 30 per cent. of maize starch and 70 per cent. of calcium and sodium phosphates. It was, in fact, one of a class of substances which is sold as "Cream of Tartar Substitute." This was an informal sample. A subsequent formal sample was genuine, but from the conversation which the sampling officer had with the vendor at the time the formal sample was

purchased, it would appear that the sale of the informal sample was not due entirely to a mistake. Further formal samples from this vendor are to be taken in due course.

This is a typical example of the state of affairs which arises when inferior materials are allowed to be sold under similar names to the articles which they are prepared to counterfeit. It would appear that it is not unusual for vendors to stock "Cream of Tartar" and "Cream of Tartar Substitute," and to sell these articles in reply to a demand for cream of tartar. Sometimes a variation in price is suggested, sometimes not, but it is rarely that the true difference in the nature of the materials is pointed out to the purchaser.

LEMONADE POWDER.—A sample, sold as "Lemonade Powder," was composed of sugar and tartaric acid flavoured with essential oil of lemons. It would appear likely that lemonade was originally diluted lemon juice flavoured with lemon peel and sweetened with sugar, and it is surely not unreasonable for a purchaser to think that a "Lemonade Powder" will be a substance from which such a lemonade may be made. It is certainly doubtful whether it is possible to prepare such a powder, but, as the present sample was prepared from tartaric acid, which is not found in lemon juice, it is obvious that in this case no attempt has been made to do so. The manufacturers have undertaken to print the following notice on their containers in type of a readable size:—"This Powder is prepared from ingredients of the highest quality, and is flavoured with Lemon and the Pure Acid of Fruits. It will be found to make an excellent substitute for lemonade."

In the present state of the law, this is as fair a declaration as could reasonably be expected. G. D. ELSDON.

# Legal Notes.

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

## CONSTANTS OF IRISH BUTTER.

ON May 16, at the Tralee Circuit Court, an appeal was heard against the decision of the Listowel District Justice, who had fined a provision merchant £1 1s. and costs for having sold butter which was certified by the Public Analyst to contain 13 per cent. of fat other than butter fat.

According to the certificate of Dr. Ryan, State Analyst, the sample contained 12 per cent. of foreign fat, and a third portion of the sample, which had been analysed by another analyst, also gave results below the limits adopted by the Public Analyst.

Mr. O'Mahony, Public Analyst for Kerry, said that he applied the Reichert–Wollny, Polenske and Kirschner tests to the sample. The Reichert–Wollny value was 22·1 and the State Analyst had obtained a similar result. The majority of Kerry butters sent to him during the same week had given values of 24 to 27. If the figure fell below 24, the presumption was that the butter was not genuine. What had struck him with regard to this particular sample was the abnormally low proportion of water (only 9·5 per cent.), from which he drew the inference that the butter was mixed with margarine or some other forms of foreign fat. He was

quite satisfied before he obtained the analytical results that the sample was "faked."*

In cross-examination, Mr. O'Mahony admitted that the City of Dublin Analyst, who had obtained practically identical analytical figures, had certified the butter as genuine. He also agreed that it had been shown in departmental investigations that samples of genuine Irish butter had given Reichert–Wollny values as low as 20·1 and 18; and that in an investigation made by Mr. George Brownlee, in 1923, in the course of which 112 samples were examined, it had been found that 54 per cent. fell below the 24 figure, and 34 per cent. below the 22 figure.

Mr. Browne (for the appellant) contended that, as there was no legal standard for the fat in butter, the certificate of the Public Analyst only raised a presumption that the butter was adulterated, and that it was open to them to prove by positive evidence that no actual adulteration had taken place. From the cross-examination it was evident that the tests for butter were not reliable, and he said that any suspicions which the figures had created would be removed by evidence for the defence.

The defendant stated that he had bought the butter in question from a farmer, from whom he had been buying butter for 20 years. This lot, which was hand-separated butter, was in a lump weighing about 39 lbs.; it was afterwards beaten into 1 lb. parcels, and the effect of this beating was that a quantity of water was beaten out of it. There had been no interference with the butter from the time he bought it until the sample had been sold to the inspector.

The Judge said that he accepted as accurate the analysis of Mr. O'Mahony; the results obtained in his three scientific tests were all practically in agreement with the figures arrived at by the other analysts. But the deduction to be drawn from these figures was another matter. Mr. O'Mahony had told them that there was considerable controversy among scientists of reputation on the subject, and the results obtained by Mr. Brownlee showed that the figures obtained in this case were not definite evidence of adulteration. From what he knew of the defendant he could not believe that he or his wife had deliberately adulterated this butter. He was positively of opinion that this butter was sold by the defendant exactly as it came from the churn. Therefore he had no hesitation, whatever, in reversing the decision of the District Justice in this case, and he allowed 40s. costs to the appellant.

* The analytical figures for the fat were as follows:—

| | O'Mahony. (Nov. 1926). | Ryan. (Feb. 1927). |
|---|---|---|
| Reichert-Wollny .. .. | 22·1 | 21·1 |
| Polenske .. .. .. | 1·6 | 3·0 |
| Kirschner .. .. .. | 17·0 | 16·0 |
| Avé-Lallemant .. .. | (not done) | +12 |

EDITOR.

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## CONTAMINATION OF MINERAL WATER WITH *B. COLI*.*

ON or about July 12, 1926, the U.S. Attorney for W. Louisiana, acting upon a report by the Secretary of Agriculture, asked for the seizure and condemnation of 329 cases of mineral water, which had been transported from the State of Texas into the State of Louisiana, and charging adulteration in violation of the Food and Drugs Act.

* U.S. Dept. Agriculture, Service and Regulatory Announcements, Bureau of Chemistry. Suppl. 223, 1927. No. 14638.

Adulteration was alleged for the reason that the article consisted wholly or in part of a filthy, decomposed and putrid substance, in that it contained *B. coli*, an organism indicative of the presence of sewage contamination.

On September 21, 1926, the claimant of the property having consented to its destruction, judgment of condemnation and forfeiture was entered, and it was ordered by the court that the water be poured out and destroyed by the United States marshal. It was further ordered by the court that the bottles be delivered to the said claimant upon the execution of a bond in the sum of $500, conditioned that they be thoroughly sterilised.

# Department of Scientific and Industrial Research.

## THE NATIONAL PHYSICAL LABORATORY.

### REPORT FOR THE YEAR 1927.*

GENERAL RESEARCH.—In addition to the usual work, research into heats of combustion of gases has been begun, and work on methods of measuring humidity has involved a study of the effect of humidity on the relative mobility of ions. In the Optics Division it is proposed to employ photoelectric methods of spectrophotometry for measurements in the ultra-violet, and in the Metallurgy Department work on methods of production of pure metals has been continued, particularly with regard to pure iron and chromium. Small quantities of impurities have been determined by means of the quartz spectrograph, and the physical structure of metals and alloys studied by X-ray methods. Dental alloys, silicon of high purity, and manganese-iron alloys free from carbon have all received attention, whilst the investigation into colour relations in alloy systems has shown the desirability for further work on the production of single crystals of the alloys. The relation between the magnetic behaviour of iron, nickel, etc., and their elastic deformation; the behaviour of copper and other metals cooled to liquid air temperature under strain, and the measurement of heat generated by plastic deformation have all been investigated; the work on the solubility of gases in metals has been continued, and the apparatus for determination of surface tension of liquid metals has now been used for tin and lead.

MAINTENANCE OF STANDARDS.—This has involved much work, particularly in connection with the International Temperature Scale.

RESEARCH FOR BOARDS AND COMMITTEES OF THE DEPARTMENT has included lubrication research, involving measurements of changes in attitude and eccentricity of a cylindrical bearing with changes of load, and the variation with temperature of the static friction on loaded lubricated surfaces for a number of lubricants, whilst the dry sliding friction of graphite on graphite has been determined at high temperatures and under reduced pressure.

RESEARCH AND TESTS FOR OTHER GOVERNMENT DEPARTMENTS.—This has included work for the Aeronautical Research Committee of the Air Ministry, and for the War Office in the high speed wind channel.

SPECIAL INVESTIGATIONS were many. *Standardisation of Scientific Glassware.*—Tests of volumetric apparatus, in conjunction with the Joint Committee for the Standardisation of Scientific Glassware, have increased in number. The following

* Obtainable at Adastral House, Kingsway, W.C.2. Price 7s. 6d. net.

proposals have been adopted by the Committee:—(i) The scale intervals on subdivided volumetric glassware, calibrated in metric units of volume, *e.g.* burettes, cylinders, etc., shall be equivalent to 1 ml., 2ml. or 5 ml., or decimal or sub-multiples of these volumes. (ii) For vessels subdivided in 1 ml. or 2 ml. intervals, or decimal or sub-multiples of these volumes, the schemes of subdivision shall be as illustrated (Fig. 1), and for 5 ml. divisions as illustrated in Fig. 2. Graduation marks must be

Fig. 1.

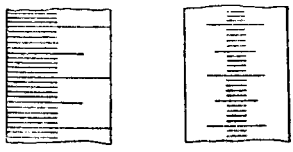
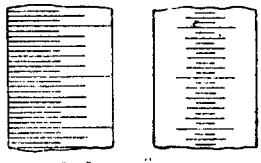



Fig. 2.




omitted from the lower portions of cylinders for Class A test, and are recommended for omission in Class B test apparatus.

*Standardisation of Hydrometers.*—Surface tension effects in the standardisation of hydrometers and verification of standards for the testing of hydrometers, according to the specification of the Institute of Petroleum Technologists, have occupied much time. In the latter tests the laboratory standards are now verified in petroleum spirit or mixtures of petroleum spirit and benzol, and for a range of specific gravities from 0·65 to 0·85 hydrometers submitted are compared against the standards in these liquids. Over the range of 0·85 to 0·95 mixtures of alcohol and water are used. The heavier oils of sp. gr. 0·95 to 1·10 are dark and viscous, and, owing to differences in surface tension and the need of avoiding test liquids necessitating non-negligible corrections, mixtures of alcohol and sulphuric acid were used for this range, since their surface tensions are not very different from those of the heavy oils, and these were found satisfactory. Corrections in the laboratory standards were determined at intervals corresponding to 0·01 changes in sp. gr.

*Work for the Alloys of Iron Research Committee* included the study of iron-chromium, iron-manganese, iron-phosphorus and iron-silicon alloys. The effect of small quantities of impurities was found to be great, and where materials had to be melted at temperatures over 1500° C. there was great difficulty in maintaining the necessary purity. In the case of iron-chromium alloys small quantities of nitrogen materially affected the structure and constitution of the alloys, and the iron-manganese system was found to require the preparation of thermal curves by special methods; hence a series of alloys of uniform structure are being prepared by annealing in hydrogen. The study of the iron-phosphorus alloys has been completed up to 30 per cent. phosphorus.

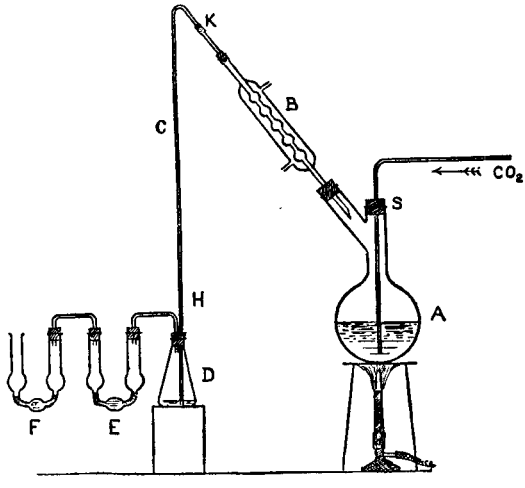
Work has also been done in *Aerodynamics* and in connection with the *William Froude National Tank.* D. G. H.

# Ministry of Health.

## DETERMINATION OF SULPHUR DIOXIDE IN FOODS.*

THE following method, which is suggested in the Report (*cf.* ANALYST, 1927, 343) for the determination of sulphur dioxide in foodstuffs, ensures that the whole of the sulphur dioxide is liberated and distilled over without oxidation. It also eliminates errors due to volatile sulphur compounds and organic acids, and economises times, in that a gravimetric determination is necessary only when very small quantities of sulphur dioxide are prevent.

Concentrated hydrochloric acid (20 c.c.) and distilled water (500 c.c.) are boiled in a current of pure carbon dioxide in the 1500 c.c. flask A, till all air is removed from the apparatus. The flask is then cooled. The sample (usually 100 grms.) is then quickly added, through a tap-funnel in the case of a liquid, and




the mixture boiled in a slow current of carbon dioxide. In certain cases (*e.g.* cornflour) the flask is first heated in a bath of boiling water in order to avoid cracking it. At the end of 1 hour (*vide infra*) the flow of water in the reflux condenser (B) is stopped, and any sulphur dioxide is then driven over into the cooled receiver D, which contains 10 c.c. of pure, sulphuric acid free, 10-volume (3 per cent.) hydrogen peroxide.

The Peligot tube, E, contains a similar solution, and the tube F, which contains 5 c.c. of a mixture of hydrogen peroxide and barium chloride solutions acidified with hydrochloric acid, acts as a guard-tube for any excess of sulphur dioxide, but should however not usually be necessary.

Rubber stoppers are used throughout. As soon as the tube at the point H is hot to the touch, the receivers are disconnected at K, the contents all washed into D, and the sulphuric acid produced titrated at room temperature with 0·1 *N* sodium hydroxide solution. Brom-phenol blue is preferable to methyl orange as indicator, since it gives a sharp colour change and is unaffected by carbon dioxide

* By Dr. G. W. Monier-Williams. (Ministry of Health. Reports on Public Health and Medical Subjects. No. 43. May, 1927. Pp. 56). H.M. Stationery Office. Price 1s. 3d. net. (*Cf.* ANALYST, 1927, 343).

and traces of volatile organic acids. If a gravimetric determination is required as a check, the barium sulphate must be precipitated and filtered in the cold, or the presence of volatile sulphur compounds may lead to fictitiously high results. The precipitate should be washed by decantation with hot water before filtration.

The method has been tested on solutions containing known amounts of sulphur dioxide, and the titration and gravimetric methods have been shown to be in satisfactory agreement. There was evidence, however, of oxidation during the distillation, as slightly low results were obtained in some cases. Acetaldehyde in amounts in which it may normally be present (*e.g.* in wines) does not affect the results, and hydrochloric, benzoic, salicylic, acetic and cinnamic acids do not pass over into the distillate from the flask. Pre-treatment with alkali (sodium bicarbonate, or sodium carbonate or hydroxide in the presence of carbon dioxide) has been previously advocated, but does not appreciably affect the results when hydrochloric acid (30 c.c.) is used instead of phosphoric acid.

Since 1 c.c. of 0·1 *N* sodium hydroxide solution corresponds with 32 parts of sulphur dioxide per million on 100 grms. of sample, the gravimetric method is recommended when the titration figure is less than 0·5 c.c.

The method has also been tested for cornflour, port wine, dried fruits, gelatin, sausages, corn syrup, sugar, jam, mustard and onions, by direct distillation with hydrochloric and phosphoric acids, both with and without alkali pre-treatment. Concordant results were obtained in all cases by the method described above, the results obtained gravimetrically and by titration being in close agreement with those obtained by independent analysts. The onions and mustard contained 1 and 2 parts per million, respectively.

With dried fruits (especially apricots) prolonged boiling in strongly acid solution is necessary to decompose the combined sulphur dioxide completely. In cases of doubt it is advisable to boil the contents of the flask for a further 30 minutes with the end of a delivery tube dipping into a small volume of slightly alkaline hydrogen peroxide and brom-phenol blue. No colour change should be produced.

J. G.

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## SULPHUR DIOXIDE IN PEARL BARLEY.

### THE PRESERVATIVES REGULATIONS AND PEARL BARLEY.

REPRESENTATIONS in regard to Public Health (Preservatives, etc., in Food) Regulations have recently been made to the Ministry of Health on behalf of members of the pearl barley trade, and in reply the following letter has been received:—

MINISTRY OF HEALTH,
WHITEHALL, S.W.1.
3rd June, 1927.

Ref. IIb. 119140/459/25.

GENTLEMEN,

I am directed by the Minister of Health to inform you that he has considered the representation made to this Department on the 28th ultimo by Mr.——— and other members of the pearl barley trade, but he is not prepared to modify the Public Health (Preservatives, etc., in Food) Regulations so as to allow permanently the presence of added sulphur dioxide in pearl barley.

He will, however, as an act of grace, in order to enable the trade to dispose of their existing stocks in the country, postpone the operation of the Regulations until 1st January, 1928, so far as they relate to the sale of pearl barley. This postponement will not, however, apply as regards importation.

# ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

## Food and Drugs Analysis.

**Determination of Amino Acids in Foodstuffs. J. Tillmans and J. Kiesgen.** (*Z. Unters. Lebensm.*, 1927, **53**, 126–131.)—Determinations of the amino acids in a number of meat extracts and sauces have been carried out by two new methods, and the results shown to agree well with those obtained by Grünhut's modification of the "formol" titration method (*ibid.*, 1919, **37**, 304). From 5 to 10 grms. of sample are dissolved in 250 c.c. of hot water, 0·3 grm. of barium chloride, and an excess beyond neutrality of 10 c.c. of 2*N* sodium hydroxide solution added, and the solution allowed to stand 3 hours in contact with ammonia. The liquid, made slightly acid with hydrochloric acid, is then decolorised with animal charcoal (which has been shown not to remove amino acids), filtered, and the residue washed with 2*N* sodium chloride solution. For the first method 50 c.c. of the liquid are diluted to 300 c.c., and 30 c.c. placed in one cylinder of the Grünhut colorimeter, and titrated with 0·1 *N* sodium hydroxide solution in the presence of 0·5 c.c. of a filtered 0·1 per cent. aqueous solution of tropaeolin O. It is matched against a similar vessel which contains *N* sodium chloride solution and enough sodium hydroxide to produce a $P_H$ value of 11·8. To the titrated solution are then added 0·5 c.c. of a 0·2 per cent. aqueous solution of neutral red, and the whole back-titrated with 0·1 *N* hydrochloric acid solution, and matched against a similar solution of $P_H$ 7. An allowance is made for any salt error. The other method depends on the fact that, whilst Willstätter's *Zwitter ion* explanation of the amphoteric nature of amino acids holds for aqueous solutions, in alcoholic solutions the acids ionise normally, as if the amino group were not present. To the above extract are added 50 c.c. of neutral 96 per cent. alcohol and 0·5 c.c. of a 0·4 per cent. alcoholic solution of thymolphthalein, and the liquid titrated with 0·1 *N* sodium hydroxide solution till the first blue colour is observed. The colour is matched with that of a solution of $P_H$ 7. J. G.

**"Formol" Titration as a Means of Distinguishing Artificial and Natural Foodstuffs. J. Tillmans and J. Kiesgen.** (*Z. Unters. Lebensm.*, 1927, **53**, 131–137.)—A modification of the Sörensen–Grünhut "formol" titration method, which gives reproducible and comparable values, has been used to distinguish natural and artificial foodstuffs. The amount of 0·1 *N* sodium hydroxide solution required to restore the rose colour to a mixture of a solution of the sample and formalin, both of which had been previously neutralised separately to phenolphthalein, was determined. Artificial lemon juices did not change colour, but natural juices (10 c.c.) required 2·0 to 2·5 c.c., except in the case of old samples which required more, probably on account of protein hydrolysis. Honey (20 grms.) required 1 to 2 c.c., and in some cases an analogy existed between this

figure and the Lund precipitation figure. The Fiehe and Auzinger reactions gave positive results for three samples whose titration figures were very low. Of the vinegars tested, only wine-vinegars gave a "formol"-titration figure, 100 c.c. requiring from 0·4 to 8·8 c.c. of the alkali solution. J. G.

**Optical Rotation of Honey. H. A. Caulkin.** (*Pharm. J.*, 1927, **118**, 544.)—Analyses of 128 samples of honey (English, Californian, Chilian, New Zealand, Canadian, and West Indian) support the contention of Franklin (*ibid.*, 1927, **118**, 52) that the B.P. limits for the rotation of a 25 per cent. solution in a 200 mm. tube (0·5° and −5°) are too narrow. In no case was there evidence of the presence of invert sugar, starch, or dextrin, and Ley's and Lund's tests, and the ash, sulphate and chloride contents indicated that the samples were genuine. The sucrose, dextrose and laevulose were also determined on four samples with particularly high rotations (−5·49° to −12·50°). J. G.

**Origin of the Colouring Matter of Beeswax and the Composition of Propolis. G. F. Jaubert.** (*Comptes rend.*, 1927, **184**, 1134–1136.)—Beeswax is naturally practically white, and obtains its yellow colour from the propolis with which it is found mixed in the hive. This colouring matter of propolis combines with certain metallic salts to form lakes, and, when thus isolated in a crystalline and pure state, was found to consist of 1·3 dihydroxyflavone or chrysine. Propolis coming from several districts in France was formed almost exclusively, from the exudation of the buds, leaves and green parts of *Populus nigra*, var. *pyramidalis*, which, in its turn, was found to contain a glucoside formed of melezitose and 1·3·dihydroxyflavone, and it may well be that, owing to the wide distribution of the poplar 1·3·dihydroxyflavone is a normal constituent of all beeswaxes. D. G. H.

**Non-volatile Acids of the Pear, Quince, Apple, Loganberry, Blueberry, Cranberry, Lemon, and Pomegranate. E. K. Nelson.** (*J. Amer. Chem. Soc.*, 1927, **49**, 1300–1302.)—Determinations by the ester distillation method show that the non-volatile acids of Bartlett pears are citric (2 parts) and malic (1 part); of Winesap apples, *l*-malic and citric (trace), the latter being absent from York Imperial apples; of loganberries, citric (about 96 per cent.) and *l*-malic (about 4 per cent.), with no isocitric acid; of blueberries, mainly citric, with a small proportion of *l*-malic; of cranberries, citric (about 80 per cent.), *l*-malic (about 20 per cent.) and benzoic (0·069 per cent.); of Californian lemons, citric and a very small amount of *l*-malic; of pomegranates, only citric; of quinces, *l*-malic with a small amount of levulinic acid. (See ANALYST, 1925, **50**, 295.) T. H. P.

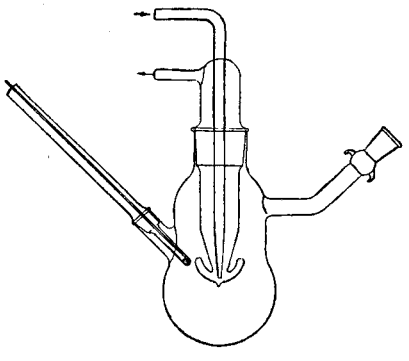
**Determination of Lactic Acid. T. E. Friedemann, M. Cotonio and P. A. Shaffer.** (*J. Biol. Chem.*, 1927, **73**, 335–358.)—A procedure is described for the determination of lactic acid in blood, muscle and sugar derivative solutions which gains considerably on other methods in simplicity, speed, convenience and reliability. The basis of the method is the oxidation of the lactic acid in boiling

sulphuric acid solution to acetaldehyde by permanganate, removal of the aldehyde, by aeration, into a large excess of sodium bisulphite solution, and titration of the combined bisulphite, as described by Clausen (*J. Biol. Chem.*, 1922, **52**, 263). The chief modifications introduced are :—(1) The form of apparatus used for the oxidation and for the collection of the aldehyde ; (2) the addition of manganous sulphate to catalyse the oxidation by permanganate ; and (3) the giving of more accurate definitions for the conditions for the titration of the sulphurous acid bound by the aldehyde. The steps in the process were separately investigated. The use of manganous salt increases the rate of oxidation and also the yield of aldehyde. The manner of its action is discussed. The effect of manganese salts, together with the form of apparatus described, simplifies and shortens the method, and makes the results more nearly quantitative and less variable. The accuracy of the Clausen titration of bound sulphite was established. The extent of interference in the determination of lactic acid of about 50 substances was determined. By the procedure described the yield of aldehyde is consistently 96 to 98 per cent. of the theoretical amount. A determination may be made in about 15 to 20 minutes. The apparatus, which is carefully described and shown in a diagram, may be constructed from ordinary flasks, test-tubes and glass tubing. H. A. Davenport and M. Cotonio (*J. Biol. Chem.*, 1927, **73**, 359–361) describe in detail and also diagrammatically an apparatus in which a condenser unit is used as an optional variation in the device described by Friedemann, Cotonio and Shaffer for the determination of lactic acid; it is in place of the Hopkins' type of condenser. The apparatus can be more compactly and conveniently arranged. P. H. P.

**Determination of Starch in Potatoes. G. Rankoff.** (*Z. Unters. Lebensm.*, 1927, **53**, 138–146.)—Previous methods for the determination of starch in potatoes are summarised and discussed critically, and sources of error indicated. The following method gives results agreeing with those obtained from the difference (100 − non-starch material), but 2 per cent. and 1 per cent. lower than the methods of Kaiser and Fellenberg, respectively. Accurate results obtained by Kaiser's method may be due to the compensation of errors. The finely ground sample (0·3 to 1·0 grm.) is well shaken with 100 c.c. of water and heated in a calcium chloride bath (b.pt. 130° C.) at 110° to 115° C. The solution is then made up to 250 c.c. at 15° C. and filtered through a dry Gooch crucible. To 50 c.c. of the filtrate are added 60 c.c. of a cold saturated solution of sodium sulphate, and enough iodine solution to colour the supernatant liquid. When the precipitate has settled it is filtered off in a Gooch crucible (prepared with pumice powder and asbestos according to the author's instructions, so as to produce rapid filtration and a clear filtrate), and washed 5 times with a solution containing 100 c.c. of saturated sodium sulphate solution and 5 c.c. of iodine solution per litre. The iodine is removed from the starch iodide precipitate by the action of 20 c.c. of sulphuric acid (1:3) at 110° to 115° C. for about 40 minutes. The starch ($S$) is then oxidised by means of potassium permanganate and sulphuric acid, and the carbon dioxide evolved ($a$) determined. Then $S = 0{\cdot}61393.a$. For the last operation

an apparatus is described in which the carbon dioxide is drawn off from the flask containing the reacting liquids, and absorbed in weighed U-tubes. This method gave satisfactory results with oxalic acid. J. G.

**Polarimetric Determination of Starch in Pastry. J. Grossfeld.** (*Z. Unters. Lebensm.*, 1927, **53**, 156–160.)—Baumann and Grossfeld's method for the polarimetric determination of starch (ANALYST, 1917, **42**, 218, 365) is applied to foodstuffs containing starch paste. The dextro-rotatory substance contained in the kernels of apricots and peaches, as determined by the author's ether extraction method, gives results considerably lower than those obtained after treatment in the autoclave. This is possibly due to the autoclave treatment causing the extraction of hemicelluloses, which are converted into sugar when heated with hydrochloric acid. The volume of the insoluble portion of the sample was also determined and allowed for in the usual way, and the revised formula for the starch content (per cent.) was then given by the expression $(B-A-0{\cdot}18)\times 5{\cdot}28$, where A and B are the rotations of the water-soluble portion after inversion and after hot hydrochloric acid treatment, respectively. J. G.



**Distillation of Coconut Oil at very Low Pressures. H. I. Waterman and J. A. Nijholt.** (*Chem. Weekblad.*, 1927, **24**, 268–269.)—The figure shows an internally-cooled receiver, placed in the distillation-flask itself, and used in the distillation of coconut oil at very low pressures. The first drops of distillate were visible at 155° C., and at 222° C. the whole of the oil (4 grms.) distilled. The flavour of the distillate was weaker than that of the original sample, but that of an undistilled residue was stronger. The chemical constants were unaltered after distillation, and no decomposition occurred. J. G.

**Composition of Maize Wax. R. L. Shriner, F. P. Nabenhauer, and R. J. Anderson.** (*J. Amer. Chem. Soc.*, 1927, **49**, 1290–1294.)—The solid matter which separates when crude maize oil is chilled during the process of refining consists of a mixture m.pt. 81–82·5° C., of the two waxes : myricyl *n*-tetracosanoate, and myricyl isobehenate. T. H. P.

## Biochemical, etc.

**The Nitrogenous Groups of Nucleic Acid. H. O. Calvery and W. Jones.** (*J. Biol. Chem.*, 1927, **73**, 73–76.)—It has been concluded by Jones and Riley (Jones, *J. Biol. Chem.*, 1916, **24**, 3) and by Hoffman (*J. Biol. Chem.*, 1927, **73**, 15) that a molecule of yeast nucleic acid contains two purine elements and two

pyrimidine elements. The possibility was left that both pyrimidine groups may be cytosine groups and this was supported by Jones and Perkins (*J. Biol. Chem.*, 1924–5, **62**, 557) who were unable to isolate uracil nucleotide. However, an examination of the solutions from which Jones and Perkins precipitated the lead salts of the nucleotides has shown a considerable amount of soluble nucleotide material; from this it has been found that the lead salt of uracil nucleotide has a specifically great solubility in dilute solutions which contain sodium acetate. Thus Jones and Perkins and also Calvery (*J. Biol. Chem.*, 1927, **72**, 27) failed to isolate uracil nucleotide from the hydrolytic products of yeast nucleic acid, owing to a loss of material. An excellent method for the decomposition of nucleic acid into its nucleotides and subsequent quantitative recovery of the products is described. The acid is split by a sufficiently prolonged (7 days) hydrolysis *with ammonia at the room temperature.* Neither purine bases nor phosphoric acid are set free, and deaminisation is not likely to occur under these conditions. The excess of ammonia can be removed before the lead salts of the nucleotides are precipitated. Guanine nucleotide, adenine nucleotide, cytosine nucleotide and uracil nucleotide were isolated and obtained in crystalline form. From 50 grms. of crude moist yeast nucleic acid, after the frequent removal of small portions for tests, 48 grms. of crude nucleotides were obtained, from which 44 grms. were obtained after the lead test. Their analyses and that of the brucine salt of uracil nucleotide are given. P. H. P.

**Micro Determination of Pentose in Yeast Nucleic Acid and its Derivatives. W. S. Hoffman.** (*J. Biol. Chem.*, 1927, **73**, 15–25.)—A micro method has been devised for the determination of pentose in free or combined form. It is based on the method of Pervier and Gortner (*Ind. and Eng. Chem.*, 1923, **15**, 1167, 1255) for the conversion of the pentose into furfural by distillation with hydrochloric acid, and on the method of Youngburg and Pucher (*J. Biol. Chem.*, 1924, **61**, 741) for the colorimetric determination of the furfural in the distillate. The method is applicable to a study of the pentoses in the body, since it gives theoretical results with compounds which contain *d*-ribose. Tables give a summary of some of the results obtained, and show that in 3-hour distillations with the use of 50 c.c. of 20 per cent. hydrochloric acid the method gives theoretical results in the case of xylose, adenosine, guanosine, adenine nucleotide and guanine nucleotide. Arabinose is only slowly converted into furfural, and the pyrimidine nucleotides are only very slowly hydrolysed. The difference between xylose and arabinose in the speed of furfural formation is due to their molecular configuration. A study of the pentose distribution in yeast nucleic acid confirms the idea which prevails, *viz.* that a molecule of yeast nucleic acid is made up of two purine elements and two pyrimidine elements. Thus, on distillation, yeast nucleic acid gives up little more than half of its pentose in 3 hours, as is to be expected. P. H. P.

**Histochemical Reaction of Lecithins. Iodophile Reaction. M. Romieu** (*Comptes rend.*, 1927, **184**, 1206–1208.)—Pure, fresh lecithin from eggs or brain appears colourless in a thin layer under the microscope, but when treated, especially

after incipient hydrolysis by dilute hydrochloric acid, either with a moderately concentrated solution of iodine in potassium iodide solution or in a closed vessel with iodine vapour, it developes a distinct mahogany or garnet colour. This reaction is not due to the presence of glucose or glycogen as impurity, but probably results from the formation of an unstable iodocholine, soluble in lecithin but insoluble in the iodine reagent. T. H. P.

**Note on the Enzyme Uricase. H. O. Calvery.** (*J. Biol. Chem.*, 1927, **73**, 77–80.)—It has been shown that extracts of animal organisms have the power of destroying uric acid. Ascoli and Izar (*Z. physiol. Chem.*, 1908–9, **58**, 529) showed that after the uric acid was destroyed it could be resynthesised by the tissues under anaerobic conditions. Later (*Z. physiol. Chem.*, 1909, **62**, 347) they showed that the enzyme from livers could synthesise uric acid from dialuric acid and urea, but not from many other substances which they tried, including allantoin, which is the chief end-product after the enzyme has destroyed uric acid. Spiers (*Biochem. J.*, 1915, **9**, 336) was unable to confirm the findings of the Italian investigators in any particular, but his work was carried out with slight variations which should have made no difference. The work has now been repeated as nearly as possible in every particular, but the colorimetric method of Benedict and Franke (*J. Biol. Chem.*, 1922, **52**, 387) was used for the determination of uric acid. A few experiments are given briefly. The work of Ascoli and Izar on the resynthesis of uric acid from its decomposition products by the uricase of the liver has not been confirmed. The results of Spiers have been corroborated. Uric acid is oxidised by uricase under precisely the conditions which Ascoli and Izar claimed were necessary for the reverse of this process. High concentrations of carbon dioxide over a period of several days destroy uricase. P. H. P.

**The Cholesterol Content of Hair, Wool and Feathers. H. C. Eckstein.** (*J. Biol. Chem.*, 1927, **73**, 363–369.)—It was previously reported that the subcutaneous lipids of man contain 0·25 per cent. of cholesterol and only traces of the phospholipids, whilst the cutaneous lipids contain 20 per cent. of the sterol and 2·5 per cent. of the phospholipid. A table now drawn up from experiments shows that the cholesterol content of the lipids in human hair, as determined by the digitonin method, exceeds that previously reported for the lipids of the subcutaneous layers, but is less than that reported for the lipids in human skin. There is more cholesterol present in the lipids from the hair of children than in those extracted from adult hair, and there is more cholesterol in the hair of children than in their blood. One-third of the lipids of rabbit and dog hair, 13·3 per cent. of those from rat hair and 10·3 per cent. of those from cat hair, are present as cholesterol. Calculated on the basis of the hair itself, the percentages for the rat and the cat are 0·57 and 0·55 per cent. respectively, as compared with 0·57 and 0·56 per cent. for the rabbit and dog respectively. The lipids of wool from sheep and lambs cannot be characterised by their high cholesterol content, since the largest amount in any of the wools analysed is only 12·5 per cent. of the total lipids, whilst as much as 41·0 per cent. is present in the lipids of rabbit hair; yet the

wool itself contains more of the sterol than do any of the samples of hair, the variation for wool, expressed as per cent. of the wool, being from 0·63 to 1·11 per cent. The cholesterol content of the lipids from feathers is also variable, the lowest figure (9·4 per cent. of the total lipids) is that obtained for duck feathers, and the highest (25·8 per cent.) is that found for turkey feathers. Calculated on the basis of the feathers themselves, the range is only from 0·26 to 0·29 per cent.

P. H. P.

**Influence of Irradiation upon Oxidation Products of Cholesterol. F. W. Schlutz, M. R. Ziegler and M. Morse.** (*J. Biol. Chem.*, 1927, **73**, 209–213.)—Cholesterol, in itself inactive, becomes a potent antirachitic agent after irradiation by ultra-violet light and the nature of this change has been investigated by many workers. It seemed probable that the ultra-violet light might cause a reaction to take place at the double bond, possibly an oxidation. The authors prepared $\alpha$- and $\beta$-cholesteryl oxides, $\alpha$-cholestantriol and hydroxycholesterol, simple oxidation products of cholesterol, in a pure state by means of mild oxidising agents, benzoyl hydroperoxide, benzoyl peroxide and hydrogen peroxide. If irradiation does cause an oxidation of cholesterol, one would not expect a deep-seated change, and therefore the milder the oxidising agent, the simpler the resulting product and the more likelihood that the product could be formed by means of ultra-violet light. $\alpha$- and $\beta$-Cholesteryl oxides, hydroxycholesterol and $\alpha$-cholestantriol, when given to rats in doses of 3 to 5 mgrms. per day, did not heal rickets, and these products could not be activated by irradiation. This definitely eliminates certain oxidation products of cholesterol as antirachitic factors. Only slight changes have been made in the original cholesterol molecule, but the power of activation by light has been destroyed. Further evidence is also furnished of the importance of the double bond in activation. It seems, according to Rosenheim and Webster (*J. Soc. Chem. Ind.*, 1926, **45**, 932), that it is not cholesterol, but a substance which they call provitamin, which is changed by irradiation. This supports the earlier conclusion by Schlutz and Morse (*Amer. J. Dis. Child.*, 1925, **30**, 199) that an impurity in cholesterol is the potent factor. P. H. P.

**Sterol Colour Reactions in their Relation to Vitamin *A*. O. Rosenheim.** (*Biochem. J.*, 1927, **21**, 386–388.)—Treatment of cholesterol with benzoyl peroxide in chloroform solution produces a chromogenic substance which gives with arsenic trichloride a blue colour, and a similar colour is obtained when arsenic trichloride is added to a solution of cholesterol in formaldehyde. On superficial inspection the blue pigment formed from cholesterol resembles that obtained in Rosenheim and Drummond's cod-liver oil reaction (ANALYST, 1926, **51**, 93), but it seemes that they are not identical, although a similarity between the carbon ring system of the sterol molecule and that of the unknown chromogen is suggested, and support is given to the view that oxidative changes of the sterol molecule may be concerned in the formation of vitamin *A* from sterols.

Lifschütz's so-called oxycholesterol is undoubtedly a complex mixture, one constituent of which gives rise to the green colour reaction. This material also

gives a blue reaction with arsenic trichloride, but the chromogen of the blue pigment is not that giving rise to the green reaction. Vegetable or animal oils which do not give the blue colour with arsenic chloride fail to give it when mixed with a purified sample of the oxidation products of cholesterol, the rapid destruction of the artificial chromogen being apparently connected with the presence of unsaturated linkages in the oils. T. H. P.

**Parent Substance of Vitamin *D*. O. Rosenheim and T. A. Webster.** (*Biochem. J.*, 1927, **21**, 389–397.)—When purified by one of various methods, cholesterol and phytosterol can no longer be rendered antirachitic by the action of ultra-violet light. That impurity present in ordinary sterols which is responsible for their activation by ultra-violet light appears to be a sterol of an unsaturated and labile type, of which ergosterol is the only known representative. Ergosterol exhibits the same characteristic absorption spectrum in the ultra-violet as non-purified cholesterol, the intensity of the absorption being, however, enormously increased. On the assumption that the impurity concerned is ergosterol, its amount in ordinary cholesterol is shown by comparative spectroscopic examination to be of the order 1:2000. Irradiated ergosterol, in daily doses of 0·0001 mgrm., cures and prevents rickets in rats on a rachitogenic diet. The conclusion seems justified that the natural parent substance of vitamin *D* is either ergosterol or a highly unsaturated sterol of similar constitution, this being converted into vitamin *D* by irradiation. T. H. P.

**Solubilities of the Antiscorbutic Factor Present in Lemon Juice. E. B. Vedder and W. E. Lawson.** (*J. Biol. Chem.*, 1927, **73**, 215–218.)—Some details of experiments on the antiscorbutic vitamin, which were carried out a number of years ago, have now been summarised. The extracts of lemon juice were tested for their efficiency on young guinea pigs which had previously been fed on a diet which included sufficient fresh green vegetables for there to be no danger of incipient scurvy. Data on the solubility of the antiscorbutic vitamin in some of the common solvents are given. Of those investigated, ethyl alcohol was found to give the purest and most uniformly curative extracts. The results with acetone were not in harmony with the work of Bezssonoff (*Compt. rend.*, 1925, **180**, 970), who reported the isolation, from cabbage juice, of a curative substance which may be recrystallised from absolute acetone or absolute alcohol. The authors found that in their experiments the acetone-soluble portion was inactive, and the acetone-insoluble portion was curative, but when a small amount of alcohol was added to the acetone both the acetone-soluble and acetone-insoluble fractions were curative. The influence of certain precipitants was studied in an effort to prepare a purer extract. Tests were carried out on aqueous solutions prepared from the alcoholic extracts, and it was found that neutral lead acetate removed the bulk of the phosphorus and sulphur from these extracts without impairing their curative value. The presence of a natural indicator, yellow in acid and reddish-brown in alkali, was shown. In the alkaline state it may have been present as a salt, since it was soluble in water and insoluble in ether; the

acid product was soluble in both water and ether. The indicator was apparently different from the one described by Bezssonoff, which was red in acid and yellow in alkali, and which he considered to be the quinone formed by oxidation of his antiscorbutic substance. P. H. P.

**Precipitation of the Antiscorbutic Factor from Lemon Juice. S. S. Zilva.** (*Biochem. J.*, 1927, **21**, 354–355.)—In the treatment of decitrated lemon juice by means of lead acetate, most of the anti-scorbutic factor is precipitated within the $P_H$ range 5·4–7·2. The preparation thus obtained shows greatly reduced proportions of total solids and sugar-content and serves as a suitable starting-point for further purification. T. H. P.

**Note on the Vitamin *A* and *B* Content of Cow's Milk. J. Outhouse, I. G. Macy, V. Brekke and A. Graham.** (*J. Biol. Chem.*, 1927, **73**, 203–208.)—A study of the vitamin *A* and *B* content of cow's milk has been made since it was of particular interest and importance to determine the comparative vitamin values of milks used in infant feeding. The milk selected was obtained from cows which were given an unchanging standard dairy ration, and so possible seasonal variation in the vitamin *A* and *B* content of the milk (due to change in the food of the cows) was eliminated. The results show that 3 c.c. of fresh raw cow's milk daily contain adequate vitamin *A* to produce satisfactory growth in the rat, but this small amount does not always give protection against secondary pathological conditions. When rats had been given a vitamin *B*-free ration until growth had ceased, the addition of 12 c.c. of milk daily caused a normal increase in weight for 4 weeks only, whereas 16 c.c. produced satisfactory growth for a period of 8 weeks. A daily quantity of 20 or 25 c.c. allowed for excellent growth, average or above, for about 12 weeks, at which time growth was interrupted. No change in the deportment of the latter group was produced when 0·4 grm. of autoclaved yeast was given daily, but the same quantity of fresh dried yeast brought about an immediate response in growth. Thus, of the 2 factors associated with the growth-promoting properties of vitamin *B*, the thermo-stable fraction is the limiting factor which prevents rats from attaining the average adult weight. The data obtained from giving these amounts of milk to rats as a source of vitamin *B* show that the ingestion of large quantities of milk causes no diminution in the amount of basal food eaten. P. H. P.

**Quantitative Differentiation of Vitamins *A* and *D*. I. H. C. Sherman and M. C. Hessler.** (*J. Biol. Chem.*, 1927, **73**, 113–120.)—In experiments carried out on rats under the conditions of the method of Sherman and Munsell (*J. Amer. Chem. Soc.*, 1925, **47**, 1639) for the quantitative determination of vitamin *A* in foods, it was found that irradiation of the animals with the mercury vapour quartz lamp did not produce large increases in the growth of animals receiving limited amounts of vitamin *A*. This was because the animals contained a nearly sufficient bodily store of antirachitic vitamin to carry them through the test period where growth was limited. A study of the bone structure, which showed few and minor deviations from the normal X-ray picture, line test and histological appearance,

confirmed this. The weight curve used as a measure of the vitamin *A* content of the food under investigation was not influenced by shortage of vitamin *D* when the food was butter fat; but was somewhat influenced when the food was carrot. The butter fat contained more vitamin *D* in relation to its vitamin *A* content than did the carrot. Irradiation, or the giving of irradiated food, to ensure an adequate supply of vitamin *D* is a wise precaution, unless the character of the food and the bodily store of vitamin *D* have been previously established. The difference this may make varies with the stock diets used. The animals which had received their limited allowance of vitamin *A* in a form most likely to involve a shortage of vitamin *D* were investigated further by quantitative determinations of body calcium, but whether or not the irradiation influenced the percentage of calcium in the body in these cases could not be positively decided, since results which showed a slight increase were not confirmed. The weight curve, under adequately controlled conditions, may be a more quantitative indication of a shortage of vitamin *D*. In the rats the factor by which femur calcium could be multiplied to obtain total body calcium was, in the mean of 56 cases, 14·14. P. H. P.

## Bacteriological.

**Thermophilic Bacteria in Milk. M. O. Eckford.** (*Amer. J. Hyg.*, 1927, **7**, 201–221.)—Thermophiles isolated from the Baltimore milk supply were all aerobic and complied, in general, with the findings of earlier investigators. Their morphological features were similar, but culturally they differed. The optimum temperature was between 50 and 60° C., and the lower limit for some of the forms isolated from milk was 42° C.; these were called " true " thermophiles, whilst those growing below this temperature were called " thermo-tolerants." The thermophilic bacteria survived pasteurisation and temperatures of 100–120° C. for 15 minutes in the De Khotinsky oil bath. It is probable that they get into milk during milking, from the cow feed, dust, hands of the milker, or more probably from the discharges of the cow, and it is likely that their presence in milk would serve as a check on the sanitary or unsanitary conditions under which the milk is produced. Of those thermophiles isolated, 66 per cent. liquefied gelatin, and 22 per cent. of these peptonised milk. They were not strongly fermentative, and only two groups fermented lactose. They are not present in canned milk, but may give pasteurised milk a high bacterial count, and cause pin point colonies at 37° C. One thermophilic proteolytic streptococcus was isolated, and the 4 obligate thermophiles isolated have not been previously described. D. G. H.

## Toxicological and Forensic.

**Relative Toxicity of Benzol and its Higher Homologues. J. J. Batchelor.** (*Amer. J. Hyg.*, 1927, **7**, 276–298.)—Toxicity in acute poisoning by intraperitoneal injection of " Hi-flash " naphtha, xylol, toluol and benzol increases in the order given, whilst in subcutaneous injection benzol produces

progressive destruction and aphasia of the whole haemopoietic system, with haemorrhages and fatty degeneration of the bone marrow, but the other solvents lead rather to a stimulation of the haemopoietic system with hyperplasia. Lesions produced in the excretory organs do not exceed what would be expected with the extreme dosage administered, and toluol, xylol and " Hi-flash " naphtha produce only negligible effects on the animal's general behaviour. Benzol thus possesses special neurotoxic and haematopoietic properties almost wholly lacking in the other solvents tested. In inhalation experiments toluol and xylol proved more toxic than benzol to animals exposed for 18 to 20 hour a day (1600 parts per million proved fatal), owing to their higher narcotic effect, although more serious damage resulted both to the central nervous system and blood-forming organs with benzol for concentrations of 1000 parts per million, approximating industrial conditions. In this case the narcotic effects of toluol and xylol are nearly absent, but even for concentrations of 466 parts per million for benzol profound leucopenia occurs and 25 per cent. loss of weight. Toluol, xylol and " Hiflash " naphtha are indicated for use whenever possible instead of benzol, owing to the much smaller industrial hazards. D. G. H.

# Water Analysis.

**Colorimetric Determination of Phosphates in Potable Waters by the Denigès Method. R. Danet.** (*J. Pharm. Chim.*, 1927, **119**, 490–491.)—The standard scales, as prepared by Denigès for his colorimetric determination of phosphates by means of stannous chloride and sulphomolybdic reagent, were found unsatisfactory, and a solution containing 0·02 grm. of phosphoric anhydride per litre may be prepared by diluting 100 times the ammonium phosphate solution used in the determination of uranium. One drop of this solution in 10 c.c. of water gives a solution containing one-tenth of a mgrm. per litre, so that a scale of standards from 0·1 to 5 mgrm. per litre may be thus rapidly prepared. D. G. H.

**Phosphorus and Arsenic Compounds of Sea-Water. W. R. G. Atkins and E. G. Wilson.** (*J. Marine Biol. Assoc.*, 1927, **14**, 609–614.)—A consideration of the analytical methods and results of previous workers indicates that much of what was formerly considered to be phosphorus in organic combination in sea-water, is really arsenic. Thus Raben used the phospho-molybdate precipitate method which is invalidated by the inclusion in the precipitate as arseno-molybdate, of arsenate produced by the oxidation of the arsenite in the sea-water during the experiment. The Denigès reaction, also, is given by phosphates and arsenates, but not by arsenites. The arsenic in oysters is probably derived from the diatoms on which they feed, and the increase in the amount found in algae with increasing depth of the sea indicates seasonal changes due probably to absorption of arsenic by plants. (*Cf. Biochem. J.*, 1926, **20**, 1223.) J. G.

**Importance of the various Factors Responsible for the Death of Fishes in Polluted Waters. H. S. Pruthi.** (*J. Marine Biol. Assoc.*, 1927, **14**, 729–739.) —This paper deals with substances that pollute water indirectly as the result of

the production of poisons, rather than with substances which are themselves poisonous. The common freshwater stickelback (*Gasterosteus aculeatus*), found near Plymouth, was chosen for the experiments, and peptone, egg albumin and milk casein were taken as the putrefying substances. These were used in Plymouth tap-water ($P_H$ value 7·0, alkali reserve 0·0005 *N*) under aerobic and anaerobic conditions, and all cultures were kept at 65° to 70° F. The $P_H$ values were determined colorimetrically, the oxygen by Winkler's method, and the " alkali reserve " by titration to methyl orange with 0·01 *N* sulphuric acid. The $P_H$ value of the water in which the fish were found was 7·5–8·0 (not corrected for the salt error), and the alkali reserve 0·0045 *N*. Outside the range $P_H$ 6·0–8·5 an increase on the acid side was more harmful to the fish than one on the alkaline side. A concentration of carbon dioxide below 10·0 to 13·0 c.c. per litre was not harmful. If other conditions are favourable, the fish can live in water the oxygen content of which is 0·25 to 0·50 c.c. per litre, and the case is cited of freshwater fish that were not affected above the concentration of 1·7 to 0·4 c.c. per litre. During putrefaction the $P_H$ value falls for a short time and then rises, probably on account of the production of basic substances. The fact that it does not fall much below $P_H$ 6·0, either in aerated or anaerobic solutions, is attributed to the low alkali reserve, which is almost that of distilled water. Solutions exposed in wide bowls gradually lost all their oxygen (6·5 c.c. per litre) within 4 days, but when it was renewed the rate of loss was greatest after a week. Fermenting casein had the highest rate of loss. The killing powers of the solutions, as measured by the dying time of three specimens in 500 c.c. of thoroughly aerated solution exposed in a bowl, at the favourable $P_H$ value of 7·0–8·0, were also studied. The solutions were most poisonous after 7 to 15 days (a little longer in the case of the aerobic solutions), after which they gradually lost their toxicities. Casein was the first to acquire toxicity and became most poisonous. The loss of toxicity was shown to be due to the volatile nature of the toxic substances, by a comparison of the killing times of the solutions with those of the distillates and residues after distillation of the putrefied solutions in both acid and alkaline conditions. Since the distillate was more toxic than the residue and less toxic than the original liquid, only some of the toxic substances are volatile, whilst the residues probably lose their toxic powers owing to the action of heat. The stage of highest toxicity was always preceded by a period of rapid oxygen consumption. It is suggested that the pressure of carbon dioxide and the rate of oxygen consumption are more important factors, especially in still water and in the lower layers of polluted water, respectively. But even at the optimum conditions, so far as these factors are concerned, the toxic by-products of putrefaction may kill the fish in as short a time as 0·5 to 6 hours. J. G.

# Organic Analysis.

**Determination of Carbonyl in Aldehydes and Ketones. G. W. Ellis.** (*J. Chem. Soc.*, 1927, **122**, 848.)—If aldehydes or ketones are allowed to react with phenylhydrazine, the carbonyl group of the former combines with the latter to

form phenylhydrazone. If an excess of phenylhydrazine be employed, the amount in excess can be determined by means of Fehling's solution, which reacts with phenylhydrazine, but not with phenylhydrazone, to produce benzene and nitrogen. From the volume of nitrogen produced, the proportion of carbonyl in the original ketone can be calculated. The method given below is an improvement on the older methods, in that the nitrogen is completely removed from solution in the reacting liquids. Two hundred and fifty c.c. of mercury are placed in a strong, short-necked, 300 c.c. round-bottomed flask, provided with a two-holed rubber stopper. Through one hole passes a siphon tube, connecting the mercury, by means of rubber-tubing, with a smaller quantity of mercury contained in a levelling reservoir. Through the other hole passes a glass capillary-tube and two-way cock, leading to a cup of 5 to 10 c.c. capacity or to the eudiometer. The flask is surrounded by a beaker containing only a little water, which can be heated to boiling. Fifty c.c. of Fehling's solution are poured on to the mercury in the flask, and the dissolved air is removed under reduced pressure by boiling the water in the beaker, the two-way cock being closed and the mercury reservoir lowered as far as possible Cold water is sprayed on to the upper part of the flask, thus increasing the vacuum still further. The evolved air is removed by opening the two-way cock and raising the mercury reservoir. The process is repeated 2 or 3 times on the Fehling's solution, and also at the end of the actual determination. The solution obtained by treating, *e.g.* 0·0619 grm. of salicylaldehyde with 0·43 grm. of phenylhydrazine hydrochloride (mixed with 0·43 grm. of anyhydrous sodium acetate) in a water bath for a few minutes, was placed in the cup and run into the Fehling's solution. The cup was rinsed in, the nitrogen evolved, as described above, was measured, saturated with aqueous and benzene vapours, in the eudiometer. The method gives fairly concordant results. For example :—*Salicylaldehyde* : Found CO 22·2, 24·8, 23·2 ; calculated, 22·9 per cent. *Acetone* : Found CO 47·3 ; calculated, 48·2 per cent. A sketch of the apparatus is given. R. F. I.

**Some Ill-defined Acids of the Oleic Series. I. Hypogaeic Acid. T. P. Hilditch and N. L. Vidyarthi.** (*J. Soc. Chem. Ind.*, 1927, **46**, 172.)—The presence of " hypogaeic acid " in arachis nut oil has been investigated by the following method. The liquid fatty acids were separated from the mixed fatty acids, and the most volatile portions converted into their methyl esters. These were hydrolysed and oxidised in alkaline solution. The only dihydroxy acid detected was 9:10-dihydroxystearic acid (m.pt. 130° C.). From this the conclusion is drawn that " hypogaeic acid," or other acid of the formula $C_{16}H_{30}O_2$, does not occur in arachis oil. The authors suggest that the acid $C_{16}H_{30}O_2$ found in marine animal oils ($\Delta$ 9:10-hexadecenoic acid) be termed *palmitoleic acid*, and that the term " hypogaeic acid " be deleted from the literature. R. F. I.

**Some Ill-defined Acids of the Oleic Series. II. Acids in Parsley Seed Oil. T. P. Hilditch and E. E. Jones.** (*J. Soc. Chem. Ind.*, 1927, **46**, 174.)—The authors have found that English parsley seed oil contains 76 per cent. of petroselinic acid, and confirm the formula given to this acid by Vongerichten and Kohler,

*viz.* α-Δ-6:7-octadecenoic acid. The unsaponifiable portion of English and French parsley-seed oil consists mainly of the liquid myristicin, $(CH_2O_2)$ (MeO) $C_6H.CH_2.CH:CH_2$. The unsaponifiable portion of German parsley-seed oil is mainly the crystallin apiole $(CH_2O_2)$ $(MeO)_2C_6H.CH_2.CH:CH_2$. R. F. I.

# Inorganic Analysis.

**Use of Methoxytriphenylcarbinols as One-colour Indicators. I. M. Kolthoff.** (*J. Amer. Chem. Soc.*, 1927, **49**, 1218–1221.)—Various polymethoxytriphenylcarbinols form useful acidimetric indicators, as either solutions or test papers, being colourless in alkaline and coloured in acid solution. 2:4:2′:4′:2″-Pentamethoxytriphenylcarbinol, for which the name *pentamethoxy red* is suggested, is colourless at $P_H=3{\cdot}2$ and reddish-violet at $P_H=1{\cdot}2$, and has very small salt and alcohol errors. One hundred c.c. of water containing 0·25 c.c. of 0·1 per cent. indicator solution requires 0·04 c.c. of 0·1 *N* hydrochloric acid to give a barely visible pink; 0·1 c.c. of the acid gives a distinct pink, which is the best end-point ($P_H$, 4·0). The indicator may be used in determining the $P_H$ of gastric juice and in the titration of ammonia and alkaloids. Phosphoric acid may be titrated as a monobasic acid, and with borax the same results as with methyl red are obtained. T. H. P.

**Determination of Cobalt. G. Spacu and J. Dick.** (*Z. anal. Chem.*, 1927, **71**, 97–101.)—Cobalt is precipitated by ammonium thiocyanate and pyridine as pink, crystalline $(CoPy_4(CSN)_2)$, which is weighed. The method is stated to be as accurate as electrolysis. Copper, cadmium, nickel, manganese, and zinc are also precipitated; methods for the separation of these metals will be published in due course. (*Cf.* ANALYST, 1925, **50**, 580.) W. R. S.

**Specific Colour Reaction for Magnesium and a Colorimetric Method for the Determination of Traces of Magnesium. I. M. Kolthoff.** (*Chem. Weekblad*, 1927, **24**, 254–255.)—The indicator "Titan yellow" ($P_H$ 12·0 to 13·0) is adsorbed by magnesium hydroxide when precipitated in a solution containing it, with the production of a colour change of yellow to dark red. Complete adsorption and a colourless solution are obtained with 50 mgrms. of magnesium and 0·5 c.c. of a 0·1 per cent. solution of indicator in 10 c.c. of liquid containing 1 c.c. of 0·1 *N* sodium hydroxide solution. The colour is modified to red-brown and orange-brown by smaller quantities of magnesium. A sensitiveness of 0·2 mgrm. of magnesium per litre is obtained by the use of 0·2 c.c. of the indicator solution with 0·25 to 0·5 c.c. of a 4 *N* sodium hydroxide solution in 10 c.c. of liquid. When the colour is to be matched, the best scale is obtained with solutions containing 4·0 to 0·4 mgrms. of magnesium per litre. The reagent is specific for magnesium, but cadmium and calcium salts deepen the colour. When calcium is present the standards are prepared in solutions containing 100 mgrms. of calcium per litre. Methods of removal of tin and aluminium (which interfere with the colour

by adsorption of the magnesium hydroxide) are given, and zinc is rendered inert by the addition of an excess of sodium sulphide solution. The method has been tested by analyses of tap-water and of salts of the alkali metals. J. G.

**Determination of Magnesium by Means of Oxyquinoline. R. Berg.** (*Z. anal. Chem.*, 1927, **71**, 23–36.) (*Cf.* next abstract.)—Magnesium yields with *o*-oxyquinoline, in ammoniacal (or alkaline tartrate) solutions, a yellowish-green crystalline precipitate ; the reaction is much more sensitive than phosphate precipitation. The precipitate has the composition $Mg(C_9H_6ON)_2.4H_2O$. The magnesium solution, which must contain sufficient ammonium chloride or acetate to prevent precipitation of the hydroxide, is treated with a few c.c. of strong ammonia, and precipitated at a temperature near the boiling-point with a 2 per cent. alcoholic solution of the reagent in small excess. This is indicated by the yellow colour of the liquid, as a result of the formation of the strongly-coloured ammonium oxyquinolate. After settling, the precipitate is collected in a Gooch or porous crucible, washed with hot, weakly ammoniacal water, and dried at 100° to 105° C. Constant weight is attained at that temperature at the dihydrate stage, corresponding to 6·98 per cent. Mg. The precipitate becomes anhydrous after 4 to 8 hours' heating at 130° to 140° C., but not without very slight decomposition. Alternatively, the precipitate may be converted into oxide by careful ignition under a layer of 2 to 3 grms. of anhydrous oxalic acid. Titration is the most convenient form of determination : the washed precipitate is dissolved in 10 per cent. hydrochloric acid, and titrated with 0·1 *N* bromate-bromide solution in slight excess ; this is measured with thiosulphate, after addition of a few c.c. of 20 per cent. potassium iodide solution. The reaction yields 5,7-dibromoxyquinoline, hence 1 c.c. of 0·1 *N* bromine = 0·000304 grm. Mg. The process affords a separation from large quantities of alkali metals. In presence of alkaline earths, magnesium is precipitated as follows :—The solution (100 c.c.) is treated with 5 to 10 grms. of ammonium acetate and a few c.c. of strong ammonia, heated to boiling, and precipitated as before. More ammonia is added to the boiling solution until it imparts a weak alkaline reaction to phenolphthalein paper. The precipitate is collected without delay and washed with hot, weakly ammoniacal, 5 per cent. ammonium acetate solution till the washings are colourless. The precipitate is dissolved in a minimum of hot dilute hydrochloric acid, and the precipitation repeated as above after addition of 1 to 2 grms. of ammonium acetate and a few drops of the reagent. The re-precipitation is always advisable for the quantitative separation from calcium, as well as for considerable quantities of barium and strontium. W. R. S.

**Oxyquinoline as a Reagent for Magnesium, Zinc and Aluminium. F. L. Hahn and K. Vieweg.** (*Z. anal. Chem.*, 1927, **71**, 122–130.) (*Cf.* preceding abstract.)—A 5 per cent. alcoholic solution of 8-oxyquinoline was used : 10 c.c. suffice for the precipitation of one millimol of tervalent, and 7 c.c. for a bivalent metal. The faintly acid or neutral solution (0·1 grm. of metal or less per 100 c.c.)

is warmed and treated with the required amount of reagent, and a little more than enough ammonium acetate to react with the free and liberated mineral acid. In the case of magnesium and beryllium, a moderate excess of ammonia must be present. The precipitates are allowed to stand for some time on the water bath. After cooling, they are collected on porous glass or porcelain and washed with *N* acetic acid, then weaker acetic acid, and finally with water; dilute ammonia is used in the case of magnesium and beryllium. The precipitates filter well; they are dried, first at 100° to 120° C., and then to constant weight at 140° to 160° C. *Magnesium*: the precipitation is stated to be the most accurate and by far the simplest of all the known processes. The presence of various salts of the alkali metals is without influence on the results. *Zinc* may be precipitated in ammoniacal or in acid acetate solution; in presence of magnesium, zinc is first precipitated from acetate solution; the filtrate is treated for magnesium with ammonia and additional reagent. *Aluminium*: the results given are very concordant, the extreme deviations being 99·95 and 100·12 per cent. W. R. S.

**Iodimetric Determination of Vanadium. J. B. Ramsey.** (*J. Amer. Chem. Soc.*, 1927, **49**, 1138–1146.)—Under no conditions of acid, iodide, and vanadium concentrations is the catalytic effect of quinquevalent vanadium on the oxidation of iodide ion by oxygen negligible, so that the iodimetric determination of vanadium does not give accurate results if carried out in presence of air. The following procedure admits of a determination accurate to within 0·1 per cent. being completed in about 15 minutes. Between 10 and 20 grms. of approximately 0·1 *N* vanadate solution and 5 c.c. of dilute sulphuric acid are placed in a 400 c.c. flask, closed with a rubber stopper fitted with an inlet tube reaching to within 1 cm. of the surface of the liquid, and with an outlet tube. Carbon dioxide is passed through the flask while the liquid is heated just to boiling, a trap containing water being then attached to the outlet tube, and the liquid cooled with water at such rate that the gas continues to bubble through the trap. The stopper is removed momentarily from the cold flask, and the desired amount of solid potassium iodide added. The gas flow is then diminished until the pressure in the flask just exceeds the atmospheric pressure, and the solution shaken to dissolve the iodide. When 5 c.c. of 6 *N* sulphuric acid and 2 to 4 grms. of potassium iodide are used the reduction of the vanadium to the quadrivalent stage is complete within two minutes from the addition of the iodide. The flask is then unstoppered and the liquid diluted to about 300 c.c. Standard sodium thiosulphate solution is run in until reduction of the liberated iodine is almost complete, the end-point being determined after addition of starch. T. H. P.

**Determination of Alkalis in Ores, Clays, etc. J. Ciochina.** (*Z. anal. Chem.*, 1927, **71**, 45.)—The finely-powdered material, contained in a steel, copper or nickel boat, is heated for 2 hours in a silica tube in a current of dry hydrogen sulphide at 500° to 600° C. The boat and tube are rinsed with cold distilled water, the solution is filtered, and the washed residue treated once more as the original material. The combined filtrates are treated with carbon dioxide, whereby the

carbonates of calcium and magnesium are precipitated. The solution is boiled for a minute, cooled, treated with excess of standard iodine solution, and well stirred. It is then acidified with 30 c.c. of hydrochloric acid (1:3), and the excess of iodine titrated with thiosulphate. The sulphide found is a measure of the alkali content. W. R. S.

**Determination of Sulphate in Presence of other Sulphur Compounds. A. Kurtenacker and R. Wollak.** (*Z. anal. Chem.*, 1927, **71**, 37–42.)—The neutral or weakly alkaline solution is left to stand for 5 minutes with sufficient formaldehyde to combine with the sulphite present. Acetic acid (10 c.c. of 20 per cent.) and 0·1 *N* iodine solution to the appearance of a faint, permanent yellow coloration, are added. A faint excess of iodine is removed by a drop of sulphate-free thiosulphate solution. The solution is diluted to about 350 c.c., and precipitated cold, while kept stirred all the time, with 0·1 *N* barium chloride, added drop by drop. After settling, the barium sulphate is collected, washed with cold water, and ignited in the usual manner. W. R. S.

**Determination of Available Chlorine in Bleaching Preparations. J. Hausner.** (*Chem. Zeit.*, 1927, **39**, 373.)—The following method is suitable for determining the available chlorine in bleaching-powder or sodium hypochlorite liquors, and can be used by an unskilled operator. It is carried out in a special glass-stoppered cylinder, 23 cm. high by 2 cm. in diameter, which is graduated on an arbitrary scale. The liquor to be tested is poured into the cylinder up to a mark, and then occupies about one sixth of the capacity of the cylinder. This portion is ungraduated. Above this mark the cylinder is graduated in divisions representing from 0·25 to 5 grms. of available chlorine per litre of the liquor under examination. A slightly acid dilute solution of sodium thiosulphate and indigo carmine is made up containing 0·85 grm. of thiosulphate, 0·14 grm. of indigo-carmine, and one or two drops of glacial acetic acid per litre. This solution is added to the bleach-liquor in quantities of a quarter of a division at a time, the cylinder being shaken after each addition, until the solution acquires a blue-green colour, when the number of grms. of available chlorine per litre of test solution is read off. The method gives results which compare very favourably with the potassium iodide and thioculphate method. It is recommended that the thio-sulphate and indigo carmine solution should be protected against bacterial decom-position by means of a preservative. R. F. I.

**Analysis of Sodium Nitrite. F. A. Höeg.** (*Z. anal. Chem.*, 1927, **71**, 102–107.)—The following modification of Klemenc's method is given as an im-provement on the well-known procedure consisting in adding the nitrite liquor to acidified permanganate solution. A round flask (400 to 500 c.c.) with a short, wide neck is fitted with a doubly perforated rubber stopper, through which pass a small separator funnel reaching nearly to the bottom of the flask, and a glass tube with tap for evacuating the air. In the flask are placed 150 c.c. of 20 per cent. sulphuric acid and 50 c.c. of 0·1 *N* permanganate. The flask is stoppered and

evacuated to 100 mm. The nitrite solution (25 c.c. of 6·9000 grms. dissolved in 1 litre) is added through the separator, which is then rinsed with a few small portions of distilled water. The closed flask is kept at 40° C. for about 5 minutes, opened, and the excess of permanganate measured with 0·04 *N* oxalic acid adjusted against the permanganate solution. This is standardised against 100 per cent. nitrite (repeatedly re-crystallised and heated to constant weight), or sodium oxalate.

W. R. S.

# Physical Methods, Apparatus, etc.

**New Glycerol Tables for Specific Gravity and Per Cent. of Glycerol. L. W. Bosart and A. O. Snoddy.** (*Ind. Eng. Chem.*, 1927, **19**, 506–510.)—Tables are given showing the apparent and true specific gravities, *i.e.* specific gravities reduced to vacuum, of mixtures of glycerol and water of varying strengths at different temperatures.

| Glycerol. Per Cent. | Apparent Specific Gravity. | | | | True Specific Gravity. | | | |
|---|---|---|---|---|---|---|---|---|
| | 15/15 °C. | 15·5/15·5 °C. | 20/20 °C. | 25/25 °C. | 15/15 °C. | 15·5/15·5 °C. | 20/20 °C. | 25/25 °C. |
| 100 | 1·26557 | 1·26532 | 1·26362 | 1·26201 | 1·26526 | 1·26501 | 1·26331 | 1·26170 |
| 99 | 1·26300 | 1·26275 | 1·26105 | 1·25945 | 1·26270 | 1·26245 | 1·26075 | 1·25910 |
| 98 | 1·26045 | 1·26020 | 1·25845 | 1·25685 | 1·26010 | 1·25985 | 1·25815 | 1·25655 |
| 97 | 1·25785 | 1·25760 | 1·25585 | 1·25425 | 1·25755 | 1·25730 | 1·25555 | 1·25395 |
| 96 | 1·25525 | 1·25500 | 1·25330 | 1·25165 | 1·25495 | 1·25470 | 1·25300 | 1·25140 |
| 95 | 1·25270 | 1·25245 | 1·25075 | 1·24910 | 1·25240 | 1·25215 | 1·25045 | 1·24880 |
| 94 | 1·25005 | 1·24980 | 1·24810 | 1·24645 | 1·24975 | 1·24950 | 1·24780 | 1·24615 |
| 93 | 1·24740 | 1·24715 | 1·24545 | 1·24380 | 1·24710 | 1·24685 | 1·24515 | 1·24350 |
| 92 | 1·24475 | 1·24450 | 1·24280 | 1·24115 | 1·24445 | 1·24420 | 1·24250 | 1·24085 |
| 91 | 1·24210 | 1·24185 | 1·24020 | 1·23850 | 1·24185 | 1·24155 | 1·23985 | 1·23825 |
| 90 | 1·23950 | 1·23920 | 1·23755 | 1·23585 | 1·23920 | 1·23895 | 1·23725 | 1·23560 |
| 89 | 1·23680 | 1·23655 | 1·23490 | 1·23320 | 1·23655 | 1·23625 | 1·23460 | 1·23295 |
| 88 | 1·23415 | 1·23390 | 1·23220 | 1·23055 | 1·23390 | 1·23360 | 1·23195 | 1·23025 |
| 87 | 1·23150 | 1·23120 | 1·22955 | 1·22790 | 1·23125 | 1·23095 | 1·22930 | 1·22760 |
| 86 | 1·22885 | 1·22855 | 1·22690 | 1·22520 | 1·22860 | 1·22830 | 1·22660 | 1·22495 |
| 85 | 1·22620 | 1·22590 | 1·22420 | 1·22255 | 1·22595 | 1·22565 | 1·22395 | 1·22230 |
| 84 | 1·22355 | 1·22325 | 1·22155 | 1·21990 | 1·22330 | 1·22300 | 1·22130 | 1·21965 |
| 83 | 1·22090 | 1·22055 | 1·21890 | 1·21720 | 1·22060 | 1·22030 | 1·21865 | 1·21695 |
| 82 | 1·21820 | 1·21790 | 1·21620 | 1·21455 | 1·21795 | 1·21765 | 1·21595 | 1·21430 |
| 81 | 1·21555 | 1·21525 | 1·21355 | 1·21190 | 1·21530 | 1·21500 | 1·21330 | 1·21165 |
| 80 | 1·21290 | 1·21260 | 1·21090 | 1·20925 | 1·21265 | 1·21235 | 1·21065 | 1·20900 |
| 79 | 1·21015 | 1·20985 | 1·20815 | 1·20655 | | | | |
| 78 | 1·20740 | 1·20710 | 1·20540 | 1·20380 | | | | |
| 77 | 1·20465 | 1·20440 | 1·20270 | 1·20110 | | | | |
| 76 | 1·20190 | 1·20165 | 1·19995 | 1·19840 | | | | |
| 75 | 1·19915 | 1·19890 | 1·19720 | 1·19565 | 1·19890 | 1·19865 | 1·19700 | 1·19540 |
| 74 | 1·19640 | 1·19615 | 1·19450 | 1·19295 | | | | |
| 73 | 1·19365 | 1·19340 | 1·19175 | 1·19025 | | | | |
| 72 | 1·19090 | 1·19070 | 1·18900 | 1·18755 | | | | |
| 71 | 1·18815 | 1·18795 | 1·18630 | 1·18480 | | | | |
| 70 | 1·18540 | 1·18520 | 1·18355 | 1·18210 | 1·18515 | 1·18495 | 1·18330 | 1·18185 |
| 69 | 1·18260 | 1·18240 | 1·18080 | 1·17935 | | | | |
| 68 | 1·17985 | 1·17965 | 1·17805 | 1·17660 | | | | |
| 67 | 1·17705 | 1·17685 | 1·17530 | 1·17385 | | | | |
| 66 | 1·17430 | 1·17410 | 1·17255 | 1·17110 | | | | |

| Glycerol. Per Cent. | Apparent Specific Gravity. 15/15 °C. | 15·5/15·5 °C. | 20/20 °C. | 25/25 °C. | True Specific Gravity. 15/15 °C. | 15·5/15·5 °C. | 20/20 °C. | 25/25 °C. |
|---|---|---|---|---|---|---|---|---|
| 65 | 1·17155 | 1·17130 | 1·16980 | 1·16835 | 1·17135 | 1·17110 | 1·16960 | 1·16815 |
| 64 | 1·16875 | 1·16855 | 1·16705 | 1·16560 | | | | |
| 63 | 1·16600 | 1·16575 | 1·16430 | 1·16285 | | | | |
| 62 | 1·16320 | 1·16300 | 1·16155 | 1·16010 | | | | |
| 61 | 1·16045 | 1·16020 | 1·15875 | 1·15735 | | | | |
| 60 | 1·15770 | 1·15745 | 1·15605 | 1·15460 | 1·15750 | 1·15725 | 1·15585 | ·15445 |
| 59 | 1·15490 | 1·15465 | 1·15325 | 1·15185 | | | | |
| 58 | 1·15210 | 1·15190 | 1·15050 | 1·14915 | | | | |
| 57 | 1·14935 | 1·14910 | 1·14775 | 1·14640 | | | | |
| 56 | 1·14655 | 1·14635 | 1·14500 | 1·14365 | | | | |
| 55 | 1·14375 | 1·14355 | 1·14220 | 1·14090 | 1·14360 | 1·14340 | 1·14205 | 1·14075 |
| 54 | 1·14100 | 1·14080 | 1·13945 | 1·13815 | | | | |
| 53 | 1·13820 | 1·13800 | 1·13670 | 1·13540 | | | | |
| 52 | 1·13540 | 1·13525 | 1·13395 | 1·13265 | | | | |
| 51 | 1·13265 | 1·13245 | 1·13120 | 1·12995 | | | | |

In every case water at the same temperature is taken as unity. A table is also given showing the rate of expansion of mixtures of glycerol and water of varying concentrations from 15° C. to 25° C., thereby giving a means for comparing all glycerol tables with each other. W. P. S.

# Reviews.

ESSAYS ON THE ART AND PRINCIPLES OF CHEMISTRY. HENRY E. ARMSTRONG, F.R.S. Pp. 276. London: Ernest Benn, Limited. 1927. Price 15s. net.

This volume consists of an introduction and seven essays reproduced from the *Encyclopaedia Britannica* and various scientific journals; they give an illuminating and stimulating survey of chemical theory and bear the impress of a master mind.

Professor Armstrong is not "dry"; but he loves water. Water flows in a deep swift stream right through the book and this, of course, involves the special consideration of its constitution and the part it plays in chemical actions. Ions (in the sense of the electrolytic dissociation theory) and particularly $P_H$ are anathema to our author, but not without reason; the arguments adduced relate to fundamental points and are forceful. One is impressed with the need of attention to the fundamentals of the subject; there are unanswered questions which are of so important a character that it is unsatisfactory to build the more delicate superstructure of the theory until the foundations are secure. Where does the energy come from; why should hydrogen chloride dissociate in water; and if *pure* water is a non-conductor, how can the electrolytic theory apply to it?

Armstrong agrees with Fitzgerald that it is risky for a chemist to apply mathematics and vigorously disputes the mathematical developments of physical

chemistry; "chemistry is an art as much as a science and the chemist is full of feeling which cannot be quantified." This feeling is admirably developed in the essay on Hydrone, but if the author could overcome his aversion and persuade a mathematician to woo the fair Hydrone, the quantification of their issue might lead to investigations of a quantitative kind which would prove or disprove the theory. The reason for the wide acceptance of the ionic hypothesis probably is that it is capable of exact quantitative presentation and yields results which can be experimentally tested, whereas the alternative, so ably urged by Armstrong, although attractive to the chemic mind is not available quantitatively.

The corrosion of metals, enzyme action, organic structures and catalysis are prominent among the other subjects critically presented. There are extreme statements, many caustic comments and quips; some rather personal references appear and much that is controversial, but this is what makes Armstrong Armstrong. The book deserves to be read and pondered, and the style of it ensures that it will be read with interest. H. E. Cox.

ARTIFICIAL FERTILISERS. By P. PARRISH, A.I.C., M.I.Chem.E., and A. OGILVIE, A.M.I.Mech.E. Volume I. Pp. 355. London: Ernest Benn, Ltd. 1927. Price 45s. net.

This work is chiefly devoted to the methods of manufacture of various artificial fertilisers and deals with the processes involved in the production of phosphatic fertilisers. No doubt, a subsequent volume will give the methods of manufacture of fertilisers containing nitrogen and potash. A short foreword by Dr. H. C. Brown contains the important statement that the greater demand for food supplies renders imperative the use of increased quantities of artificial fertilisers, and also demands a correct knowledge and appreciation of their application to the soil. This statement might well be expanded and allusion made to the necessity for supplying phosphatic and other fertilisers at the lowest possible price, so that a more extended use might become possible by the farmer and the price still permit of a margin of profit for the manufacturer.

At least one-half of the present volume is occupied with the history, chemistry, and manufacture of superphosphate, the technological details concerned with its manufacture being very completely described. The first chapter consists of a summary of historical notes concerning artificial fertilisers, but, somewhat unlike the orthodox opening chapter of volumes devoted to agricultural science, it contains statistical information. In the authors' opinion, the annual supply of North African phosphate now equals in quantity that of the United States of America, a condition of affairs which would have been deemed to have been highly improbable but a few years ago. The seizure by the British Navy during the war, of the Island of Nauru, which had, until that time, belonged to Germany, gave an enormous supply of the richest phosphatic deposits in the world to the British Empire, and the author comments that so far England has not taken her quota of the raw phosphate allowed to her under the Nauru Island Agreement Act of

1919. No doubt, the reason for this is that it has not been possible to deliver Nauru phosphate here at a price which can compete with the price of North African phosphate. The mining and treatment of phosphatic rock is dealt with in a chapter which is not only instructive, but rendered interesting owing to a profusion of illustrations. The matter on the manufacture of superphosphate must to-day be considered of great importance, for plans and descriptions of continental factories and plant are given. Our manufacturers were, but two years ago, informed by an officially appointed Committee that continental manufacturers of superphosphate had adopted new and up-to-date plant, which had been one means of aiding them to compete successfully with the home producer. It therefore behoves everyone connected with the production of soluble phosphates for agricultural purposes to make a careful study of foreign methods and foreign plant, so that no possible opportunity of reducing the cost of production is overlooked.

Various methods of screening are explained and depicted, and the necessity for the fine grinding of raw phosphate before acidulation is emphasised. The cost of grinding is more than compensated by the saving of acid; also the final product is drier and less acid. A useful table is appended to this chapter, which shows the amount of sulphuric acid required by various substances occurring in a given quantity of phosphate rock.

A chapter devoted to compound manures deals largely with mixing plant, several systems being described and illustrated. Tables are given showing the average composition of compound fertilisers sold for application to specific crops, and the author, while acknowledging that such mixtures cannot be invariably the best on every class of soil, suggests that they may be used with benefit until the individual farmer has discovered a better formula by manurial experiments on his own land. One cannot but express agreement with this view, but the authors are perhaps not aware of the very considerable number of farmers who do now require a compound fertiliser of a composition which differs from the adopted formula of the merchant, and which therefore requires special preparation and mixing.

To obviate the setting of compound fertilisers it is suggested that the remedy lies in re-grinding after an interval and resieving. Practically no mention is made of the many organic substances of manurial value which have been used for this purpose and which do appear to help in the prevention of hard setting after purchase. The chapter is brought up-to-date by the inclusion of an article on both potassic superphosphate and potassic basic slag.

The authors' view, that bone meal should pass the $\frac{1}{8}$ inch mesh sieve, and steamed bone flour the 50 mesh, is interesting to-day, as there has been a tendency to place these products on the market in a coarse condition, and a considered statement from an authority who represents the manufacturing side of this industry is therefore important.

Articles follow on basic slag and on the handling of raw material, and the book terminates with a discussion on the trend of future developments of the industry,

the author concluding by pointing out the necessity for continued attention to research work and the expediency of following up the suggestions of such workers.

It is inconceivable that anything but benefit can result from the publication, not only of the details of manufacture, but also of costings, even if the latter can only apply to a particular works. There is no question that the industry concerned needs every help, if it is to recover from its present depressed condition.

The arrangement of the book is clear, typographical errors are almost absent, and the illustrations, which are profuse, are well executed.

F. W. F. ARNAUD.

LES RELATIONS ENTRE LES CONSTANTES DES MATIÈRES GRASSES. Par JACOB LUND; traduit de l'Allemand par N. CHARLIER et E. TCHETCHEROFF. Pp. 109. Gembloux: Imprimerie J. Duculot, Editeur. 1926.

In 1922, two papers were published, one in England by Pickering and Cowlishaw (*J.S.C.I.*, 1922, **41**, 74T), and the second in Germany by Lund (*Z. Unters. Nahr. Genussm.*, 1922, **44**, 113–187), describing the relations existing between various constants of fatty oils. These researches have been continued by Lund and form the subject of this monograph.

After dealing in a general manner with the analysis of oils, the author describes the effect of experimental conditions on the specific gravity and refractive index of an oil, the influence of impurities on various constants, and the difference between the constants yielded by oils and by the fatty acids prepared from them. He then derives a series of equations connecting each constant with other constants for a wide range of oils. In every case the equation is linear in character.

While most investigators of the analysis of oils and fats have devised new methods to deal with each new problem which has confronted them, the author of this monograph has attempted to make greater use of existing methods by a strict correlation of the results they yield. The ease with which such correlation is obtained, and the accuracy with which the equations fit the figures for widely differing oils, lead one to the view that many of the constants determined in the analysis of an oil have the same meaning and are redundant. If, on the other hand, new constants can be found, which are related by equations other than straight line equations, the application of the methods of the author of this monograph will yield information of the utmost value for the determination of the nature and proportion of the constituents of a fatty mixture. In the discovery of such constants will lie important future developments in oil analysis.

K. A. WILLIAMS.