# THE ANALYST

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

AN Ordinary Meeting of the Society was held at the Chemical Society's Rooms at Burlington House on Wednesday, October 5th, 1938, the President, Professor W. H. Roberts, in the chair.

Certificates were read in favour of:—W. J. Blackie, M.Sc., A.I.C., E. Collins, M.A., F.I.C., R. F. Corran, Ph.D., M.Sc., A.I.C., L. A. Dauncey, B.Sc., A. V. Delaporte, B.A.Sc., Chem.E., E. C. Dodds, M.V.O., D.Sc., Ph.D., M.D., F.R.C.P., S. Druce, B.Sc., J. W. E. Harrisson, P.D., Ph.M., S. H. Jenkins, M.Sc.Tech., Ph.D., F.I.C., S. Rajagopal Naidu, M.B.E., B.A., M.B., B.S., M.Sc., D.I.C., F.I.C., S. C. Ray, B.Sc., M.B., B.S., D.P.H., F. B. Richardson, M.A., R. W. Richardson, F.I.C., J. W. Stillman, B.S., M.A., Ph.D., E. T. Terry, A.I.C., A. L. Vale, B.Sc., F.I.C., A. G. R. Whitehouse, M.Sc., Ph.D., F.I.C.

The following were elected members of the Society:—A. Andrew, B.Sc., A.R.T.C., A.I.C., N. J. Chalabi, B.A., C. C. Harris, B.Sc., A.R.C.S., F. D. M. Hocking, M.B., B.S., M.Sc., F.I.C., A.C.G.F.C., C. O. Tattersall, B.Sc., A.R.C.S., F.I.C.

The following papers were presented and discussed:—"A New Colour Reagent for Lead and its Use as an Indicator in the Titration of Various Cations and Anions," by B. S. Evans, M.C., D.Sc., F.I.C.; "A New Iodine Method for the Determination of Starch, Part V. The Starch in Leaf Material," by J. J. Chinoy, M.Sc., Ph.D., F.I.C.; "The Determination of Cobalt in Animal Tissues," by K. J. McNaught.

## Obituary

### GEORGE NEVILL HUNTLY

HUNTLY was born in 1866 and as a boy was educated at Richmond Grammar School (Surrey). In 1885 he won a scholarship to what was then the Normal School of Science (later known as the Royal College of Science), South Kensington, and obtained his Associateship Diploma with Honours in Chemistry. He later attended King's College, London, graduating B.Sc. at London University.

In 1894 he was elected a Fellow of the Institute of Chemistry, later served for six years on the Council, and for ten years was examiner in inorganic chemistry.

For some three years he was demonstrator and assistant to Tuson at the Royal Veterinary College, Camden Town, and afterwards became a demonstrator at King's College.

Subsequently he established a consulting practice; it is of peculiar interest to note that, although in 1905 he took the Institute's qualification in the analysis of foods and drugs, he did not adopt that branch of work, but specialised in mineral, metal and fuel analysis. At different times he occupied laboratories in various parts of London, at one time sharing a laboratory with the writer. Some few years ago, however, he transferred his equipment to his home at Putney, and fitted up a laboratory there for his consulting work.

In addition to his private practice he was Gas Examiner under the Metropolitan Gas Acts, and also held other similar appointments outside the Metropolitan area. His services were in demand as far away as the Island of Jersey.

Huntly joined the Society in 1909, served twice on the Council, and was Vice-President in 1919–20. He took an active part in the work of the Analytical Methods Committee of the Society, and the loss of his collaboration will be greatly felt. He also took a keen interest in the affairs of the University of London, and had been for many years a representative of the Science Faculty on the Standing Committee of Convocation.

Although his professional work kept him very busy, he often found time to attend meetings of the scientific societies to which he belonged, and he was responsible for two valuable papers on calorimetry (*J. Soc. Chem. Ind.*, **1910**, **29**, 917; ANALYST, **1910**, **35**, 444; *id.*, **1915**, **40**, 41).

Huntly was a clear thinker and an excellent teacher, an expert glass-blower and a clever manipulator; he was scrupulously careful and accurate in his work, yet he had a keen appreciation of the limits of experimental error, and in a paper on bomb calorimetry he was scornfully critical of the unattainable degree of accuracy claimed by some workers in estimating the calorific value of fuel. He was a good linguist, and used his excellent knowledge of German for abstracting and translating on behalf of *Nature*, work that he carried on for several years.

In his younger days he was a keen lawn tennis player, and exceptional length of limb must have given him a substantial advantage in the game. He was very fond of the mountains and spent many holidays in the Alps. He was a good conversationalist, sociable and companionable; although neither a player nor a singer he had a keen and critical appreciation of the musical classics. He had strong political convictions and was a staunch member of the National Liberal Club.

He died on the 2nd August, 1938, leaving a widow and three children.

HERBERT F. STEPHENSON

# The Determination of the Organic Acids in Silage Extracts and Bacterial Cultures

By A. M. SMITH, Ph.D., D.Sc., A.I.C.

(*Read at the Meeting of the Scottish Section, April* 14, 1938)

Various biochemical changes take place during the ensilage of crops, and the usual foodstuffs analysis requires to be supplemented by an examination of the decomposition products in order to obtain a proper estimate of the food value of the silage. The colour and odour of the material are a useful guide to temperature changes which have taken place and to palatability, and the *p*H value of the juice is usually an important index of the nature of the decomposition; but an analysis of the aqueous extract is necessary to provide reasonably precise information on the breakdown of proteins and carbohydrates.

Silage is usually a very heterogeneous mixture and, however the sample may be taken at the farm, from a particular position or depth, it is important to secure a representative sub-sample in the laboratory for analysis. This is conveniently done by reducing the sample to pieces about an inch in length by means of large shears and mixing the material thoroughly. A suitable quantity is taken for a moisture determination, and a total nitrogen determination is carried out on the dry matter. Portions of 100 g. of the fresh material are also shaken for 3 to 4 hours with 200 ml. of $CO_2$-free water in wide-necked bottles, and the aqueous extract is obtained therefrom by squeezing through fine muslin and filtering. A small quantity of this filtrate is taken for a *p*H determination, for which purpose the quinhydrone electrode is suitable.

The analysis of the aqueous extract is based on Foreman's discovery[1] that not only ammonia but the three classes of amines are neutral to phenolphthalein in solutions containing about 85 per cent. of alcohol by volume and that their salts, and also most of the common amino acids, may be titrated quantitatively with alkali in such an alcoholic solution. A suitable procedure, therefore, is to make up 60 ml. of the extract to 200 ml. with neutralised 90 per cent. alcohol and filter off the precipitate of protein and colouring matter. The filtrate varies in colour from light to dark brown and is quite stable. The total acid groups in this solution (A) are determined by diluting 10 ml. with 40 ml. of 90 per cent. alcohol and titrating with *N*/10 sodium hydroxide solution, using phenolphthalein as indicator. Five times the amount of alkali required for this titration (B) is added to 50 ml. of solution A, which is then distilled in steam for about 6 to 7 minutes, to remove alcohol and volatile bases. The distillate is collected in standard acid and the amount of bases is determined by titration, alizarin being used as indicator. The removal of the alcohol by distillation leaves in the residue an alkalinity due to liberation of the amino groups, and titration of this alkalinity, with phenolphthalein as indicator, gives a measure of the total amino acids present. The figures for volatile bases and amino acids, in terms of nitrogen, may then be compared with the contents of crude and true protein in the silage to obtain an insight into the

type of proteolysis that has predominated. In samples of good silage, as is discussed below, there is almost invariably more amino acid than volatile base.

There remain to be considered the volatile and non-volatile organic acids in the extract. The usual procedure adopted to determine the total volatile acids is to continue the addition of sulphuric acid after the determination of the amino acids, until the total mineral acid added is equivalent to the original alkali (B) required for the total acidity, and then continue the steam-distillation until successive 100-ml. portions of the distillate give reasonably constant titration values. On account of the rather heterogeneous mixture of substances present in a silage extract, however, the volatile acids may be considerably over-estimated by this method. Lactic acid, for example, is partly volatile in steam and may influence the result. To give one instance, it was found that the addition of about 5 ml. of $N/10$ lactic acid to a quantity of silage extract increased the figure for volatile acids from 1·87 to 2·11 ml. of $N/10$ alkali. The simplest procedure, therefore, is to neutralise and concentrate the total distillate to small bulk, add the appropriate amount of acid and re-distil in steam. The amount of lactic acid or other interfering substances now present is small and unlikely seriously to affect the results. This method has been used by Allen and Harrison[2] in the examination of bacterial cultures. If the second steam-distillation is carried out at constant volume, according to Dyer,[3] 99 per cent. of the butyric acid comes over in the first 400 ml. and 97 per cent. of the acetic acid in the first 1000 ml.

A selection of results from the examination of bacterial cultures, obtained during a study of different strains of lactobacilli separated from silage, is given in Table I to illustrate the necessity for a second distillation. There are three samples containing about 0·1 per cent. and three with about 0·2 per cent. of acetic acid, and there are varying amounts of lactic acid in each set so that the ratio of lactic acid to acetic acid varies between wide limits.

TABLE I

DISTILLATION OF VOLATILE ACIDS FROM BACTERIAL CULTURES

| Sample No.: | 32 | 7 | 25 | 16 | 4 | 2 |
|---|---|---|---|---|---|---|
| Lactic acid, per cent. .. .. .. | 0·25 | 1·77 | 3·34 | 0·37 | 1·54 | 3·11 |
| Acetic acid, per cent. .. .. .. | 0·12 | 0·11 | 0·11 | 0·22 | 0·19 | 0·22 |
| Ratio of lactic to acetic acid .. .. | 2·1 | 16·2 | 31·5 | 1·7 | 8·0 | 13·9 |
| Distillate, ml. of $N/10$ alkali required | | | | | | |
| (*a*) First { 600 ml. .. .. | 16·2* | 8·7 | 16·6 | 27·5* | 14·6 | 17·9 |
| (*b*) First { total .. .. | 17·4 | 10·2 | 20·6 | 29·3 | 15·9 | 20·0 |
| (*c*) Second, total .. .. .. | 14·0 | 6·7 | 12·4 | 25·8 | 12·0 | 13·7 |
| $a/c$ .. .. .. | 1·16 | 1·3 | 1·3 | 1·06 | 1·2 | 1·3 |

* 700 ml.

It will be observed that the total first distillates (*b*) (usually 1000 to 1200 ml.) require considerably larger amounts of alkali than the second distillates (*c*), particularly where the ratio of non-volatile to volatile acids is high. The figures in (*a*) represent the number of ml. of alkali required for the first 600 or 700 ml. of the first distillate and, as the ratios $a/c$ indicate, give, as a result of a compensation of errors, a fairly close approximation to the real values for volatile acids when the amount of lactic acid is not too large.

When a second distillation is carried out, however, it is possible to estimate the individual acids by adopting either Dyer's[3] or Wiegner's[4] technique. In the former, the distillation is carried out at a constant volume of 150 ml. and the first and second 100-ml. portions of the distillate are titrated. It is then possible to calculate the actual amounts of acetic acid and butyric acid present from their distillation constants, 30·75 and 69·88, respectively. In the latter, the solution is made up to 200 ml., and 100 ml. is distilled and titrated; the residue is made up to 200 ml. and a second 100 ml. is distilled and titrated, and finally a third 100 ml. is obtained by the same method. From the three titrations it is possible to calculate the amounts of acetic and butyric acids present, the distillation constants being respectively 36·59 and 72·77. A few data in Table II show that these two methods give results that agree very well and compare favourably with the figures obtained by collecting and titrating about 1000 ml. of distillate.

TABLE II

PERCENTAGE OF TOTAL VOLATILE ACIDS (AS ACETIC ACID) IN SECOND DISTILLATES

| | | Calculated* (see footnote) | |
|---|---|---|---|
| Sample | Direct | Dyer | Wiegner |
| 11 | 0·124 | 0·126 | 0·126 |
| 14 | 0·085 | 0·087 | 0·090 |
| 16 | 0·220 | 0·230 | 0·219 |
| 28 | 0·141 | 0·148 | 0·151 |
| 31 | 0·083 | 0·086 | 0·092 |

* Dyer:—Acetic acid = $K(69{\cdot}88 - {}^{t_1}/_{T})$; butyric acid = $K({}^{t_1}/_{T} - 30{\cdot}75)$,
where $K = {}^{T}/39.13$; T = total acidity = $t_1^2/t_1 - t_2$
$t_1$ and $t_2$ are respectively the 1st and 2nd 100 ml. titration values.
Wiegner:—Acetic acid = $3{\cdot}962(t_2 + t_3) - 1{\cdot}3724t_1$
Butyric acid = $-1{\cdot}992(t_2 + t_3) + 2{\cdot}0461t_1$
$t_1$, $t_2$, $t_3$ are the 1st, 2nd and 3rd 100 ml. titration values.

An estimate of the amount of lactic acid present in the silage extract may be made by subtracting the sum of the volatile and amino acids from the figure for total acidity, but there are sometimes large discrepancies, as shown by Smith and Comrie,[5] and it is better to determine the lactic acid directly in a separate portion of the extract or culture. The basis of the method is the oxidation of the lactic acid to acetaldehyde which is distilled into bisulphite solution, and the following technique was found to be rapid and reliable.

A small excess of caustic soda is added to a measured quantity (5 to 25 ml.) of the aqueous extract, which is then evaporated to about one-third of its original volume to get rid of volatile substances. The solution is neutralised with sulphuric acid, made up to about 100 ml. in a 200-ml. flask, and heated in a water-bath to 46 or 47° C. with 0·5 ml. of 20 per cent. copper sulphate ($CuSO_4.5H_2O$) solution. A further volume of 9·5 ml. of the copper sulphate solution is added, and the mixture is kept at 47° C. for 10 minutes; 10 ml. of a 30 per cent. suspension of calcium hydroxide are then added, and the mixture is made up to 201 ml. with warm water and kept at 47° C. for 10 minutes with occasional shaking. These quantities may be altered according to the nature of the extract. A large flocculent precipitate is

formed by this procedure, which is similar to that employed by Troy and Sharp[6] in the analysis of milk; filtration is rapid and gives a colourless filtrate that should react negatively to the Molisch test. A definite volume (5 to 25 ml.) of the filtrate, containing from 2 to 10 mg. of lactic acid, is neutralised with sulphuric acid in a distilling flask, and a solution containing manganous sulphate and phosphoric acid is added so that the final total volume of 50 ml. is 0·1 *M* with respect to phosphoric acid and contains 1 per cent. of manganese sulphate, as recommended by Friedemann and Kendall.[7] The liquid is heated to boiling-point and then *N*/300 permanganate solution is added, drop by drop, and the distillate is collected in 10 ml. of approx. *N*/20 sodium hydrogen sulphite solution diluted to a suitable volume and kept cool. The contents of the distilling flask become permanently yellowish-brown after 15 to 30 minutes and the distillation is stopped. Excess of *N*/40 iodine solution is added to the sulphite solution and the excess is immediately titrated with *N*/100 thiosulphate, starch being used as indicator. Owing to the instability of the sodium hydrogen sulphite it is necessary to carry out a blank experiment each time, *i.e.* a second flask with the sulphite is placed alongside the first during the distillation and the same volume of iodine is introduced into each of the two flasks. The acetaldehyde collected is then simply equivalent to the difference between the two thiosulphate back titrations; 1 ml. of *N*/100 thiosulphate ≡ 0·45 mg. of lactic acid.

The results in Table III are typical of many experiments and show that the recovery of lactic acid from pure solutions is of the order of 92 per cent. and from silage extracts or bacterial media about 85 per cent.

TABLE III

RECOVERY OF LACTIC ACID FROM DIFFERENT SOLUTIONS

| Solution | Lactic acid taken | Lactic acid found | Recovery |
|---|---|---|---|
| | mg. | mg. | Per Cent. |
| Zinc lactate .. .. .. .. | 3·02 | 2·79 | 92·4 |
| ,, ,, .. .. .. .. | 6·04 | 5·54 | 91·8 |
| ,, ,, .. .. .. .. | 11·64 | 10·87 | 93·5 |
| Extract A .. .. .. .. | — | 3·54 | — |
| ,, ,, + 2·91 mg. lactic acid .. | 6·45 | 6·03 | 85·5 |
| Culture 18 .. .. .. .. | — | 8·85 | — |
| ,, ,, + 1·36 mg. lactic acid .. | 10·21 | 10·00 | 84·5 |

It is probable that the heavy precipitate of copper hydroxide adsorbs some of the lactic acid but, in view of the uncertain sampling errors in handling material such as silage, it is felt that the recovery is adequate for work of this type, and the factor 1·17 has been employed in calculating the final results.

In the calculation of the results it must be borne in mind that fresh silage may contain as much as 80 per cent. of moisture and that the loss of weight in drying may include between 70 and 80 per cent. of the volatile acids and from 30 to 60 per cent. of the volatile bases. Unless these volatile constituents are also determined in the dry matter a correction cannot be made, so that the figure for dry matter is too low, and this in turn affects the calculation of the amounts of the various constituents present. Except in abnormal cases, however, the discrepancies are

of minor importance because the bases (calculated as crude protein) and the volatile acids seldom exceed 1 per cent. of the fresh silage. Incidentally, it may be noted that the presence of organic acids in the dried material is liable to augment the ether extract figure to a considerable extent.

The interpretation of the results is based mainly upon two comprehensive series of analyses by Watson and Ferguson[8] and Smith and Comrie.[5] Generally speaking, the results of these two investigations agreed closely in respect of the usefulness of the *p*H value of the silage in estimating its quality. Ensilage invariably leads to an increase in the organic acids and non-protein nitrogen and, from the point of view of feeding, it is desirable that the formation of butyric acid should be avoided and that the protein breakdown should give amino acids rather than volatile bases. It was shown that the critical point lay near the *p*H value 4·5. Below *p*H 4·5, there is usually more lactic acid than volatile acids and practically no butyric acid, and the ratio of amino acids to volatile bases is also greater than unity and increases with increase in acidity. Above *p*H 4·5, the amount of volatile acids rises rapidly, butyric acid predominating, and that of lactic acid decreases; at the same time the production of bases increases and the amount of amino acids tends to decrease.

The *p*H of silage may be controlled by the addition of mineral acids or molasses to the crop during ensiling. The former reduce all the biochemical changes so that little volatile acid or base is formed, whilst the latter effects a considerable increase in the lactic acid and a reduction in the volatile bases. These methods are invaluable when the total nitrogen of the crop is high (over 2·5 per cent. of the dry matter), for it is difficult to produce good silage by the ordinary processes from such material. Apart from young grass, however, silage crops usually contain less than 2·5 per cent. of nitrogen, and good silage may be made, without any addition, when adequate precautions are taken in packing the material.

SUMMARY.—A description of a technique, suitable for determining the quantities of lactic and volatile acids, volatile bases and amino acids in silage extracts and bacterial cultures, is given and followed by a brief statement on the interpretation of the results.

## REFERENCES

1. F. W. Foreman, *Biochem. J.*, 1920, **14**, 451; 1928, **22**, 208.
2. L. A. Allen and J. Harrison, *Biochem. J.*, 1935, **29**, 2471.
3. D. C. Dyer, *J. Biol. Chem.*, 1917, **28**, 445.
4. G. Wiegner, *Anleitung zum quantitativen agrikulturchemischen Praktikum*, Berlin, 1926.
5. A. M. Smith and A. Comrie, *J. Agric. Sci.*, 1938, **28**, 203.
6. H. C. Troy and P. F. Sharp, *Cornell Univ. Agric. Exp. Stat.*, 1935, *Mem.* 179.
7. T. E. Friedemann and A. I. Kendall, *J. Biol. Chem.*, 1929, **82**, 23.
8. S. J. Watson and W. S. Ferguson, *J. Agric. Sci.*, 1937, **27**, 1.

EDINBURGH AND EAST OF SCOTLAND
COLLEGE OF AGRICULTURE

*May*, 1938

# The Determination of Acid in Wool

By J. BARRITT, B.Sc., A.R.C.S., A.I.C., H. H. BOWEN, F. L. GOODALL, M.Sc., and A. WHITEHEAD

(*Read at the Meeting, November 2nd*, 1938)

The importance of simple and accurate methods for the determination of acid in wool may be realised from the attention the subject has received and the large amount of investigation applied to it. Despite this, however, the work so far carried out has not resulted in establishing any one method as absolutely reliable or as more reliable than others.

Many of the earlier methods relied upon the neutralisation of the acid present in the material by an excess of a weak alkali—the excess of alkali being determined. Such methods are inaccurate, since the wool readily absorbs alkali and the amount absorbed, and therefore the accuracy of the acid determination, will depend upon the nature and the amount of the excess of alkali used.

One of the earliest methods of determining acid in wool was to add an excess of ammonia, but this procedure has been criticised by Lloyd[1] owing to the absorption of ammonia by wool and the uncertainty of any blank made to allow for such absorption. Similarly, Barritt[2] has criticised the triethanolamine method of Trotman and Gee,[3] though the absorption of the base in this method is much less serious than in the ammonia method. The sodium bicarbonate extraction method of Meunier and Rey[4] is a similar method and suffers from the same inherent defect.

Hirst and King[5] modified a suggested method of Woodmansey[6] for the determination of sulphuric acid in wool. They digested the acid material with an aqueous suspension of magnesium carbonate, and determined the soluble sulphate in the extract as barium sulphate. This method is limited to the determination of sulphuric acid and, further, any sodium or calcium sulphate present would be returned as sulphuric acid. Calcium carbonate is also used in a method due to Trotman and Gee,[3] and the carbon dioxide liberated by the acid is expelled by boiling and absorbed in baryta. The method was found to give values similar to those obtained with the sodium acetate, sodium bicarbonate or triethanolamine method, provided the time of distillation was not too long.

In our opinion the methods mentioned above suffer from obvious defects or are too lengthy in operation to merit detailed study in an exploration for a reliable and rapid method for the determination of acid in wool, and accordingly attention was directed to three methods which have been subjected to less criticism than those already mentioned.

It is of interest to note that when the work was started each of the three methods was in routine use in the laboratories of at least two of the present authors.

These methods are:—(*a*) The sodium terephthalate method of Hirst and King[5]; (*b*) the sodium acetate distillation method of Trotman and Gee[3]; (*c*) the pyridine method of Barritt.[2]

All three methods are claimed to give satisfactory recovery of any acid present. It appeared desirable, however, to determine whether or not the methods

gave comparable results, and, if not, which method could be considered the most reliable in respect both of absolute accuracy and of reproducibility in the hands of different operators. Comparative tests were therefore undertaken and, except where otherwise stated, the tests described were carried out independently in the separate laboratories controlled by the present authors.

The essential features of the respective methods are described below.

(*a*) THE SODIUM TEREPHTHALATE METHOD.—A definite volume of a standard sodium terephthalate solution (0·1 *N*) is added to the sample (*e.g.* 2 g.) which has been thoroughly wet out in distilled water. The sample and liquor are heated to 60° C. and allowed to stand for two or three hours. An aliquot portion of the liquor is withdrawn, and an excess of standard sulphuric acid (0·1 *N*) is added to it. The precipitated terephthalic acid is filtered off, and the excess of sulphuric acid in the solution is determined by titration with sodium hydroxide solution (0·1 *N*), bromophenol blue being used as indicator.

For certain dyed materials which show a tendency to bleed, the determination may be carried out in the cold, the steeping being allowed to proceed overnight.

(*b*) THE SODIUM ACETATE DISTILLATION METHOD.—(i) *Mineral Acid.*—The sample is wet out in a little distilled water in a 500-ml. distilling flask, and 0·5 to 1 g. of sodium acetate is added. The sample and sodium acetate solution are steam-distilled and about 650 ml. of distillate are collected.

The distillate is titrated with 0·1 *N* sodium hydroxide solution, phenolphthalein being used as indicator. Stress is laid on the importance of making the water in the steam generator alkaline so that no carbon dioxide is collected in the distillate. A blank determination without the sample is made in the apparatus.

(ii) *Organic Acids* (*e.g.* acetic or formic acid).—For such acids it is only necessary to steam-distil the samples without adding sodium acetate. The usual blank determination is made.

(*c*) THE PYRIDINE METHOD.—The sample of wool (*e.g.* 2 g.) containing acid is wet out by shaking in 190 ml. of distilled water, and 10 ml. of a 10 per cent. solution of pyridine are added, so that the final pyridine concentration is approximately 0·5 per cent. The contents of the flask are well shaken and allowed to stand for at least one hour. Suitable aliquot portions are withdrawn and the extracted acid is determined by titration with 0·1 *N* sodium hydroxide solution (free from carbon dioxide), phenolphthalein being used as indicator.

Of the three methods, that using sodium terephthalate differs from the other two inasmuch as its results are obtained by back-titration.

The sodium acetate method appears to offer a marked advantage over the other two methods, in that since it is a distillation method the actual determinations are carried out in a colourless liquor and no interference takes place from any dyes present in the pattern under examination. One disadvantage of the method is that it requires rather bulky apparatus, whereas the other two methods require only the usual volumetric apparatus.

The pyridine method, as originally recommended, is unquestionably the simplest in operation, but its accuracy has been criticised by Eavenson and Creely,[7] who report that the recovery of acid present is considerably reduced by any drying process to which the wool has been subjected after contact with the

acid. These authors recommend the use of higher temperatures, in the neighbourhood of 100° C., for the aqueous pyridine treatment of the pattern under test. Both of these points will be discussed later.

At the outset of the investigation one of us (H.H.B.) had already completed comparative tests of the sodium acetate distillation and sodium terephthalate methods on a variety of wool patterns, containing respectively acetic, formic and sulphuric acids, and obtained markedly different acid values by the two methods. The value obtained by sodium acetate distillation was invariably much higher than that given by the sodium terephthalate method, the ratio of the former to the latter varying from 1·45 to as much as 2·3.

As a preliminary the remaining three of us carried out comparative tests by preparing their own acid wool by treatment of commercially scoured woollen materials of unknown alkali-content with a known quantity of sulphuric acid (about 4 per cent.) and determining the amount of acid recovered from the cloth by the three methods. Table I gives the respective results, expressed as a percentage of the acid added, of which some has been used up in neutralisation of the alkali in the wool and some left in the treatment liquor.

TABLE I

| Method | Recovery of added acid | | |
|---|---|---|---|
| | J.B. Per Cent. | F.L.G. Per Cent. | A.W. Per Cent. |
| Sodium terephthalate .. .. | 53·8 | 47·0 | 55·0 |
| | 54·8 | 45·9 | — |
| | — | 48·5 | — |
| Sodium acetate .. .. .. | 62·5* | 51·0 | 73·0 |
| | 59·8* | 52·3 | 71·0 |
| | 60·0* | 49·2 | 67·3 |
| | | | 67·3 |
| Pyridine .. .. .. .. | 56·0 | 56·9 | 67·3 |
| | 56·0 | 57·4 | 64·3 |
| | — | 53·6 | 64·3 |

Comparison of the above results shows that the sodium terephthalate method invariably gives results rather lower than those given by the other two methods, but the difference between the sodium terephthalate method and sodium acetate distillation method was not so pronounced as in the results previously obtained by one of us (H.H.B.).

Since general considerations would suggest that the pyridine method could not possibly return more acid than is actually present (the only effective criticism of the pyridine method so far put forward is that it *fails* to give complete recovery of acid), it was felt that further work on the sodium terephthalate method should not be pursued unless serious defects were found in either or both of the other methods.

It may be remarked that already at this stage we had found substantial variation in the blank values which are integral parts of the sodium acetate distillation method. For example, the values marked by an asterisk in the above table were the results of deducting from the observed acid values an average blank value of 0·56 per cent. derived from the five blank figures 0·77, 0·67, 0·23, 0·25, and 0·89 per cent. Obviously such variation may be very serious.

It was now decided that comparative independent trials should be made, on one and the same sample of acid wool, using materials treated respectively with sulphuric acid, formic acid and acetic acid, these acids being chosen as those most commonly used in commercial treatment of wool. The measurements were made by the pyridine method only, using a period of one hour's steeping in the pyridine liquor, as originally recommended, and also with steeping over-night.

Adequate quantities of commercially scoured cloth were treated by one of us (F.L.G.) for one hour at boiling temperature with the amounts of acid shown in Table II. The quantities of acid found by the individual authors in the material and in a commercially dyed green material of unknown acid-content are reported in Table II as the mean of two or more determinations in each instance.

TABLE II

| Treatment | J.B. | | H.H.B. | | F.L.G. | | A.W. | |
|---|---|---|---|---|---|---|---|---|
| | One hour Per Cent. | Over-night Per Cent. | One hour Per Cent. | Over-night Per Cent. | One hour Per Cent. | Over-night Per Cent. | One hour Per Cent. | Over-night Per Cent. |
| Sulphuric acid, 3·93 per cent. | 2·20 | 2·36 | 2·26 | 2·58 | 2·08 | 2·16 | 2·60 | 3·06 |
| Formic acid, 4·53 per cent. .. | 0·46 | 0·52 | 0·64 | 0·81 | 0·76 | 0·74 | 1·15 | 1·15 |
| Acetic acid, 1·58 per cent. .. | 0·13 | 0·18 | — | 0·35 | 0·14 | 0·17 | 0·60 | 0·75 |
| Green cloth .. .. .. | 1·90 | 1·96 | 1·90 | 2·10 | 1·83 | 1·90 | 2·08 | 2·20 |

It will be seen that although each investigator was using samples taken from the same laboratory-treated patterns, very divergent results were obtained, particularly with the two organic acids. On the other hand, this divergence was much less evident with the commercially-dyed green cloth, with which reasonably concordant results were obtained in all four laboratories.

Since two of us (H.H.B. and F.L.G.) had already obtained very concordant results with the pyridine method on a large number of commercial samples (to be described in full later), it was considered that the discrepancy in values found on the laboratory material might have arisen from a non-uniform distribution of acid in the cloth, caused, probably by the difficulty of drying evenly a large piece of cloth under laboratory conditions. On this view the better agreement in the results for the commercial pattern could be attributed to the probability that this difficulty of drying and consequent uniform acid distribution did not arise with the commercial cloth dried in the full open width on a tentering machine.

In order to check this point it was decided to repeat the tests on fresh material, treated in the laboratory with the various acids by one of us (F.L.G.), conditioned, accurately sampled and weighed into 2-g. samples and distributed by another of us (J.B.).

The conditioning, sampling and weighing were carried out as follows:—The piece of acidified cloth was allowed to condition for several days in a room maintained at 70 per cent. relative humidity, and all weighings of the samples were made in this room.

A strip, about one inch in width, was first removed from the edges of the cloth, and the residual material was divided into sixteen equal areas. Each of these smaller areas was divided into small, approximately equal portions, the

number of portions being arranged so that sixteen of them weighed approximately 2 g.

The samples used in the determination of acid (2 g.) were obtained by taking one small portion of cloth from each of the sixteen zones, their combined weight being adjusted to 2 g. either by trimming the portions or by adding fragments derived from the various zones.

The average values found are given in the table below.

TABLE III

| Treatment | J.B. | | H.H.B. | | F.L.G. | | A.W. |
|---|---|---|---|---|---|---|---|
| | One hour Per Cent. | Over-night Per Cent. | One hour Per Cent. | Over-night Per Cent. | One hour Per Cent. | Over-night Per Cent. | One hour Per Cent. |
| Sulphuric acid, 3·79 per cent. | 2·25(3) | 2·32(3) | 2·06(1) | 2·36(1) | 2·24(3) | 2·37(2) | 2·32(6) |
| Formic acid, 4·64 per cent. .. | 0·55(3) | 0·60(3) | 0·53(1) | 0·59(1) | 0·57(3) | 0·61(2) | 0·53(3) |
| Acetic acid, 2·57 per cent. .. | 0·15(3) | 0·20(3) | 0·17(1) | 0·19(1) | 0·19(3) | 0·23(2) | 0·17(6) |

(The figures in brackets refer to the number of determinations made.)

Table III shows clearly that the precautions taken with regard to sampling and conditioning had resulted in much better agreement between the four laboratories. In our opinion the figures showed the pyridine method to be reliable from the point of view of reproducibility, and this view was confirmed by the results obtained independently on a number of commercial cloths as given in detail in Table IV. Certain of the samples were not available to two of us (H.H.B. and A.W.). The acid in the sample is calculated as sulphuric acid.

TABLE IV

| Sample marked | J.B. Per Cent. | H.H.B. Per Cent. | F.L.G. Per Cent. | A.W. Per Cent. |
|---|---|---|---|---|
| A2 | 0·10 | — | 0·20 | — |
| A4 | 0·33 | — | 0·42 | — |
| A5 | 0·26 | — | 0·35 | — |
| B12 | 2·32 | 2·30 | 2·37 | 2·31 |
| B13 | 2·26 | 2·35 | 2·32 | — |
| B14 | 2·15 | 2·25 | 2·23 | 2·20 |
| B25 | 2·65 | 2·80 | 2·68 | 2·70 |
| B26 | 2·16 | 2·25 | 2·21 | 2·31 |

The close concordance shown by these figures indicated definitely that the pyridine method in the hands of several operators could give reproducible results, and the experiments as a whole indicated that accurate sampling and conditioning of laboratory prepared samples is of great importance in investigating the acid-content.

No evidence had as yet been produced, however, to show that the pyridine method would return *all* the acid actually present in the material, and at this stage it was decided to make comparative trials of the pyridine and sodium acetate distillation methods, since the latter had given rather higher values in general in the work summarised in Table I.

The scheme first adopted was as follows:—Adequate quantities of undyed material were made *slightly* acid with sulphuric, formic and acetic acids, respectively, the cloths being treated at boiling temperature with sufficient quantities

of the acids to produce an acid reaction when a sample so treated was digested with 25 times its weight of distilled water for 30 minutes at 40° C. The "acid reaction" obtained corresponded to a decrease of 0·3 to 0·5 in the *p*H of the incubated extract as compared with the *p*H of the original distilled water similarly treated. The slightly acid cloths so prepared were conditioned, sampled and weighed in 2-g. portions as previously described. The acid-content of the samples as received was then determined in each laboratory by both the pyridine and the sodium acetate distillation methods.

For each method of determination, 2-g. samples of each of the acid cloths were then treated separately in a 40:1 liquor ratio for 1 hour at boiling temperature with a further 2·5 per cent. of the acid originally present in the samples. For the two organic acids this treatment took place under a reflux condenser. The total acid in both cloth and liquor was then determined together. By this method of test it was hoped to be able to determine whether or not the two methods investigated would return all the *additional* acid introduced.

Most of us had already found the sodium acetate distillation method to be subject to variation in blank values and in actual values, which it was thought might be due to variation in the rate of distillation and in the volume of liquor present in the distilling flask. In addition some of us had frequently obtained low results by this method, and it was therefore arranged to continue distillation until much more than the 650 ml. of distillate specified by the original authors of the method were collected, the times required to obtain any particular volume of distillate and the average volume of liquid present in the distilling flask throughout the distillation being noted.

The results obtained for the distillation method are given in Table V.

TABLE V

| | Volume of distillate | Time of distillation | Total acid found (*a*) before addition Per Cent. | Total acid found (*b*) after addition Per Cent. | Difference Per Cent. | Recovery Per Cent. |
|---|---|---|---|---|---|---|
| J.B. | | | | | | |
| *Sulphuric acid cloth; 2.45 per cent. of added acid* | | | | | | |
| 1. | 650 ml. | 37 mins. | 1·12 | 3·15 | 2·03 | 83 |
| 2. | 650 ,, | 38 ,, | 1·10 | 3·12 | 2·02 | 82 |
| 3. | 780 ,, | 40 ,, | 1·10 | 3·24 | 2·14 | 87 |
| *Formic acid cloth; 2·5 per cent. of added acid* | | | | | | |
| 1. | 650 ml. | 38 mins. | 0·21 | 2·37 | 2·16 | 86 |
| 2. | 650 ,, | 35 ,, | 0·18 | 2·18 | 2·00 | 80 |
| *Acetic acid cloth; 2·5 per cent. of added acid* | | | | | | |
| 1. | 650 ml. | 37 mins. | 0·11 | 2·40 | 2·29 | 92 |
| 2. | 650 ,, | 35 ,, | 0·11 | 2·37 | 2·26 | 90 |
| | Average volume in distilling flask: 100 ml. | | | | | |
| H.H.B. | | | | | | |
| *Sulphuric acid cloth; 2·5 per cent. of added acid* | | | | | | |
| 1. | 710 ml. | 60 mins. | 0·76 | 2·7 | 1·94 | 78 |
| 2. | 730 ,, | 60 ,, | 0·76 | 2·7 | 1·94 | 78 |
| 3. | 780 ,, | 60 ,, | 0·76 | 2·7 | 1·94 | 78 |
| | 1950 ,, | 150 ,, | 1·52 | 3·63 | 2·11 | 84 |

TABLE V—*continued*

| | Volume of distillate | Time of distillation | Total acid found (*a*) before addition Per Cent. | Total acid found (*b*) after addition Per Cent. | Difference Per Cent. | Recovery Per Cent. |
|---|---|---|---|---|---|---|
| H.H.B. | | | | | | |
| *Formic acid cloth; 2·5 per cent. of added acid* | | | | | | |
| 1. | 780 ml. | 60 mins. | 0·53 | 2·55 | 2·02 | 81 |
| 2. | 790 ,, | 60 ,, | 0·53 | 2·58 | 2·05 | 82 |
| 3. | 790 ,, | 60 ,, | 0·53 | 2·55 | 2·02 | 81 |
| | 2040 ,, | 150 ,, | 1·10 | 3·34 | 2·24 | 90 |
| *Acetic acid cloth; 2·5 per cent. of added acid* | | | | | | |
| 1. | 730 ml. | 60 mins. | 0·54 | 2·13 | 1·59 | 64 |
| 2. | 730 ,, | 60 ,, | 0·54 | 2·37 | 1·83 | 73 |
| 3. | 700 ,, | 60 ,, | 0·54 | 2·25 | 1·71 | 68 |
| | 1930 ,, | 150 ,, | 1·11 | 3·3 | 2·19 | 88 |
| Average volume in distilling flask: 200 to 250 ml. | | | | | | |
| F.L.G. | | | | | | |
| *Sulphuric acid cloth; 2.45 per cent. of added acid* | | | | | | |
| 1. | 660 ml. | 50 mins. | 0·54 | 2·03 | 1·49 | 61 |
| 2. | 660 ,, | 55 ,, | 0·54 | 2·40 | 1·86 | 76 |
| | 1020 ,, | 85 ,, | 0·74 | 2·77 | 2·03 | 83 |
| 3. | 660 ,, | 45 ,, | 0·54 | 1·86 | 1·32 | 54 |
| | 1310 ,, | 95 ,, | 0·88 | 2·65 | 1·77 | 72 |
| 4. | 700 ,, | 45 ,, | 0·54 | 1·78 | 1·24 | 51 |
| | 1350 ,, | 95 ,, | 0·88 | 2·65 | 1·77 | 72 |
| *Formic acid cloth; 3·11 per cent. of added acid* | | | | | | |
| 1. | 690 ml. | 50 mins. | 0·33 | 2·30 | 1·97 | 63 |
| | 1050 ,, | 80 ,, | 0·42 | 2·83 | 2·41 | 77 |
| 2. | 660 ,, | 50 ,, | 0·33 | 2·25 | 1·92 | 62 |
| | 1210 ,, | 95 ,, | 0·45 | 2·06 | 2·61 | 84 |
| | 1820 ,, | 135 ,, | 0·59 | 3·52 | 2·93 | 92 |
| *Acetic acid cloth; 2·52 per cent. of added acid* | | | | | | |
| 1. | 610 ml. | 50 mins. | 0·41 | 1·64 | 1·23 | 49 |
| | 1250 ,, | 100 ,, | 0·69 | 2·36 | 1·67 | 66 |
| | 1870 ,, | 145 ,, | 0·93 | 2·91 | 1·98 | 78 |
| 2. | 700 ,, | 50 ,, | 0·41 | 1·87 | 1·45 | 57 |
| | 1350 ,, | 100 ,, | 0·69 | 2·53 | 1·84 | 72 |
| | 1940 ,, | 145 ,, | 0·93 | 2·92 | 1·99 | 79 |
| Average volume in distilling flask: 250 to 300 ml. | | | | | | |
| A.W. | | | | | | |
| *Sulphuric acid cloth; 2·5 per cent. of added acid* | | | | | | |
| 1. | 600 ml. | 90 mins. | 0·72 | 3·01 | 2·29 | 91 |
| 2. | 600 ,, | 105 ,, | 0·74 | 3·08 | 2·34 | 93 |
| *Formic acid cloth; 2·5 per cent. of added acid* | | | | | | |
| 1. | 600 ml. | 105 mins. | 0·46 | 2·69 | 2·23 | 89 |
| 2. | 600 ,, | 90 ,, | 0·46 | 2·67 | 2·21 | 88 |
| *Acetic acid cloth; 2·5 per cent. of added acid* | | | | | | |
| 1. | 600 ml. | 90 mins. | 0·60 | 3·15 | 2·55 | 102 |
| 2. | 600 ,, | 90 ,, | 0·60 | 3·18 | 2·58 | 103 |
| Average volume in distilling flask: 200 to 225 ml. | | | | | | |

It is noteworthy that only one of us (A.W.) obtained recovery values of a reasonably high standard, whilst the values obtained by two of us (H.H.B. and F.L.G.) are very low indeed, even when distillation was prolonged until the distillate was three times as large as the 650 ml. originally specified. It may be remarked that in the latter tests the blank experiments were also continued for a correspondingly increased time. Pronounced variations were also found in acid values obtained by each of us in tests on identical material, *i.e.* on the acidified cloths before treatment with any additional acid. No explanation of the variable nature of the results can be found in consideration of time or rate of distillation or volume of liquor in the distillation flask, but the variation is so large as to make it clear that the method is unreliable and cannot be recommended for general use. It may be added that one of us (F.L.G.) obtained positive values by the method for acid-content on a material known to be definitely alkaline.

The pyridine method gave more promising results throughout, as the values shown in Table VI indicate.

TABLE VI

| | J.B. | H.H.B. | F.L.G. | A.W. |
|---|---|---|---|---|
| *Sulphuric acid patterns*— | Acid added: 2·45 per cent. Per Cent. | Acid added: 2·5 per cent. Per Cent. | Acid added: 2·45 per cent. Per Cent. | Acid added: 2·5 per cent. Per Cent. |
| Original acid content .. | 0·56, 0·61, 0·54 } 0·57 | 0·54 | 0·64, 0·65 } 0·645 | 0·69 |
| After treatment .. | 2·98, 2·96, 2·96 } 2·96 | 2·94, 2·98, 2·94 } 2·95 | 3·02, 2·99, 3·01 } 3·01 | 3·01 |
| Acid recovered .. | 2·39 | 2·41 | 2·365 | 2·32 |
| Recovery .. .. | 97·5 | 96·4 | 96·5 | 92·8 |
| | Acid added: 2·5 per cent. Per Cent. | Acid added: 2·5 per cent. Per Cent. | Acid added: 3·11 per cent. Per Cent. | Acid added: 2·5 per cent. Per Cent. |
| *Formic acid patterns*— | | | | |
| Original acid content .. | 0·22, 0·22, 0·21 } 0·22 | 0·18 | 0·30, 0·29 } 0·30 | 0·28 |
| After treatment .. | 2·85, 2·77, 2·75 } 2·79 | 2·67, 2·71, 2·71 } 2·70 | 3·50, 3·50, 3·38 } 3·46 | 2·78 |
| Acid recovered .. | 2·57 | 2·52 | 3·16 | 2·50 |
| Recovery .. .. | 102·6 | 100·8 | 101·6 | 100·0 |
| | Acid added: 2·5 per cent. Per Cent. | Acid added: 2·5 per cent. Per Cent. | Acid added: 2·52 per cent. Per Cent. | Acid added: 2·5 per cent. Per Cent. |
| *Acetic acid patterns*— | | | | |
| Original acid content .. | 0·07, 0·09, 0·08 } 0·08 | 0·06 | 0·19, 0·20 } 0·20 | 0·15 |
| After treatment .. | 2·55, 2·50, 2·48 } 2·51 | 2·58, 2·46, 2·46 } 2·50 | 2·59, 2·68, 2·62 } 2·63 | 2·56 |
| Acid recovered .. | 2·43 | 2·44 | 2·43 | 2·41 |
| Recovery .. .. | 97·2 | 97·6 | 96·4 | 96·4 |

With one exception recovery values of 96 per cent. or over were obtained throughout by the pyridine method and, in addition, attention may be drawn to the concordant results obtained by each laboratory in determining the original acid-content of the materials sampled.

Besides giving a satisfactory reproducibility in the hands of different operators, the pyridine method was thus shown to be capable of returning all the additional acid introduced, but further investigation was required on several points in connection with the method before it could be regarded as thoroughly reliable. These were:

(*a*) *A possible zero error in the method.*—Since most of our experimental results by the pyridine method were higher than corresponding results by the distillation method, this possible error did not appear likely to be very large, but it was considered worthy of investigation.

(*b*) *The effect of drying after contact with acid.*—Mention has already been made of the criticism of Eavenson and Creely[7] that the amount of acid recovered is materially reduced according to the conditions of drying the material after contact with the acid. This led them to recommend extraction with pyridine at temperatures approaching boiling-point, but this recommendation would, in our opinion, lead to difficulties with some dyed materials for a third reason, *viz.:*

(*c*) *Coloration.*—This is due to the bleeding of dye into the pyridine solution where coloured materials are being examined.

(*a*) *A possible zero error in the method.*—Any investigation of this question required a supply of wool of a satisfactory degree of purity. This was only available to two of us and, as the method of investigation differed in both instances, each is given in full here.

One of us (J.B.) treated raw 64's Cape wool in three changes of warm benzene, followed by air drying and removal of foreign matter by opening out the locks. The sample was extracted in a Soxhlet apparatus with alcohol and then washed for some days in distilled water, the water being changed two or three times daily. The wool was finally dried in the air and conditioned at 70 per cent. R.H. It had an ash-content of 0·55 per cent., and a 1 : 50 extract with distilled water showed a *p*H of 6·4.

Samples of this wool were refluxed with 3 per cent. sulphuric acid for 1 hour, and the acid-content in wool and liquor were determined together by the original method (1 hour's steeping). In three successive experiments the acid-content amounted to 2·86, 2·92 and 2·85 per cent., equivalent to an average recovery of 94·3 per cent.

A similar set of three experiments, in which the acid in the wool and the liquor was determined separately, gave the following results:

| | | | |
|---|---|---|---|
| Acid in wool, per cent. .. | 2·19 | 2·17 | 2·03 |
| Acid in liquor, per cent. .. | 0·68 | 0·72 | 0·82 |
| Total acid, per cent. .. .. | 2·87 | 2·89 | 2·85 |

Another of us (F.L.G.) used commercially scoured yarn which was purified by the following method:

Forty g. of yarn were treated for 30 minutes at 80° C. in 500 ml. of water containing 6 ml. of conc. hydrochloric acid. The yarn was washed off in distilled

water and extracted in a Soxhlet extractor with distilled water for 2 hours, after which the ash-content was determined. If the ash-content was above 0·05 per cent. the above procedure was repeated, but this repetition was not usually necessary.

The yarn was then immersed in 500 ml. of water containing 20 ml. of pyridine, heated to 60° C., and allowed to stand overnight, after which it was washed off with distilled water and extracted for 3 hours in a Soxhlet apparatus with a mixture of 50 ml. of water, 50 ml. of methylated spirits and 2 to 5 ml. of pyridine. The yarn was dried and extracted with methylated ether in a Soxhlet apparatus for 3 hours.

The result of this treatment is a wool which has an ash-content less than 0·05 per cent., and acid-content (by the pyridine method) less than 0·1 per cent., and shows *p*H 5·0 to 5·8 on digestion in 25 times its weight of distilled water, which similarly treated alone shows a *p*H of the order of 5·6.

The actual samples used for the determinations which follow had the following characteristics:—ash-content, nil; *p*H 5·20 (distilled water 5·6); acid-content 0·06 per cent. by the standard pyridine method.

Samples of this wool were treated for 45 minutes under reflux with sulphuric acid, the solution was cooled and the acid in the wool and the liquor together was determined. The following figures were obtained:

| | Acid added Per Cent. | Acid recovered Per Cent. | Recovery Per Cent. |
|---|---|---|---|
| 1. | 0·223 | 0·22 | 98·9 |
| 2. | 0·441 | 0·41 | 92·9 |
| 3. | 0·882 | 0·85 | 96·3 |

The figures given under "acid recovered" are, of course, values obtained after deducting from the observed final values the 0·06 per cent. of acid found in the original cloth.

The work of one of us (J.B.) was therefore carried out on a purified wool which deviated from an ideal standard of purity in having a very slight alkali-content, as indicated by the *p*H value of a water extract and its ash-content. On this wool the pyridine method gave 94 per cent. recovery of acid added to the extent of about 3 per cent. The work of another (F.L.G.) was carried out on a wool from which inorganic matter had been removed and which, owing to its method of preparation, was in fact slightly acid. With such wool the pyridine method gave recovery values of more than 92 per cent. with much smaller amounts of added acid.

These values are sufficient to indicate that if a zero error exists at all, it is of very small magnitude.

(*b*) *Effect of drying after contact with acid.*—The significant experiments made by Eavenson and Creely[7] refer to three 2·5-g. samples of purified yarn, wetted out in warm water, squeezed, placed in flasks and treated respectively with 5, 10 and 15 ml. of 0·1 *N* sulphuric acid, followed by drying in a current of air at about 20° C., for 3 days, after which the yarn was quite dry. The contents of the flask were then digested for 1 hour at 55° C. with 0·5 per cent. pyridine solution, and the acid was determined in the usual manner; recovery values of 79·5, 85·5 and 88·0 per cent. respectively, were obtained. The yarn in question was purified[8] by scouring the greasy wool under commercial conditions with petroleum naphtha and water,

followed, after spinning, by extraction of the yarn with carbon tetrachloride, and then with alcohol. No ash determinations were made, but it is probable that after the treatment described the yarn would have an ash-content of the order of 1 per cent., which might have a significant effect in reducing the amount of recoverable acid.

Our own investigation of the effect of drying on the pyridine method of determination was carried out in two stages, in the first of which one of us (H.H.B.) was unable to take part.

Commercially scoured white flannel was treated under dyeing conditions with 2·5 per cent. sulphuric acid, 5 per cent. formic acid, and 6 per cent. acetic acid, respectively, and after this treatment the acidified samples were dried at room temperature and then conditioned and sampled as described previously. Acid determinations were made on the cloths as received, and it was intended to repeat the determinations on all the cloths after subjecting them to further drying at higher temperatures and for varying periods.

The amounts of organic acid present in the respective patterns were found, however, to be extremely small (Table VII), and it was decided that heating experiments on these patterns would be of little value. Accordingly attention was confined to the patterns treated with sulphuric acid, where the amount of residual acid was appreciable.

TABLE VII

| | Cloth treated with acetic acid Per Cent. | Cloth treated with formic acid Per Cent. |
|---|---|---|
| J.B. .. .. | 0·17 | 0·23 |
| F.L.G. .. .. | 0·18 | 0·24 |
| A.W. .. .. | 0·26 | 0·32 |

Two sets of such sulphuric acid patterns were available, and the results shown in Table VIII were obtained.

TABLE VIII

| | | Sulphuric acid found | | |
|---|---|---|---|---|
| | | J.B. Per Cent. | F.L.G. Per Cent. | A.W. Per Cent. |
| 1. | (*a*) Before heating .. .. .. | 1·54 | 1·55 | 1·75 |
| | (*b*) After heating for 3 hours at 110° C. | 1·56 | 1·58 | 1·82 |
| 2. | (*a*) Before heating .. .. .. | 2·37 | 2·38 | 2·45 |
| | (*b*) After heating for 3 hours at 110° C. | 2·40 | — | 2·45 |

These figures show values obtained after a drying procedure which was common to all the three investigators. In addition each investigator made a variety of other tests (*e.g.* 2½ hours at 90 to 100° C., 5 hours at 100° C., 5 hours at 80° C., 2 hours at 120° C., 3 hours at 140° C.), and the recovery of acid after each of these treatments was in no instance lower than the values set out above.

It was now evident that the recovery of acid by the pyridine method was substantially unaffected by considerable variation in conditions of heating and drying after the initial air drying employed in the preceding experiments.

There remained the possibility that the actual air drying gave rise to error. It was accordingly arranged to supply to each investigator samples of flannel made slightly acid with sulphuric acid and then conditioned, sampled and weighed as previously. Each investigator should then

(1) Determine the amount of acid present.

(2) Add a known amount of the order of 3 per cent., boil for 1 hour under a reflux condenser, and determine the acid present in liquor and wool together.

(3) Treat as in (2) and evaporate to dryness on the water-bath before determining the acid present.

(4) Treat as in (3), but dry for an extended period as in the first stage of our work on this aspect of the problem.

The results obtained are given in Table IX.

TABLE IX

| | J.B. Acid added: 3·00 per cent. | | | H.H.B. Acid added: 3·00 per cent. | | |
|---|---|---|---|---|---|---|
| | Acid found Per Cent. | Acid recovered Per Cent. | Recovery Per Cent. | Acid found Per Cent. | Acid recovered Per Cent. | Recovery Per Cent. |
| 1. Original pattern .. | 0·20 | — | — | 0·245 | — | — |
| 2. Treated with acid only | 3·26 | 3·06 | 102 | 3·18 | 2·935 | 97·8 |
| 3. Treated with acid and evaporated to dryness .. | 3·07 | 2·87 | 95·6 | 2·96 | 2·715 | 90·5 |
| 4. As (3) but dried for 3 hours at 110° C. .. | 2·92* | 2·72 | 90·6 | 2·89 | 2·645 | 88·2 |

| | F.L.G. Acid added: 2·72 per cent. | | | A.W. Acid added: 3·00 per cent. | | |
|---|---|---|---|---|---|---|
| | Acid found Per Cent. | Acid recovered Per Cent. | Recovery Per Cent. | Acid found Per Cent. | Acid recovered Per Cent. | Recovery Per Cent. |
| 1. Original pattern .. | 0·17 | — | — | 0·18 | — | — |
| 2. Treated with acid only.. | 2·77 | 2·60 | 96·6 | 3·31 | 3·13 | 104·3 |
| 3. Treated with acid and evaporated to dryness.. | 2·77 | 2·60 | 96·6 | 3·13 | 2·95 | 98·3 |
| 4. As (3) but dried for 3 hours at 110° C. .. | 2·73 | 2·56 | 94·1 | 3·00 | 2·92 | 94 |

* Heated at 110° C. for 1 hour only.

Two of us (H.H.B. and J.B.) found that some degree of charring occurred when the patterns were dried by either of the methods described above. One of us (F.L.G.) noticed slight yellowing of the edges of the flannel cuttings after heating at 110° C., but did not consider this serious. In view of these considerations and having regard to the findings of Harris, Mease and Rutherford,[9] that concentrated solutions of sulphuric acid at 25° C. can react "irreversibly" with wool, that is, in such a manner that the acid-treated wool no longer liberates the whole of its content of $SO_3$ on subsequent prolonged treatment with water or alkaline solutions, we consider that the values given in Table IX indicate a very satisfactory recovery of all the available acid before and after drying.

(*c*) *Coloration of the liquor.*—It will be seen from the results given under the previous heading that for routine determination in mill practice, there is no necessity to deviate from the specification originally laid down by Barritt of treating the pattern under test with pyridine solution for 1 hour at room temperature. It is natural that more rapid and slightly more accurate results will be obtained by raising the temperature at which the pyridine treatment is carried out, but in our opinion the use of higher temperatures is an unnecessary refinement and may involve a risk of dye bleeding into the pyridine solution. We have experienced some difficulty in the application of the method, as originally put forward, to certain heavily coloured acid-dyed materials. This difficulty appears to be associated chiefly with heavily acid-dyed cloths, which contain relatively small amounts of acid by virtue of some treatment after dyeing which has removed some of the acid left in the dyeing process.

The method as originally put forward has been found to be practicable with strongly coloured solutions, where the colour of these solutions is not such as will mask the development of the pink colour of phenolphthalein at the end of the titration. With red or violet solutions satisfactory end-points have been obtained by using thymol blue instead of phenolphthalein. In our experience this indicator gives the same result as phenolphthalein, but the colour-change, *viz.* from yellow to blue, is quite different and can readily be detected in reddish or violet solutions.

SUMMARY.—An examination of three of the methods in general use for the determination of acid in wool has been carried out in each of four independent laboratories.

In initial experiments the sodium terephthalate method was found to give lower acid values than those obtained by either the sodium acetate or the pyridine method. Since it did not appear possible that the pyridine method could return more acid than is actually present, work on the sodium terephthalate method was not pursued.

The results obtained by the sodium acetate distillation method were irregular and the method is influenced appreciably by a large blank correction which was found to be erratic.

The results obtained by the four investigators by the pyridine method on commercially dyed and laboratory acid prepared cloth agreed well. Work was therefore directed to an examination of possible defects in the method, such as (i) degree of recovery of acid present; (ii) degree of recovery of acid from wool dried at high temperatures; (iii) titration difficulties in coloured liquors. It has now been shown that the recovery of acid from wool, whether dried at low or high temperatures, is almost complete (of the order of 90 to 100 per cent.) under conditions as originally described; *i.e.* the sample is thoroughly wetted out in 0·5 per cent. pyridine (100 ml./1 g. of wool) and allowed to stand for one hour at room temperature.

The difficulty of determining the end-point of the titration in red or violet liquors with phenolphthalein as indicator may be overcome by substituting thymol blue for phenolphthalein.

Our thanks are due to members of our respective staffs for assistance in this

work. For permission to publish the paper, acknowledgment is made to the following:

The Wool Industries Research Association (J.B.).
The Bradford Dyers Association, Ltd. (H.H.B.).
The Geigy Colour Co., Ltd. (F.L.G.).
The British Cotton & Wool Dyers Association, Ltd. (A.W.).

ADDENDUM.—Since submitting the above account we have read with much interest the paper by Trotman and Bramley on "The Determination of Acidity in Knitted Woollen Goods."[10]

In this paper Trotman and Bramley state (1) that the pyridine method "does not always give the whole of the acid present," and (2) that in their opinion, "the only method applicable to both dyed and undyed goods is distillation with sodium acetate and titration of the acetic acid distilled."

We wish to make the followed observations on these two statements.

(1) This statement in its literal interpretation is true, since very small quantities of acid may be removed on repeated extraction of the acid-containing wool. This is to be expected, since the acid will be distributed between the wool and the pyridine but chiefly in the latter. That these quantities are, however, small may be seen from the following data obtained by carrying out the acid determination in the usual way, the sample being squeezed on a glass filter between the extractions.

ACID IN WOOL

| | Sample I Per Cent. | Sample II Per Cent. | Sample III Per Cent. | Sample IV Per Cent. | Sample IV (overnight) Per Cent. |
|---|---|---|---|---|---|
| First extraction .. | 2·33 | 2·28 | 2·48 | 2·18 | 2·32 |
| Second extraction .. | 0·05 | 0·08 | 0·08 | 0·17 | 0·09 |
| Third extraction .. | 0·03 | 0·04 | 0·04 | 0·07 | — |

The additional acid obtained in the second and third extractions in the above experiments is less than the values quoted by Trotman and Bramley, which we attribute to their use of a 0·1 per cent. solution of pyridine, instead of the 0·5 per cent. solution specified in the original method. When considerable quantities of acid are present in wool a low concentration of pyridine is not sufficient to extract substantially all the acid, as is shown by the following values obtained by using various concentrations of pyridine on thoroughly sampled acid wool.

| Concentration of pyridine Per Cent. | Acid found in cloth Per Cent. |
|---|---|
| 0·05 | 1·86 |
| 0·1 | 2·21 |
| 0·5 | 2·53 |

We would remark that the results obtained by Trotman and Bramley in comparing the sodium acetate and pyridine methods appear to be erratic, which may be due partly to the low concentration of pyridine used. In the absence of data as to the method employed in the purification of the wool and its acid or alkali content, it is not possible to draw definite conclusions from their results as to the reliability of either method.

(2) This opinion is based upon the admitted advantage that the sodium acetate distillation method gives a colourless titration liquor. Since we have failed to obtain consistent results by this method, we have preferred to modify the pyridine method in such a manner that this difficulty is avoided by suitable choice of indicator; the procedure has been proved to be satisfactory in the examination of a large number of commercially dyed materials.

## References

1. L. L. Lloyd, *J. Soc. Dyers & Col.*, 1914, **30**, 12.
2. J. Barritt, *J. Text. Inst.*, 1935, **26**, 87T.
3. S. R. Trotman and G. N. Gee, *J. Soc. Dyers & Col.*, 1932, **48**, 321.
4. L. Meunier and G. Rey, *Rev. Gen. Mat. Col.*, 1924, **28**, 66.
5. H. R. Hirst and A. T. King, *J. Text. Inst.*, 1926, **17**, 101T.
6. A. Woodmansey, *J. Soc. Dyers & Col.*, 1918, **34**, 172.
7. A. Eavenson and J. W. Creely, *Amer. Dyes. Rep.*, 1936, **25**, 719.
8. Private communication to F.L.G.
9. M. Harris, R. Mease and H. Rutherford, *Amer. Bureau of Standards J. of Research*, 1937, **18**, 343.
10. S. R. Trotman and A. Bramley, ANALYST, 1938, **63**, 408.

WOOL INDUSTRIES RESEARCH ASSOCIATION, LEEDS
THE BRADFORD DYERS ASSOCIATION, LTD., BRADFORD
THE GEIGY COLOUR CO., LTD., BRADFORD
THE BRITISH COTTON AND WOOL DYERS ASSOCIATION, MANCHESTER

*May 22nd*, 1938

## Discussion

Mr. S. R. TROTMAN, in a written communication, said that the wool used in his experiments had been purified by the oleic acid process recently described by Trotman and Stocker[10] (*J. Text. Inst.*, 1938, 148T; Abst., ANALYST, 1938, 758). Table IV in his paper showed that with comparatively small percentages of acid the results of the pyridine and sodium acetate processes agreed well, but that when 4 or 5 per cent. of acid were present the pyridine results were low and a second extraction was necessary. The authors of the present paper stated that a large blank correction was required in the sodium acetate process and that this was found to be erratic. In his experience the correction had always been between 0·4 and 0·6 ml. of $N/10$ alkali; to be on the safe side, he deducted 0·6 ml. from the alkali titration number. Two points were of importance: (i) freedom of the steam from carbon dioxide, (ii) rate of distillation, which should be about 45 minutes for 650 ml. of distillate. The acidity given in Table V in his paper[10] by the distillation method in September, 1937, was 3·07 per cent. Since then the sample had been in an air-tight receiver. On October 29th, 1938, he had re-determined the acidity by the distillation method and had found 2·97 per cent. In the investigation of complaints of skin irritation, etc., where dyed goods were concerned, the determination of acidity was admittedly difficult and must involve the titration of a coloured solution except when the sodium acetate method was used.

Dr. H. E. COX said that he would like to pay tribute to the valuable work done by the authors on a matter of considerable practical importance. He had some experience of the methods described and hitherto had preferred the distillation method; he found it reasonably accurate, and it had the advantage that it could be combined with other tests and determinations. He thought, however, that the most important practical point was to establish what acid was present, and what was a proper limit for such acid. So long as one did not know what amount of acid should be regarded as a maximum the accuracy of the determination within 0·1 per cent. or so was not material. Dr. Cox further asked the authors as to their

experience of the development of acidity in dyed and undyed woollen fabrics due to the action of light. Also, did they think that the acidic groups in the acid azo dyestuffs contributed materially to the titratable acidity? In his experience the acidity of a dyed wool was often lower than that of an undyed—perhaps sulphited—garment, yet complaint would be made of the acidity in the upper garment but not of that in the under-garment. He hoped, in view of its industrial importance, that the authors would continue their investigations into the wider aspects of the subject.

Mr. C. E. SAGE asked whether any tests had been made with wool "pulled" by fellmongers, as distinct from "clipped" wool, and said that the alkaline conditions under which the former was stripped caused the development of not only ammonia but a considerable amount of amino substances, which might not always be removed in subsequent treatment.

Mr. J. BARRITT, replying to Mr. Trotman's written communication, said that the discrepancy in the results obtained by the sodium acetate and pyridine methods referred to by Mr. Trotman would appear to arise from the use of too weak a solution of pyridine, this being emphasised with samples containing up to 5 per cent. of acid; this point was elaborated in the addendum to the paper, and it should be noted also that in commercially finished materials the amount of residual acid did not reach 5 per cent. Referring to the blank correction, he said that they had been unable to obtain uniform blanks, and their experiments had covered the conditions laid down with respect to freedom from carbon dioxide and rate of distillation. In attempts to solve this difficulty variations in the rates of distillation had been made and were discussed in the paper. The sodium acetate method had a marked advantage, inasmuch as the titration was conducted in a colourless solution, but in their experience this advantage was discounted by the difficulties in obtaining reproducible values and by the low recovery of acid. The difficulty in the determination of the end-point in certain coloured solutions, when using the pyridine method, was overcome by the use of thymol blue as indicator, and during the examination of a large number of samples the authors had not come across a material in which the acid could not be estimated satisfactorily by this method.

Replying to Dr. Cox, Mr. Barritt said that the nature of the acid in the material might be deduced by making *p*H determinations of a water extract (1 g. in 50 ml.) and of this extract after tenfold dilution. The original *p*H value, coupled with the difference on dilution, enabled a distinction to be drawn between organic and strong mineral acids. The amount of sulphuric acid present in finished woollen material rarely exceeded 2·8 per cent., and 3 per cent. might perhaps be regarded as an upper limit. The quantities of formic and acetic acids found in materials were usually less than 1 per cent. Wool on exposure to light developed an acid reaction which could be followed by *p*H measurements. Some of this acid was in the first instance sulphurous acid, as might be shown by the formation of azo sulphite on wool dyed with certain azo dyestuffs. Undyed wool might contain more acid than a dyed wool, for one of two reasons: (i) The wool might have been sulphur-bleached, *i.e.* treated with sulphur dioxide or a solution of sulphurous acid. From such material up to 1 per cent. of sulphur dioxide could be expelled by distilling the wool with dilute acids. The whole question of the condition of the sulphur dioxide absorbed during bleaching of wool was somewhat complicated; it was discussed in detail by Elsworth and Phillips (*J. Text. Inst.*, 1938, **29**, 219T). (ii) A sample of undyed carbonised wool might, if not washed off or neutralised, contain up to 7 per cent. of sulphuric acid, but would not reach the consumer in that state.

He (Mr. Barritt) could confirm all that Mr. Sage had said regarding the conditions in the pulling of fleeces, but he had not made any special tests with the two types of raw wool.

# Analysis of Commercial Lead

By E. A. COAKILL, A.I.C.

The following scheme has been devised for the determination of the impurities commonly occurring in commercial lead, the main objects in view being to avoid the use of unwieldy quantities, such as are generally recommended, without a corresponding sacrifice of accuracy, and to enable an examination to be carried out with greater speed. A number of the methods are not new and have been included merely for the sake of completeness, others have been modified, and in one or two instances the reagent used is practically all that remains unchanged in the original method.

Regarding the possibility of the interference of any one of the elements with the determination of another, it was found that when the process was used for its specific purpose, namely, the determination of impurities in quantities usually occurring in commercial lead, either no trouble was experienced or any interference could be inhibited.

Preparation of the Sample.—A transverse section, about half-an-inch in thickness, is cut from the centre of a pig, the sample for analysis is obtained by making saw cuts with a hack saw blade, the sawings are picked over with an electro-magnet to remove any iron, and 25 g. (or 50 g. if the lead is very pure and other quantities *pro rata*) are weighed and transferred to a beaker. The metal is dissolved in 120 ml. of dilute nitric acid (1 volume of sp.gr. 1·42 to 2 volumes of water), and the solution is gently boiled to expel oxides of nitrogen; except in rare instances the solution will be found to be quite bright, but in the event of a slight opalescence having formed the solution must be allowed to remain on a hot plate until the supernatant liquid is bright, after which it is usually possible to syphon off most of the clear liquid and filter the remainder, which contains the hydrated stannic oxide, on a retentive paper. The small precipitate is washed with 2 per cent. nitric acid only, the washings are added to the main filtrate, and the hydrated stannic oxide is retained to be examined later for possible traces of antimony. To the clear, nearly boiling solution of lead nitrate are added 40 ml. of hot dilute sulphuric acid (1 volume of sp.gr. 1·84 to 3 volumes of water), the precipitate of lead sulphate is cooled rapidly and filtered off on a Buchner funnel, about three washes with 1:15 sulphuric acid are given, and the total filtrate is in a shallow beaker of the "Baco" type until dense fumes of sulphuric anhydride appear. When cool the residue is diluted with water and made up to 100 ml. in a graduated flask. Four ml. of this solution correspond with 1 g. of the original metal and in the following determinations quantities are expressed in grams. Comparison in Nessler cylinders is usually required, and it is necessary that the standards should be treated in the same manner as the unknown solution; in every instance, therefore, the starting point of the standard should be dilute sulphuric acid equal in quantity to that present in the assay. A still more satisfactory "blank" may be obtained by taking 25 g. of assay lead, in which the very minute amounts of impurities have been carefully determined, and treating this in the manner already indicated.

DETERMINATION OF SILVER, ON 5 GRAMS.—The measured quantity is transferred to a small beaker, a few drops of phenolphthalein solution are added, the free acid is neutralised with dilute ammonia and then the solution is brought back to faint acidity with acetic acid and cooled; then ten to twelve drops of a 10 per cent. aqueous solution of gum arabic are added. Six ml. of acetone are put into a Nessler cylinder, followed by 0·125 ml. of a saturated acetone solution of *p*-dimethylaminobenzal-rhodanine (this is sufficient for 0·1 mg. of silver, and is about 10 drops as delivered from an average 1-ml. pipette), the solution suspected of containing silver is then poured into the cylinder and the contents are well mixed. The orange colour of the reagent is changed to a more or less red tint, according to the amount of silver present, and this is estimated by adding standard silver nitrate solution to a blank prepared in exactly the same manner. It should be noted that the smaller the excess of the reagent added the more sensitive is the test, and it is hardly necessary to mention that special care must be taken to exclude chlorides during all operations.

DETERMINATION OF COPPER, ON 2 GRAMS.—The portion for the determination is put into a Nessler cylinder, free acid is neutralised with ammonia and the liquid is brought back to faint acidity with acetic acid, after which 0·5 ml. of 10 per cent. aqueous solution of gum arabic is added, followed by 4 to 10 drops of a 0·25 per cent. alcoholic solution of rubeanic acid according to the amount of copper present. A blank is prepared in the same manner and standard copper solution, which must contain no free mineral acid, is added until equal intensities of colour are obtained.

DETERMINATION OF BISMUTH.—*Method* 1 (*approximate*) *on* 2 *Grams*.—The requisite amount is transferred to a Nessler cylinder, and 2 ml. of a 20 per cent. solution of potassium iodide (containing a few drops of a saturated aqueous solution of sulphur dioxide)* are added. Standard bismuth solution is then added to a blank, prepared in the same way, until the colours match correctly.

It may be of interest to record that recently a doubt arose concerning the bismuth-content of a consignment of oxide of lead supplied to the Railway Company by a well-known firm of manufacturers; the latter made their analyses by the tedious B.S.I. method and the Railway Company by that just quoted, with very comparable results. As will be seen from the tabulated results the figures are low, for it is almost impossible to prevent the occlusion of a portion of bismuth in the bulky precipitate of lead sulphate, and to prevent this the following method has since been devised and is recommended to supersede Method 1.

*Method* 2.—Five g. of lead are dissolved in 30 ml. of dilute nitric acid (1 volume of sp.gr. 1·42 to 4 volumes of water) the solution is diluted to about 80 ml. with hot water, and dilute ammonia (sp.gr. 0·94) is added until most of the free acid is neutralised but no permanent precipitate is formed, about 5 ml. being required. Dilute ammonium carbonate solution (one volume of saturated solution to 3 volumes of water) is then added to the boiling solution, 1 ml. or less at a time until a small precipitate of possibly 0·2 to 0·25 g. remains after boiling for a quarter of a minute; without delay the precipitate is filtered on a smooth surface paper, washed several

* See ANALYST, 1938, **63**, 107, for notes on the use of sodium hypophosphite in place of sulphurous acid.

times with hot water, removed from the paper to the original beaker with a strong jet of water followed by 10 ml. of (1 : 3) sulphuric acid and boiled briskly for a few minutes. The liquid, which should not be more than 30 ml. in volume, is filtered to remove lead sulphate and made up to a definite volume, and an aliquot portion is examined for bismuth in the manner already described, dilution to at least 50 ml. being recommended should the solution be strongly coloured; by this means the whole of the bismuth is separated from the lead and, if necessary, the result may be obtained within an hour.

TIN.—The question of the most satisfactory method of determining a trace of tin which may have escaped precipitation, and therefore observation, during the original solution of the metal, is one that has not yet been satisfactorily solved, although tin may be detected comparatively simply as follows:—Strong hydrochloric acid is added to a portion of the solution suspected to contain tin until about 25 per cent. by volume is present, after which a coil of iron wire is introduced and heat is applied until a brisk action is obtained. When it is judged that the reduction is complete, the solution, which still contains the iron wire, is cooled, preferably in an atmosphere of an inert gas, rapidly filtered to remove copper, antimony, etc., and mixed with a few drops of an aqueous solution of cacotheline; a more or less pronounced violet colour is obtained in the presence of tin, but unfortunately it is not usually sufficiently permanent to allow of its use for quantitative purposes, apart from the fact that the inevitable, even though short exposure to air may oxidise part or all of the stannous compound before the addition of the reagent.

More recently, dithiol has been used for the detection of tin, and whilst this appears to work quite well qualitatively, I have been unable to obtain reliable quantitative results with it.

The strength of acid recommended for dissolving the metal for the main analysis is such that a mere trace of tin would not be precipitated, and consequently, it is desirable to test, say, 50 g. of lead specially for that element by dissolving it in the least possible excess of nitric acid (sp.gr. 1·42) diluted with five times its volume of water (theory requires about 31 ml.). The hydrated stannic oxide, after separation, may be dealt with by one of the usual methods, it being borne in mind that it will almost certainly contain lead and possibly antimony. It is recognised that this is by no means an ideal method, and it is hoped that before long it may be possible to recommend a reasonably reliable colorimetric one.

DETERMINATION OF ANTIMONY, ON 5 GRAMS.—To the solution contained in a small beaker is added about 0·1 g. of tartaric acid, a few drops of phenolphthalein solution and 50 per cent. sodium hydroxide solution (weight/volume) from a pipette until the reaction is just alkaline and then four drops in excess. The beaker is then placed on the hot plate and treated with 5 drops of sodium sulphide solution (made by saturating 5 per cent. sodium hydroxide solution with hydrogen sulphide and afterwards adding 20 to 25 per cent. of its volume of the original sodium hydroxide solution). After digestion near the boiling-point for about ten minutes the coagulated sulphides are filtered off on a 7 cm. paper of close texture, and the filtrate is collected in a Nessler cylinder, cooled, and treated with 10 drops of a 10 per cent. aqueous solution of gum arabic and 2 ml. of hydrochloric acid (1 : 1),

and the yellow to orange colour is compared with that of a similarly prepared standard of known antimony-content. The comparison should be made as quickly as possible, owing to the fact that after some minutes colloidal sulphur may begin to appear, and the smaller the excess of sodium sulphide that has been used and the colder the solution the longer will its appearance be delayed. It has been found that the most satisfactory method of preparing the solution for comparison is to have a known amount of antimony present in the form of a standard solution (say 0·1 to 0·2 mg.) while still in the alkaline condition and to form the colour by the addition of the acid as with the assay. It has been observed that on adding standard antimony solution after the acid, that is, in the presence of the liberated hydrogen sulphide, the colour obtained is of a somewhat browner tint than that normally produced, but that the small additions necessary to obtain equal intensity of the standard and the sample do not materially affect the shade, provided these additions are not excessive.

Under the heading of "Preparation of the Sample" mention was made of the possibility of a small precipitate of hydrated stannic oxide which might also contain antimony, and this may be dealt with by the above method with slight modifications as follows:—The precipitate is ignited at as low a temperature as possible and fused with two or three pellets of potassium hydroxide in a silver crucible, the melt is extracted with water, the solution is acidified with hydrochloric acid, and from this point the procedure is as described above, except that sodium oxalate in the proportion of 0·1 g. per mg. of tin must be introduced to inhibit the action of that element; this must be done prior to the final addition of acid.

Determination of Arsenic, on 2·5 Grams.—The appropriate volume of solution is transferred to a Nessler cylinder, free acid is neutralised with strong sodium hydroxide solution, the minimum amount of methyl red being used as indicator, and the solution then adjusted with very dilute sulphuric acid until faintly acid. The liquid is then cooled to 20° C. or below, one ml. of Denigès' reagent (10 g. of ammonium molybdate in 100 ml. of water poured into 300 ml. of (1 : 1) sulphuric acid) is added, followed by 4 drops of a 2 per cent. solution of stannous chloride in 10 per cent. hydrochloric acid, the liquid being well stirred after each addition. A blue colour is produced in the presence of less than 0·005 mg. of quinquevalent arsenic, but the maximum intensity is not attained until the lapse of some minutes after the addition of the stannous chloride. The standard, containing quinquevalent arsenic, is prepared in the same way, and the stannous chloride is added to it and to the assay solution within a few seconds of each other; the quantity of arsenic likely to produce a depth of colour not very divergent from the sample is usually 0·005 to 0·01 mg., and comparison is made by diluting the more highly coloured solution. It must be remembered that differences of temperature, acidity, etc., between the sample and the standard render unreliable what is otherwise an accurate determination. The blue colour is also produced by phosphates, but none of the impurities commonly occurring in lead appears to affect the result.

Determination of Iron, on 2 Grams.—Five ml. of (1 : 1) hydrochloric acid and 5 ml. of 10 per cent. potassium thiocyanate solution are added to the sample contained in a Nessler cylinder, and the pink (or red) colour is matched by adding

standard ferric iron solution to a blank solution similarly prepared. If desired, the colour due to ferric thiocyanate may be extracted by shaking with amyl alcohol; this procedure renders the test more sensitive. Thioglycollic acid may also be used for the determination of iron, but my experience of this reagent is not very extensive and the following details are given with reserve:—One ml. of 10 per cent. tartaric acid solution is added to the sample, which is then made just alkaline with ammonia, and, when cool, is treated with 5 drops of thioglycollic acid (90 per cent.), and the colour, which may be lilac to violet, according to the quantity of iron present, is matched as described above.

ZINC, ON 2 TO 5 GRAMS (according to the amount of impurity expected).—A method for the determination of zinc by means of diphenylthiocarbazone was published by Hibbard,[1] but separation from traces of lead was somewhat tedious and a speedier method was therefore sought. After a large number of experiments had been made it was found that a solution of 0·05 g. of diphenylthiocarbazone in 100 ml. of chloroform containing 4 per cent. by volume of thioglycollic acid not only preserved the reagent from oxidation but also inhibited the interference of lead sufficiently to render its complete removal unnecessary, and it is upon this fact that the following procedure is based:—The quantity of the solution to be examined is transferred to a small, deep beaker, heated to boiling-point, saturated with hydrogen sulphide to remove lead and other heavy metals as far as possible, and filtered, the concentration of sulphuric acid being sufficient without any adjustment to prevent the precipitation of the zinc. The filtrate is heated until the volume is reduced to about one-half, or until all the hydrogen sulphide has been driven off, and then cooled, and about 0·5 ml. of 5 per cent. tartaric acid solution is added, followed by ammonia until the reaction is just alkaline, the minimum quantity of bromocresol blue being used as indicator. The solution is again cooled and transferred to a 50-ml. stoppered cylinder, 5 ml. of chloroform and 1 ml. of the reagent prepared as described are added, the cylinder is shaken gently, and the aqueous portion, which will now probably be faintly acid, is adjusted with very dilute ammonia until just alkaline. The cylinder is then shaken vigorously until no further colour change takes place. A blank solution prepared in the same way as the assay, of the same alkalinity and containing exactly the same amount of reagent, should be available, and to this is added standard zinc solution with vigorous shaking after each addition until the colours of both standard and sample are the same. The comparison may be made either during or immediately after shaking and the strength of the standard solution should be varied according to the amount of the impurity present. One ml. of the reagent is sufficient to inhibit the action of 0·4 mg. of lead and for the determination of about 0·1 mgm. of zinc; but it must always be in excess and more should be added before the colour, originally green, approaches a true red. Sometimes sufficient iron may be present to produce a colour with the thioglycollic acid contained in the reagent, in which event it will be first noticed in the aqueous portion when it is finally made alkaline. As the amount of iron present will then be known, the same quantity is added to the blank, in the form of standard iron solution, and the aqueous portion is adjusted by means of very dilute ammonia or sulphuric acid until it shows no more than a faint pink colour, which is maintained, by further adjustment if necessary, until

the conclusion of the test. In practice it is probable that the amount of iron present in most leads will not be sufficient to cause any interference.

CADMIUM.—Whilst there are a number of reagents by means of which coloured precipitates may be obtained with cadmium, all suffer either from insufficient sensitivity, or lack of adaptability in minute quantities or both. For the determination of cadmium, therefore, it would seem that a larger quantity of metal must be examined, and this element determined by one of the recognised means, until such time as a reliable colorimetric method can be recommended.

The following figures will serve as an indication of the accuracy to be expected. The first series of tests were made on assay lead to which were added impurities

TABLE I

PURE LEAD, WITH IMPURITIES ADDED

(All figures in parts per million)

| Impurity | Analysis No. | Amount of impurity added | Amount of impurity recovered |
|---|---|---|---|
| Silver | 1 | 20 | 17 |
| | 2 | 20 | 18 |
| | 3 | 40 | 35 |
| | 4 | 40 | 36 |
| Iron by thioglycollic acid | 1 | 10 | 10 |
| | 2 | 20 | 20 |
| | 3 | 30 | 31 |
| | 4 | 50 | 50 |
| Iron by thiocyanate | 1 | 10 | 10 |
| | 2 | 20 | 22 |
| | 3 | 30 | 31 |
| | 4 | 50 | 51 |
| Copper | 1 | 40 | 41 |
| | 2 | 50 | 49 |
| | 3 | 50 | 50 |
| | 4 | 60 | 58 |
| Bismuth Method 1 | 1 | 40 | 33 |
| | 2 | 50 | 36 |
| | 3 | 80 | 67 |
| | 4 | 120 | 90 |
| Bismuth Method 2 (separate quantity) | *a* | 50 | 50 |
| | *b* | 100 | 100 |
| | *c* | 200 | 197 |
| Arsenic | 1 | 2 | 2 |
| | 2 | 4 | 4 |
| | 3 | 6 | 6 |
| | 4 | 8 | 8 |
| Antimony | 1 | 20 | 21 |
| | 2 | 40 | 41 |
| | 3 | 40 | 42 |
| | 4 | 80 | 81 |
| Zinc | 1 | 8 | 8 |
| | 2 | 10 | 10 |
| | 3 | 20 | 19·5 |
| | 4 | 40 | 39·5 |

in the form of standard solutions, the volume of which was allowed for in the original solution in dilute nitric acid. A sample of the assay lead was treated in exactly the same manner, but without the addition of any impurities, and was used as a blank when making colour comparisons in order to counter the effect of any trace of impurity that it might contain.

The second series was obtained by using low grade lead (the impurities in which had been determined by the foregoing process) and adding known amounts of standard solutions as in the first series; in some instances, especially where dealing with the higher concentrations, the quantities taken for examination were a half or even a quarter of those recommended.

It will be noticed that the results for bismuth by Method 1 are unreliable and that little more than 80 per cent. of the quantity added could be recovered; on the other hand the results by Method 2 may be regarded as quite satisfactory.

TABLE II

IMPURE LEAD, WITH IMPURITIES ADDED

(All figures in parts per million)

| Impurity | Analysis No. | A Original amount of impurity | B Impurity added | C Total impurity found | D Added impurity recovered |
|---|---|---|---|---|---|
| Silver | 1 | 5 | 20 | 23 | 18 |
| | 2 | 5 | 40 | 44 | 39 |
| Iron by thioglycollic acid | 1 | 3·5 | 10 | 13·5 | 10 |
| | 2 | 1·5 | 20 | 22 | 20·5 |
| Iron by thiocyanate | 1 | 3·5 | 10 | 13 | 9·5 |
| | 2 | 1·5 | 20 | 20 | 18·5 |
| Copper | 1 | 25 | 40 | 67 | 42 |
| | 2 | 42 | 20 | 63 | 21 |
| Bismuth Method 1 | 1 | 142 | 20 | 156 | 14 |
| | 2 | 126 | 40 | 160 | 34 |
| Bismuth Method 2 (separate quantity) | *a* | 152 | 50 | 202 | 50 |
| | *b* | 152 | 200 | 350 | 198 |
| Arsenic | 1 | 1 | 4 | 5 | 4 |
| | 2 | 1 | 8 | 9 | 8 |
| Antimony | 1 | 21 | 20 | 42 | 21 |
| | 2 | 14 | 40 | 56 | 42 |
| Zinc | 1 | 6 | 10 | 16 | 10 |
| | 2 | 6 | 20 | 26·5 | 20·5 |

Column D is C—A and in theory should equal B.

A few remarks concerning the storage of standard solutions may be added. It has been found that very dilute solutions do not retain their original strength for long periods and that, generally speaking, it is more satisfactory to prepare "stock" solutions, containing 1 mg. per ml. of the element concerned, to store these in the dark, and to dilute to one-tenth the strength for use (with arsenic, and

possibly zinc, to one-hundredth). It is claimed that after a fair trial the foregoing process will be found to yield reasonably accurate results and to effect a very considerable saving of time and reagents when compared with other methods which involve the use of very large quantities of metal.

In conclusion I wish to thank the following members of the Research Department, London, Midland & Scottish Railway, Mr. W. P. Henderson, Chief Chemist, Dr. P. Lewis-Dale, late Chief Chemist, and Mr. G. W. Jones, Assistant Chief Chemist, for reviewing the text, and Mr. N. I. Hafley for his assistance in carrying out some of the very large number of experiments that were found to be necessary.

REFERENCE

1. P. L. Hibbard, *Ind. Eng. Chem., Anal. Ed.*, 1937, **9**, 127.

RESEARCH DEPARTMENT
LONDON, MIDLAND & SCOTTISH RAILWAY
STONEBRIDGE PARK

*April*, 1938

# Determination of Magnesium in Biological Materials

## Interference of Manganese

BY J. DUCKWORTH, D.SC., AND W. GODDEN, B.SC., A.R.C.S., F.I.C.

PRIOR to performing certain mineral balance studies with sheep it was decided to investigate the suitability of the hydroxyquinoline method for the determination of magnesium in foodstuffs and excreta. In the course of this investigation it was noticed that the precipitate of magnesium oxyquinolate as obtained from ammoniacal solution, when standing on the water-bath for the precipitate to granulate, changed from the original pale yellow colour to a much darker shade with certain foodstuffs analysed. Spectrographic examination* of these dark precipitates showed the presence of manganese. This discoloration still occurred even when the magnesium was first precipitated as its double ammonium phosphate prior to precipitation as its oxyquinolate. It is, of course, well known to metallurgical chemists that manganese is precipitated as the double ammonium phosphate but, owing to the low concentration of manganese in most biological materials, this fact appears to have been overlooked by biological chemists.

In the ordinary way, and particularly in blood analysis, we prefer to determine magnesium indirectly by colorimetric estimation of the phosphorus in the magnesium ammonium phosphate. The method employed is cited by Godden,[2] and calls for no further comment here. Whether magnesium is determined gravimetrically (the method we were investigating) as its oxyquinolate, or by bromination of its oxyquinolate, as several authors recommend, or indirectly by determination of the phosphorus in the magnesium ammonium phosphate, it appears obvious that manganese may be a source of error. This fact becomes of greater importance since it has been suggested[1] that a connection exists between

* We are indebted to Dr. R. L. Mitchell of the Macaulay Institute for this examination.

manganese and magnesium metabolism. Further reference will be made later to this point.

## Experimental

In view of the above preliminary findings it became necessary to determine the magnitude of the error likely to be introduced by the presence of manganese when determining magnesium and to devise some method of avoiding this interference.

To test the magnitude of the error a bulk sample of plasma was prepared from oxalated bovine blood, and to 5-ml. aliquot portions were added 1-ml. volumes of manganous chloride solutions of different strengths. The magnesium was then determined, in each instance in duplicate, by the indirect method, *i.e.* precipitation of the double phosphate and colorimetric estimation of the phosphorus present. The results are given in Table I. The ratio values given in column 4 of this table are obtained from the amounts of manganese added and the phosphorus increments found owing to the manganese contamination. The interference of manganese is apparent when as little as 1 mg. of manganese is added per 100 ml. of plasma, but the formation of the manganese ammonium phosphate is not quantitative even with 10 mg. of manganese added. Were it quantitative the ratio would be 1 : 1·77.

As it seemed possible that these results might have been due to the presence of other substances in the plasma, a similar series of determinations was carried out on aqueous solution of pure magnesium and manganese salts. The results are shown in Table II, all results again being in duplicate.

### Table I

### Interference of Manganese in the Determination of Magnesium in Plasma

(All results expressed in mg. per 100 ml. plasma)

| Magnesium present | Manganese added | Magnesium found | P:Mn ratio |
|---|---|---|---|
| 2·39 | 0·00 | 2·39 | — |
| 2·39 | 1·00 | 2·64 | 1:3·13 |
| 2·39 | 2·00 | 3·02 | 1:2·54 |
| 2·39 | 4·00 | 3·93 | 1:2·04 |
| 2·39 | 6·00 | 4·67 | 1:2·05 |
| 2·39 | 8·00 | 5·53 | 1:2·00 |
| 2·39 | 10·00 | 6·51 | 1:1·90 |

These data, although obtained with smaller amounts of added manganese, support the results shown in Table I.

In view of these results it was of importance to find some method for overcoming this interference due to manganese. Since the ratio of P : Mn was never 1 : 1·77, either the manganese was not completely precipitated or it did not come down as manganese ammonium phosphate. It would be useless, therefore, to try to determine manganese colorimetrically, *e.g.* by the method of Richards,[3] in another portion, and thus make the necessary allowance for it in the magnesium determination. After various trials the following simple technique was found to be satisfactory.

TABLE II

INTERFERENCE OF MANGANESE IN THE DETERMINATION OF MAGNESIUM IN AQUEOUS SOLUTIONS

(All results expressed in mg. per 100 ml.)

| Magnesium present | Manganese added | Magnesium found | P:Mn ratio |
|---|---|---|---|
| 2·00 | 0·00 | 1·98 | — |
| 2·00 | 0·40 | 2·14 | 1:2·14 |
| 2·00 | 0·80 | 2·28 | 1:2·14 |
| 2·00 | 1·00 | 2·28 | 1:2·68 |
| 2·00 | 1·20 | 2·40 | 1:2·30 |
| 2·00 | 1·40 | 2·49 | 1:2·31 |

METHOD.—Several adequate techniques exist for the precipitation of magnesium ammonium phosphate from ash extracts and biological fluids such as blood plasma or serum, and no useful purpose would be served by recapitulating them here. It is sufficient to follow any usual technique and to obtain this precipitate, contaminated with manganese phosphate, in a centrifuge tube of suitable size. The supernatant liquid is poured off after centrifuging, and the precipitate is washed once with Holzapfel's reagent* (*loc. cit.*).[2] The precipitate is then dissolved in 1 ml. of *N* sulphuric acid and 0·5 ml. of 2 *N* sodium hydroxide solution are added to neutralise the solution. A few crystals of sodium acetate are added to buffer the solution during the subsequent oxidation, and the tube is placed in a boiling water-bath. After a few minutes the tube is removed and rotated between the fingers to effect complete mixing, and then 0·75 ml. of bromine water is added. The tube is returned to the boiling water-bath for 7 to 8 minutes. A further 0·75 ml. of bromine water is added, and the tube is again heated for a similar period. The manganese is thus converted into its insoluble hydrated oxide. The tube is cooled in ice water and, when cold, is treated with 1 ml. of ammonia (sp.gr. 0·880). This is followed by 1 ml. of 5 per cent. ammonium hydrogen phosphate solution, and after its contents have been well mixed the tube is stoppered and allowed to stand overnight. The following day the precipitate is washed by centrifuging as described for the method[2] and the phosphorus is determined in the usual manner. The manganese hydroxide is soluble in the molybdic acid used and does not interfere in the Fiske–Subbarow method.

In Table III are shown a typical set of results in which ox serum was used, the calcium having been first determined and removed by the Clark–Collip method† (*loc. cit.*).[2]

* Holzapfel's reagent is prepared by mixing 580 ml. of 96 per cent. alcohol, 320 ml. of water, 100 ml. of amyl alcohol and 30 ml. of concentrated ammonia (sp.gr. 0·880).

† The Clark–Collip method (*J. Biol. Chem.*, 1925, **63**, 461) for determining calcium in serum is as follows:—Measure 2 ml. of half-saturated ammonium oxalate solution into a 5-ml. conical centrifuge tube and add 2 ml. of the serum. Twist the tube between the fingers, mix the liquids, and set aside for 4 hours. Centrifuge. Pour off the supernatant liquid into a dry test tube and put it aside for the estimation of magnesium. Invert the centrifuge tube in a rack to drain for 5 minutes. Dry the mouth and inside of the tube walls with filter paper, and then wash the precipitate and the tube walls with 2–3 ml. of 0·6 % ammonia. Centrifuge, drain and dry as before. Then blow 2 ml. of *N* sulphuric acid on to the precipitate. Place the tube in a boiling water-bath for 1 minute, and finally titrate with 0·01 *N* potassium permanganate solution.

TABLE III

DETERMINATION OF MAGNESIUM IN SERUM IN THE PRESENCE OF ADDED MANGANESE

(All results expressed in mg. per 100 ml.)

| Magnesium present | Manganese added | Magnesium found |
|---|---|---|
| 2·08 | — | 2·08 |
| 2·08 | 1·00 | 2·08 |
| 2·08 | 2·00 | 2·08 |
| 2·08 | 4·00 | 2·07 |
| 2·08 | 6·00 | 2·14 |
| 2·08 | 8·00 | 2·12 |
| 2·08 | 10·00 | 2·13 |

The purification of the magnesium ammonium phosphate by washing and centrifuging is somewhat difficult if large amounts of manganese are present. In the last three tests in Table III the slightly high values are probably caused by the difficulty experienced in washing the somewhat bulky precipitates, although even the extreme values are near the limits of accuracy for the magnesium method. The ratio of manganese to magnesium in these latter tests, however, is higher than is known to occur in foodstuffs or normal blood serum, and this difficulty is not likely to arise in practice.

In a further test of the method three rabbits were used. A blood sample was taken from each, and immediately afterwards the animal received by stomach tube a solution of manganous chloride giving either 1 or 2 g. of the anhydrous salt per kilo. of body weight. Further blood samples were taken at varying intervals, and in all the samples the magnesium content of the whole blood was determined, with and without the removal of manganese by the method outlined. The results are shown in Table IV.

TABLE IV

EFFECT OF ORAL ADMINISTRATION OF MANGANOUS CHLORIDE

(All blood magnesium values are in mg. per 100 ml. of whole blood)

| Rabbit No. | Magnesium values: Old method | Magnesium values: New method | Difference | Time after administration mins. | Dosage per kilo. of bodyweight g. |
|---|---|---|---|---|---|
| 1 | 7·40 | 7·40 | 0 | 0 | — |
| | 11·10 | 9·25 | 1·85 | 55 | 1·0 |
| | 12·06 | 10·18 | 1·88 | 105 | 1·9 |
| 2 | 6·99 | 6·99 | 0 | 0 | — |
| | 13·36 | 8·35 | 5·01 | 15 | 1·0 |
| 3 | 7·18 | 7·18 | 0 | 0 | — |
| | 18·20 | 10·10 | 8·10 | 22 | 2·0 |

The figures shown in Table IV fully support those in the preceding tables. The values in the column headed "Difference" are an indication of the extent to which the orally administered manganese has got into the blood stream in the case of each rabbit, with the passage of time, and hence has vitiated the determination

of magnesium by the old method. They indicate that in any experiments dealing with the effect of manganese on magnesium metabolism, attention must be paid to the possible presence of manganese in any blood samples being analysed. In this connection attention may be drawn to the recent work of Blakemore *et al.*,[1] in which the authors investigated the effect of lethal and sub-lethal doses of manganese chloride, administered to rabbits, on the level of the serum magnesium. They report a rise of serum magnesium with lethal and a fall with sub-lethal doses, but they do not appear to have taken into account the possible presence of marked amounts of manganese in the blood of their experimental animals.

DISCUSSION.—Richards[4] records values for manganese in pasture grass up to 0·120 per cent. on a dry matter basis and Blakemore *et al.* quote values as high as 0·132 per cent. In view of the fact that the colorimetric method is widely used for the determination of magnesium in pasture grass and in the light of our results given above, it would appear essential that some procedure be adopted for the removal of manganese prior to the determination of magnesium in such materials. The same warning applies to the analysis of blood samples in experiments in which manganese salts are being administered. The method as outlined appears to us to offer a simple and rapid procedure for overcoming the difficulty.

REFERENCES

1. F. Blakemore, J. A. Nicholson and J. Stewart, *Vet. Rec.*, 1937, **49**, 415.
2. W. Godden, *Imp. Bur. Anim. Nutr.*, 1937, *Tech. Commun. No.* 9.
3. M. B. Richards, ANALYST, 1930, **55**, 554.
4. Rowett Institute, unpublished data.

THE ROWETT RESEARCH INSTITUTE
ABERDEEN

*June* 9, 1938

# 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.*

## COLORIMETRIC DETERMINATION OF CHROMIUM IN PLANT ASH, SOIL, WATER AND ROCKS

OWING to the fact that many citrus trees are grown in soils that lie on the chromiferous rocks of the South African Bushveld Igneous Complex, and in view of recent researches on the toxic effect of traces of chromium,[1] it was decided to investigate to what extent chromium is taken up by the plants. Chromium occurs in the rocks as the mineral chromite and probably also as a chromium-bearing magnetite. Weathering of the rock may partly decompose these minerals, thus bringing the chromium into a form in which it may become available for the plants.

The first difficulty was to find an accurate method for the determination of very small quantities of chromium in plant ash, soil and water. After a few preliminary experiments it was decided to base a method on the work of Kuchinski and Zavoskaya[2] and of van der Walt[3].

From 5 to 10 g. of plant material are ashed in a large platinum crucible in an electric muffle furnace. After cooling, 10 ml. of hydrofluoric acid and 4 drops of

conc. sulphuric acid are added, and the mixture is evaporated to dryness on a sand-bath, to remove all the silica (citrus leaves contain much silica). Six g. of potassium pyrosulphate are then added and the mixture is fused over a small flame until the melt is clear. It was found advisable to add part of the flux and fuse until frothing stopped, and then to add the rest of the flux. After the fusion is complete the mass is cooled and dissolved in 150 ml. of dilute hydrochloric acid (1:9). Plant ash containing little aluminium yields a melt readily soluble in water or in acid even more dilute than 1 : 9. We found it advantageous at this stage to add 2 ml. of a 5 per cent. ferric sulphate solution in order to get an easily filterable precipitate in the following step.

The solution thus obtained is diluted to 200 ml., a few drops of conc. nitric acid are added, the liquid is boiled for one minute and ammonia (1:1) is added, drop by drop, until precipitation is complete, the *p*H being kept as low as possible, *i.e.* until litmus just turns blue. The precipitate is washed thoroughly with hot 2 per cent. ammonium sulphate solution and dissolved in sulphuric acid, and the precipitation and washing are repeated. The precipitate is returned to the original beaker, the small amount adhering to the filter-paper being dissolved in hot dilute sulphuric acid. The main precipitate is dissolved in sufficient sulphuric acid to bring the total quantity to 4 ml. of conc. acid. (This solution should now contain no chlorides and no manganese, or at most a trace.) The liquid is diluted to 80 ml., heated to boiling, and treated with 5 drops of conc. nitric acid, 1 ml. of a 2·5 per cent. solution of silver nitrate and 20 ml. of a freshly-prepared 10 per cent. solution of ammonium or potassium persulphate. The beaker is covered and its contents are boiled for ten minutes. The final volume should be not less than 70 ml. After cooling, iron, and aluminium are precipitated by adding to the cold solution solid sodium carbonate, a little at a time, until a slight excess is present. The contents of the beaker are transferred, without filtration, to a 100-ml. graduated flask, made up to the mark with water, and filtered through a dry filter into a dry stoppered flask.

Fifty ml. of the filtrate are placed in a 100-ml. stoppered graduated cylinder, 10 ml. of sulphuric acid (1:1) and 5 ml. of a freshly-prepared 0·1 per cent. solution of diphenyl-carbazide are added, and the volume is made up to 70 ml. The violet colour of the solution is then compared in a colorimeter with that of a standard chromium solution treated in the same way.

As a standard, a potassium dichromate solution, 1 ml. of which contains 0·002 mg. of chromium, is used. Usually 3 or 4 ml. of the standard are acidified, the reagent is added, and the mixture is then made up to 70 ml.

The method, with obvious modifications, is also applicable to soils and waters. Analyses of soils gave values of 0·2 to 0·4 per cent. of $Cr_2O_3$; citrus leaves, 2·5 to 7·5 p.p.m. of chromium; natural waters 0·0005 to 0·04 p.p.m. of chromium.

Micro-Determination of Chromium in Rocks.—The colorimetric method described above has been applied to the determination of chromium in certain rocks. In the procedure outlined any chromite, such as is present in pyroxenite and other basic rocks, is completely decomposed.

The finely powdered sample is decomposed with hydrofluoric acid, followed by fusion with potassium pyrosulphate and solution in dilute hydrochloric acid. After removal of the chlorides and manganese the chromium is oxidised to chromate with ammonium persulphate in the presence of silver nitrate. The presence of traces of manganese in the sulphuric acid solution of the ammonia precipitate is of no consequence, as the slight pink colour resulting from the persulphate oxidation of small amounts of manganese will hardly be noticeable in the aliquot part subsequently taken for analysis. Iron is removed from the sulphuric acid solution by means of sodium carbonate. After acidification with sulphuric acid and addition of diphenyl-carbazide, the violet colour is matched with that of a standard solution of chromium as previously described.

The following amounts of chromium (as $Cr_2O_3$) were obtained by the macro[3,4] and micro methods:

| Rock, etc. | Macro method Per Cent. | Micro method Per Cent. |
|---|---|---|
| Quartz-sericite .. | — | 0·07 |
| Soapy schist .. | 0·31 | 0·29, 0·30 |
| Pyroxenite .. .. | 0·42 | 0·42 |
| Tremolite .. .. | 0·50 | 0·47 |
| Serpentine .. .. | — | 0·48 |
| Pyroxenite .. .. | 0·81 | 0·81, 0·82 |
| Soil concentrate .. | 1·42 | 1·39 |
| Pyroxenite .. .. | 2·70 | 2·66 |
| Soil concentrate .. | 9·70 | 9·64 |
| Chromite .. .. | 45·4 | 44·9 |

We are indebted to Dr. B. W. Marloth for several analyses and for the reading of the proofs of this article.

## References

1. A. J. van der Merwe and F. G. Anderssen, *Farming in S. Africa*, 1937, 12, 439.
2. P. K. Kuchinski and N. V. K. Zavoskaya, *Chem. Zentr.*, 1934, **1**, 2457.
3. C. F. J. van der Walt, ANALYST, 1938, **63**, 176.
4. W. F. Hillebrand and G. F. Lundell, *Applied Inorganic Analysis*, 1929, p. 410.

C. F. J. VAN DER WALT
A. J. VAN DER MERWE

DIVISION OF CHEMICAL SERVICES
DEPARTMENT OF AGRICULTURE AND FORESTRY
BELVEDERE STREET, PRETORIA

---

## A NOTE ON AVOCADO PEAR OIL

THE oil of the avocado pear (*Persea americana* Mill. = *Persea gratissima* Gaertn.) is now an article of commerce. The flesh of this fruit is a very rich source of oil, which may constitute as much as 75 per cent. of the total solids of the flesh. It is difficult to obtain details of the method of extraction, but there is reason to believe that it is carried out commercially on the dried flesh by means of a suitable fat solvent. The oil finds use in the cosmetic industry, frequently supported by claims of a surprising nature. These may refer, moreover, albeit generally in vague terms, to the high vitamin-content of the oil. Since carrying out the work on which the following note was based, our attention has been called to an article in the *Chemist and Druggist* (March 19th, 1938, **128**, 347), in which a number of such statements are made. Some of them appear to have originated from papers referred to by us below, others from sources that we cannot trace. The article in question gives a number of cosmetic formulae involving use of the oil.

As we are interested in natural sources of fat-soluble vitamins, it seemed to us worth while to examine a sample of avocado pear oil. Through the courtesy of Messrs. P. Samuelson & Co., a sample of Californian origin was obtained and has been investigated for its content of three fat-soluble vitamins. Although we had no reason to doubt the genuineness of the oil, it seemed desirable also to establish its similarity with those samples on which chemical analyses had been reported in the literature, and we therefore submitted it to some of the usual quantitative tests for vegetable oils.

In order to compare our results with those reported previously, a search of the literature has been made; scanty information has been found. The figures given in the table are quoted by Winton and Winton[1] as having been published by

| Figures from | S.G. 60° F. | Ref. Ind. $n_D$ | Sapon. value | Iodine value | Acid value | Unsap. matter Per Cent. | Reichert–Meissl value | Acetyl value |
|---|---|---|---|---|---|---|---|---|
| Albro (minimum) | — | 1·4664 (25° C.) | 177 | 85 | 8 | — | 3·8 | 11·3 |
| Albro (maximum) | — | 1·4664 (25° C.) | 178 | 88 | 12 | — | 4·0 | 11·3 |
| Authors (single sample) | 0·918 | 1·4725 (21° C.) | 180 | 86 | 0·4 | 1·49 | — | — |

Albro.[2] An article containing fifteen references to previous work has been published by Liberalli,[3] but the number of the journal containing it does not appear to be accessible in this country. Reference to various volumes of abstracts has revealed a few other publications, mostly in American journals and originating either from California or Hawaii. The reports do not appear to be consistent among themselves in their findings as to vitamin-content, nor is it possible without detailed study of the originals to judge the validity of the vitamin tests carried out. Moreover, many of them date back several years, so that the results were not expressed in international units and cannot be converted into them.

Broadly speaking, claims have been made for vitamin D "in liberal quantities" (Weatherby[4]), and for richness in vitamin A (Weatherby, *et al.*[5]), to be met by a conflicting statement (Robbins and Bilger[6]) that no vitamin A is present. The last-mentioned authors, however, appear to agree with Weatherby when they refer to the oil's strong antirachitic properties. Statements as to the vitamin E content of the oil are occasionally encountered in commercial publicity matter, but no sustaining evidence has been found in the scientific literature.

Prior to submitting the sample to any biological tests, we carried out certain determinations that might throw light on its vitamin-content. We have, of course, not been concerned to look for water-soluble vitamins in the oil, in spite of the occasional appearance of statements that some vegetable oils contain "all the vitamins" or of similar claims to immunity from natural laws.

The sterol-content of the unsaponifiable matter was found to correspond with 0·625 per cent. by weight of the original oil. These sterols were examined spectrophotometrically and showed general absorption in the ultra-violet, with no noticeable inflection at 282$m\mu$ or at 271$m\mu$, the position of the main ergosterol bands. If, nevertheless, the absorption at 282$m\mu$, which corresponded with a value of $E^{1\%}_{1\,cm.} = 2{\cdot}9$, were taken, unjustifiably, as entirely due to ergosterol, this would have constituted 0·9 per cent. of the total sterols, corresponding with 24 parts per million in the oil. This can therefore be taken as a long way above the true figure for content of pro-vitamin D. It would appear that this oil would only be susceptible of very slight antirachitic activation under the influence of ultra-violet light.

Spectrophotometric examination was also made of the total unsaponifiable matter; no band at 328$m\mu$ was revealed, and this is in accordance with the fact that vitamin A itself is never present in vegetable oils. There is general absorption in the neighbourhood of 328$m\mu$, not, in our opinion, due to vitamin A.

Judging by the colour of the oil, its content of carotene was low, and it is therefore doubtful whether a biological test for vitamin A would reveal any activity unless the oil were fed at so high a level as to upset the general balance of the basal diet. Moreover, a test on the unsaponifiable matter, after it had been freed from sterols, with the standard antimony trichloride reagent of the British Pharmacopoeia, showed only a very transient blue colour of low intensity, confirming

our view that the amount of biologically active carotinoids present in the oil itself can be taken as negligible.

The statement that avocado pear oil contains vitamin E was viewed by us with scepticism; this has been confirmed by a test on animals. Four virgin rats, receiving vitamin E-free diet from weaning, were used according to the technique described by us elsewhere.[7] Each animal received 112·5 mg. of the oil for ten days, making a total dose of 1·125 g. None of the animals had a litter. The oil has, therefore, shown itself to be completely inactive at the level tested. From our knowledge of the ratio of dose and response of vitamin E, we estimate that the mean fertility dose of this sample of oil cannot possibly have been less than 5 g. and may have been any greater amount up to infinity. The oil, therefore, did not contain more than 1/10th the amount of vitamin E in a good sample of wheat-germ oil, with the probability that vitamin E was completely absent.

Finally, a sample of the oil has been examined for vitamin D by the line test, carried out according to our standard practice. A preliminary test indicated very slight activity in a daily dose of 112·5 mg. This test was then repeated on eleven animals, while two other groups, of eleven animals each, received daily doses of 0·45 and 0·75 international unit of the International Standard Preparation. The average healing shown by the animals receiving the oil was very considerably below that shown by the animals receiving 0·45 unit. This means that the oil itself cannot have contained more than 4 international units of vitamin D per gram, and probably contained less. A more exact assay could only be obtained by feeding a more concentrated solution of the unsaponifiable matter, after its separation from the oil, for the dose tested by us represents the maximum amount of an oil that can be conveniently fed daily in a biological assay. Although this oil would be quite valueless as a medicinal or commercial source of vitamin D, it is perhaps of some scientific interest that we have found it to contain a little vitamin D. This is presumably to be attributed to solar irradiation of traces of pro-vitamin D.

We should like to express our thanks to Mr. F. E. Read, A.I.C., and Miss M. I. Stern, A.I.C., for the chemical analyses of the oil, to Miss B. E. Stern for the spectroscopic examinations, and to Miss E. Allchorne, who superintended the biological tests for vitamins.

A. L. Bacharach
E. Lester Smith

Biochemical and Analytical Departments
Glaxo Laboratories, Ltd.
Greenford, Middlesex

## References

1. A. L. Winton and K. B. Winton, *The Structure and Composition of Foods*, Vol. II, 534. (Wiley, New York; Chapman & Hall, London, 1935.)
2. F. W. Albro, *Ann. Rep. Cal. Avocado Ass.*, 1917, 92.
3. C. H. Liberalli, *Bol. assoc. brasil. farm.* (1935), **16**, 57, 61.
4. L. Weatherby, *Calif. Avocado Assoc. Yearbook* (1930), **1930**, 100.
5. L. S. Weatherby, J. E. Tontz and R. Y. Watson, *J. Home Econ.*, 1929, **21**, 360.
6. R. C. Robbins and L. N. Bilger, *Hawaii Agr. Expt. Sta. Rept.*, 1934, **1933**, 23.
7. A. L. Bacharach, *Nature*, 1938, **142**, 35; *Biochem. J.*, 1938, **32**, 1298.

---

## WARNING TO USERS OF SECOND-HAND STEEL CASKS AND SIMILAR RECEPTACLES FOR STORAGE OF FOOD

Attention has been drawn to the danger that may possibly arise from the use of second-hand containers for foodstuffs. An outbreak of poisoning involving severe peripheral neuritis has recently occurred in South Africa which has been attributed to the contamination of edible oil with tricresyl-phosphate. This contamination is thought to have arisen through the use as containers for the oil of vessels which had previously contained this substance. Tricresyl-phosphate

is a colourless, almost odourless and tasteless oil, insoluble in water and non-volatile in steam, with a boiling-point of about 440° C. It is used on a large scale as a plasticiser for cellulose lacquers and varnishes and is distributed in 40-gallon steel casks, and possibly in smaller containers of various sizes. Its residues are not easily removed from metal casks, drums or cans unless a solvent is used, and it might, in these circumstances, easily be mistaken for an edible oil. Users of second-hand containers are advised to take the greatest care to ensure that these do not contain residues of tricresyl-phosphate, which may contaminate food.

*Test for Tricresyl-phosphate, etc.*—A simple test for phenolic compounds in edible oil is as follows:—Five drops of the oil mixed with 10 drops of alcohol and a fragment of potassium hydroxide (about 0·1 to 0·2 g.) are heated together until the alkali has dissolved and the oil is saponified. About 5 ml. of water are added, which should give a clear solution. A weak solution of diazotised *p*-nitraniline* is added, care being taken that the solution remains alkaline. If tricresyl-phosphate was present in the original oil the liquid becomes red owing to the formation of an azo-dye. It is advisable to carry out a control test on a sample of oil known to be pure. The limit of the test is about 0·01 per cent. of tricresyl-phosphate in an edible oil.

EDITOR

# Official Appointments

THE Minister of Health has approved the following appointment:

F. G. D. CHALMERS as Public Analyst for the County Borough of Dudley, in place of H. Silvester, resigned (September 17th, 1938).

The Minister of Agriculture and Fisheries has approved the following appointments since February 26, 1937:

J. THOMPSON as Agricultural Analyst for the County of Berkshire, in place of J. A. Voelcker, deceased.

W. W. TAYLOR as Agricultural Analyst for the County Borough of Nottingham, in place of S. R. Trotman.

S. R. TROTMAN as Deputy Agricultural Analyst for the County Borough of Nottingham.

T. R. HODGSON as Agricultural Analyst for the County Borough of Bury, in place of T. J. Hutchinson, deceased.

ERIC VOELCKER as Agricultural Analyst for the Counties of Bucks, Isle of Ely, Middlesex, Northants, Oxford and Yorks' East Riding, in place of J. A. Voelcker, deceased.

F. G. D. CHALMERS as Agricultural Analyst for the County Boroughs of Dudley and West Bromwich, in place of H. Silvester, retired, and Deputy Agricultural Analyst for the County Borough of Coventry, in place of W. T. Rigby.

W. T. RIGBY as Agricultural Analyst for the County Borough of Coventry, in place of A. Bostock Hill, deceased.

D. D. MOIR as Deputy Agricultural Analyst for the County of Surrey.

* *Diazotised p-nitraniline solution.*—A small quantity of *p*-nitraniline is shaken with about 10 ml. of cold water, the clear yellow solution is filtered or decanted off and one drop of acetic acid is added. Sodium nitrite solution (about 5 per cent.) is gradually added, with shaking, until the solution becomes practically colourless.

# Notes from the Reports of Public Analysts

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

## CITY AND COUNTY OF KINGSTON UPON HULL

### City Analyst's Report for the Year 1937

Of the 2257 samples examined under the Food and Drugs Act, 887 were taken informally.

Milk Curds.—In this part of the country milk curds are prepared from skimmed milk, but elsewhere may be whole milk curds containing the due proportion of fat. It is becoming increasingly common to add a small proportion of wheat flour, to give "the required consistency." What this may mean is uncertain, since flour is not required to make the article "fit for carriage or consumption." The addition of flour is therefore an adulteration, but the declaration of its presence by label—a practice now commonly followed in Hull—is usually a sufficient disclosure to the purchaser.

Vegetable Fats containing Water.—The sale of vegetable fats containing a high proportion of water (15 per cent.) as ready-mixed cooking fats is a recent departure. For a long time lard substitutes have generally, by common consent, been water-free fats. The declaration of the presence of water in some of the products apparently legalises the addition.

Nigerian Ginger.—For the first time for many years ground ginger that had been partly exhausted has been on sale here. Enquiries indicated that the product was probably Nigerian ginger which, in the process of cleaning, had been soaked in water for a long period. The figures obtained were:—moisture, 12·40; fibre (less ash), 3·20; total ash, 2·15; ash sol. in water, 0·80; ash insol. in acid, 0·40; ether extract, 5·10; alcohol extract, 4·96; cold-water extract (B.P.), 8·20; ash of cold-water extract, 1·44; alkalinity ($K_2O$) of ash of cold-water extract, 0·50 per cent.

The Director of the Imperial Institute informed me that the question of the preparation of Nigerian ginger was under investigation, and that the Nigerian authorities were taking immediate steps to remedy the fault. The Corporation therefore decided to draw the attention of the sellers to the matter, but to take no further action for the present.

Washing of Eggs.—It has been stated in Court that dealers wash eggs to remove dirt from the shells. Experiments were therefore made with new-laid eggs which were washed in water and in various acid liquids, rubbed with an abrasive, etc. Water-washing of the eggs produced no change that could be mistaken (under the ultra-violet lamp) for the effects of treatment that would remove the marks of origin. Rubbing with acid and with an abrasive produced dark areas, and acid treatment without rubbing generally caused a blotchy appearance of the shell, or characteristic patches of uneven shades of colour.

All the eggs in 5 parcels sold as "English Eggs" showed either rubbed areas, remains of ink marks of a reddish colour, or a patchy appearance due to washing in acid. Fines of £6 11s. 6d. and £4 14s. 6d. were inflicted on two persons concerned in these cases.

Arnold R. Tankard

# Ministry of Health

## SALE OF FOOD AND DRUGS

### EXTRACTS FROM THE ANNUAL REPORT FOR 1937–1938

AND

### EXTRACTS FROM THE REPORTS OF PUBLIC ANALYSTS FOR THE YEAR 1937

APPOINTMENT OF ANALYSTS.—During the year 1937 approval was given to the appointment of 28 Public Analysts in England.

ADULTERATION.—The total number of samples of food and drugs reported on by Public Analysts in England and Wales was 151,370; this is an increase of 4932 on the number for 1936 (itself a record) and an increase of over 20 per cent. on the number in 1927. Of these samples, 8401 (5·5 per cent.) were reported against, as compared with 5·3 per cent. in 1936.

PRESERVATIVES.—Of 389 samples that contravened the Regulations, 211 related to sausages, usually for failure to declare the permissible quantity of sulphur dioxide. Boron preservatives were found in samples of sausages, butter, cheese, chocolate biscuits and a few other articles, and the presence of formaldehyde was reported in one sample of milk, one of cheese and two of cream. Samples of meat and meat products, coffee and chicory extract contained sulphur dioxide contrary to the Regulations. One sample of lard, one of canned fruit and one of sausages contained benzoic acid, and two samples of tomato sauce contained benzoic acid in an amount that would have been permissible if declared. A fine of £10 and £3 3s. costs was imposed for the sale of sausages containing boron preservative in addition to an excessive and undeclared amount of sulphur dioxide.

MILK.—Of the 82,357 samples examined by Public Analysts, 434 "appeal-to-cow" samples were below the presumptive standard, and of the remaining samples, 5673 (7·0 per cent.) were adulterated or not up to standard. A fine of £15 and £5 costs was imposed for the sale of milk containing 6 p.p.m. of formaldehyde. One sample was reported to be contaminated with embrocation. Twenty-three samples of skimmed or separated milk were reported against owing to the presence of added water or deficiency in non-fatty solids.

CONDENSED AND DRIED MILK.—Of 1210 samples of condensed milk, 18 were reported against, and adverse reports were made on 5 of 186 samples of dried milk.

CREAM.—Adverse reports were made on 32 of 1864 samples. One sample of tinned cream, labelled "Rich Cream," contained only 21·6 per cent. of fat, and a sample of "Double Cream" contained 31 per cent. of fat. A fine of £10 and costs was imposed on the vendors of a sample of cream containing formaldehyde. One sample contained boric acid and two of tinned cream were contaminated with tin.

BUTTER AND MARGARINE.—Of 7445 samples of butter, 84 contained excess of water, 4 contained boron preservative, and 11 consisted wholly or in part of margarine. Thirty-three of 2470 samples of margarine were wrongly labelled, and 4 contained excess of water.

LARD AND OTHER FATS.—Adverse reports were made on 31 of 2679 samples of lard; 29 consisted wholly or in part of substitute fats, one contained 1·5 per cent. of free fatty acids and one contained 65 p.p.m. of benzoic acid. Thirty-seven of 638 samples of suet were reported against mainly on account of an excessive proportion of starchy matter (usually rice flour). Of 632 samples of dripping, 13 contained excess of water or of free fatty acids or both. The vendor of one sample of dripping, which consisted entirely of another fat, was fined.

CHEESE.—Of 1543 samples, 72 were reported against, mainly owing to deficiency of fat. One sample of "cooking" cheese contained only 0·7 per cent. of fat. Some samples of "cream" cheese had been made from whole milk and

others from skimmed milk. Two samples contained boric acid and 1 formaldehyde, and 1 of wrapped cheeses was contaminated with tin. Several samples, sold under fancy names, were made wholly or partly from fat other than milk-fat.

BREAD AND FLOUR.—A "starch reduced bread" contained a higher proportion of carbohydrates than was stated on the label. Eighteen of 1386 samples of flour were unsatisfactory. A number of samples contained potato starch, and in one case a fine of £15 and 25 guineas costs was imposed on the manufacturer.

JAM AND MARMALADE.—Of 1825 samples, 169 were reported against, mainly for a deficiency in fruit or soluble solids; 40 contained excess of preservative, 1 contained mould and yeast, and 1 pieces of glass.

VINEGAR.—Of 1943 samples, 233 were reported against, 75 for deficiency in acetic acid. Two contained 3·05 and 2·43 per cent. of zinc acetate respectively. Most of the other adverse reports were due to the sale of artificial vinegar as "malt" or "table" vinegar.

SPIRITS AND BEER.—Of 1684 samples, 50 of whisky, 17 of rum, 10 of gin, and 1 of brandy were below the correct strength. Three of the samples of whisky contained wine and a sample of brandy contained 69 per cent. of neutral spirit. Of 561 samples of beer, 31 were unsatisfactory. Twenty-seven (of which 19 were taken by one local authority) were contaminated with lead, one contained excess of preservative and one contained disinfectant.

MISCELLANEOUS ARTICLES OF FOOD.—Nearly 36,000 samples of other foods were examined. Forty-nine adverse reports related to the sale of pigs' liver as sheep's or lambs' liver, and 3 to the sale of ox liver as sheep's or lambs' liver. All these samples were taken by one local authority, and in most of the cases proceedings were successfully instituted against the vendors. Samples of potted meat contained excess of water and some contained starch. In one case haddock was sold as hake and in another megrim was sold as lemon sole.

Contamination by lead was reported in 16 samples of canned fish, and 94 samples, mainly sild, contained tin. Samples of canned fruits, vegetables and soups were also contaminated with tin. Lead was reported in 7 samples of curry powder, and in 1 of corned beef, and copper was found in one sample each of cider, mincemeat, tomato soup, sweets, celery, sauce and yeast extract. Contamination with arsenic was reported in samples of apples, grape juice, gelatin and confectionery.

Fourteen samples of sago consisted wholly or partly of tapioca, a sample sold as barley consisted of sago, and a sample sold as tapioca contained 50 per cent. of sago. Samples of rice contained talc, and mineral "facing" matter was also found in split peas. Seven samples of mustard contained starchy matter, such as wheaten flour, and one contained sulphur dioxide. Several samples of pepper contained magnesium carbonate and some contained starchy matter such as rice flour. The vendor of one sample containing 32 per cent. of rice starch and an artificial colouring matter was fined £10 and £4 4s. costs.

Of 23 samples of sugar, 17 contained tin and 2 excessive quantities of sulphur dioxide. One sample of icing sugar consisted of sodium phosphate and starch.

Four samples sold as coffee contained chicory, and 4 sold as "pure coffee extract" contained nearly 14 per cent. of extraneous matter (other than sugars) not derived from coffee.

Thirty-eight samples of confectionery were unsatisfactory. Two contained 5 p.p.m. of arsenic, one contained 1/30 grain and another 1/50 grain per lb.

As in previous years a number of "cream" cakes and pastries were reported against on account of the filling being wholly or partly fat other than milk-fat, and in some cases descriptions such as "Fresh Cream" and "Real Cream" were applied to samples which did not contain genuine cream. Adverse reports were also made on samples of chocolate rolls and other chocolate cakes deficient in cocoa.

Some samples of ground almonds consisted wholly or partly of other nuts, and

some contained added sugar or starchy matter. A sample sold as semolina consisted of coloured ground rice. Several samples sold as bread and butter were found to be bread and margarine.

A sample of non-alcoholic raisin wine contained 17 per cent. of alcohol. A sample of non-alcoholic raspberry wine contained no raspberry juice, and a sample described as a "Concentrated Essence of Green Ginger Wine" consisted of a solution of tartaric acid coloured with caramel and slightly flavoured with ginger. Several samples of lemon squash contained no lemon juice, and a sample of "lemon pop" contained 6·3 per cent. of proof spirit.

DRUGS.—Of the 6695 samples examined, 340 were adulterated or not up to standard. Ten samples of camphorated oil were deficient in camphor, and one contained chiefly petroleum instead of olive oil. Two contained arachis oil and one excess of camphor. Some samples of aromatic spirit of ammonia contained incorrect proportions of ammonia or ammonium carbonate or both.

Two samples of cod-liver oil had excessive acid value, 1 contained 1 per cent. of water, and 2 did not comply with the B.P. standard for vitamin A activity. One sample of malt extract and one of malt extract and cod-liver oil were deficient in proteins.

Five samples of Glauber's salt consisted of Epsom salts, 1 contained 18 p.p.m. of lead, and some were reported against on account of the anhydrous salt present. Among other articles on which adverse reports were made were samples of Parrish's Food, Easton's Syrup, paregoric, various preparations of magnesia, Seidlitz powder, liquorice powder, lime water, tartaric acid, glycerin and various kinds of tablets and lozenges.

# Department of Scientific and Industrial Research

## REPORT OF THE FOOD INVESTIGATION BOARD FOR THE YEAR 1937*

SIR FRANK SMITH resigned from the Board during the year, and Sir Joseph Barcroft was appointed to be Chairman in his place. Dr. F. F. Blackman, Professor Hilditch and Sir T. H. Middleton were re-appointed members of the Board for a period of four years.

Two Special Reports were published—one by R. B. Haines on "Microbiology in the Preservation of Animal Tissues,"† and the other by C. H. Lea on "Rancidity in Edible Fats"‡ (*cf.* ANALYST, 1938, 660).

EXPEDITION TO THE DOMINIONS.—The expedition undertaken in 1936 (*cf.* ANALYST, **1937**, **62**, 795) to secure data on the performance of modern methods in the carriage of foodstuffs overseas under refrigeration received the co-operation of all concerned, and the results obtained are now under consideration.

STORAGE OF FOODSTUFFS IN THE UNITED STATES AND CANADA.—A visit was paid by the officers of the Low Temperature Research Station to the United States and Canada. Broadly speaking, the impression gained is that while this country is in no way behindhand in research on the handling and storage of foodstuffs, the application of science in this field is not so forward here as in the United States. There the application of scientific methods was very striking, as was also the readiness of the leading industrialists to spend large sums of money in developing new processes and introducing new products to the public.

* H.M. Stationery Office, 1938. Price 4s. net.
† Report No. 45. H.M. Stationery Office. Price 2s. net.
‡ Report No. 46. Price 3s. 6d. net.

NUTRITIVE VALUE OF FOOD STORED BY MODERN METHODS.—The Board consulted the Medical Research Council on this question and received the following report:

"There is still so much to be learnt about food and nutrition that it is impossible, in the present state of knowledge, to state categorically whether or not stored foods are as nutritious as fresh foods. Nor is it feasible to undertake research with a view to answering this question directly. Many years of intensive work on large groups of human beings would be entailed, and even then there would be little prospect of arriving at a definite decision, for, with further knowledge of the effects of dietary factors on nutritional processes, it would be found that the results of the first few years' studies would have to be reconsidered in the light of fresh evidence. Nevertheless, useful information can be obtained by comparing the chemical composition of stored foods with that of fresh foods, and the general conclusion can be accepted that relatively little loss of known constituents occurs in foods stored by modern methods. Moreover, experiments have been carried out on animals in which satisfactory nutrition has been maintained with diets composed solely of stored foods. The available evidence therefore suggests that modern methods of storing foods cause little depreciation in their nutritive value; in fact, it may be said that food of good initial quality that has been stored by the best modern methods is likely to be superior in many respects to similar food that, though still technically fresh, is in reality stale. One substance of important biological significance, especially associated with fresh fruit and vegetables, namely, vitamin C or ascorbic acid, is well known to be easily destroyed by heat, applied either in ordinary cooking or in canning; to a less extent, vitamin $B_1$ is liable to be similarly affected."

RANCIDITY IN THE FAT OF COLD-STORED HERRINGS.—In an investigation, still in progress, for the Herring Industry Board, it has been found that the development of rancidity in the fat of herrings during cold storage is due to certain enzymes in the flesh, and experimental evidence suggests that these are made more active by common salt. For this reason, herrings that have been frozen in brine need careful washing and glazing before they are stored, and there would be a clear advantage if freezing could be carried out satisfactorily in air, so that contact with brine would be avoided. It is this possibility that is being explored by the use of plant provided by the Herring Industry Board.

COLD-STORAGE OF FISH.—The report states that during the last ten years, it is clear that there has been an increase in the care with which fish are handled and stored in ice at sea on trawlers, especially on long distance trips. Storage in ice will keep fish fresh for from 10 to 12 days, a period which covers some two-thirds of the trips made by British trawlers. Beyond this period, however, even with the most careful handling, ice is powerless to keep fish fresh, owing to the ability of bacteria to multiply at the temperature of melting ice, and some more powerful method is required. The method that has been worked out is that of freezing the fish in brine at a temperature of $-4°$ F., and of storing them at the same temperature, or, better still, at a temperature of $-22°$ F. Treated in this way, white fish retain their original freshness for at least six months; in fact, lemon soles have been kept in a highly palatable condition for so long as two years. The experiment demonstrates how slow is the deterioration of properly frozen and stored white fish; in fact, the process converts a highly perishable article into one which is relatively imperishable.

The industry is now seriously considering the commercial possibilities of brine-freezing and cold storage. The report recommends the freezing of that part of the trawler's catch which cannot be landed in 12 days. It is emphasised, however, that the fish should be absolutely fresh when it is frozen. "Nothing," the report states, "would be more detrimental to the prospects of the successful industrial use of the process than the sale to the public of inferior frozen fish." This matter lends special importance to work carried out at the Torry Research Station which promises to yield a quick and reliable test for freshness that could be applied equally well both to wet and to frozen fish.

GAS-STORAGE OF FRUIT.—A discovery of practical importance has been made. It appears that, in the stage before the attainment of full maturity at which they are often gathered, apples exhibit, on exposure to concentrations of carbon dioxide up to 15 per cent., a temporary increase in activity which may amount to as much

as 100 per cent. The practical bearing of this fact is that, in all forms of cold storage, both on land and at sea, the possibility of carbon dioxide accumulating to levels injurious to the fruit is greater in the period immediately after the fruit has been put into store, especially if it is warm and is on that account respiring rapidly, than had hitherto been thought possible. In gas-storage, in particular, where all possible precautions are taken against accidental leakage, the desirable concentration of carbon dioxide may be obtained more rapidly than had been anticipated, with a consequent risk of it being over-stepped. Gas-storage, which is now so successful with apples and pears, is being applied to other fruits. An experiment on the gas-storage of strawberries showed that in an atmosphere consisting of 10 per cent. of carbon dioxide, 10 per cent. of oxygen and 80 per cent. of nitrogen, the growth of fungal rots could be retarded to some extent without detriment to the fruit. The effect of this atmosphere persisted after removal to indoor temperature, namely, 65° F.

Physiology of Rigor Mortis.—If meat is to be properly kept the flesh must be acid and in a dead animal this can only be assured if there is an ample reserve of glycogen present in the muscles at the moment of death. This can be assured by proper preparation of the animal for slaughter, and in the few days preceding slaughter and actually on the slaughtering floor. The present practice of resting animals for at least 24 hours before slaughter after a fatiguing or exciting journey is essential. It would seem desirable, however, to allow animals a ration of readily absorbed food, such as cane-sugar or glucose, rather than to withhold food completely during this period, otherwise there is danger of supplies of carbohydrate falling short. "The ideal method of slaughtering would appear to be electrical stunning of the quietly-resting animal, thus avoiding all excitement and any struggling." The liberation of lactic acid from muscle glycogen is particularly heavy during exertion of short duration, and while the animal is alive it passes directly into the blood and is lost during subsequent bleeding. In the human subject the content of lactic acid in the blood during rest is about 0·01 per cent., and it is unusual for it to exceed 0·04 per cent. even during the heaviest exercise. Values between 0·05 and 0·06 per cent. in oxen and of 0·05 to 0·11 per cent. in pigs have been observed, representing an appreciable loss of acid that would otherwise have contributed to the fall in the *p*H of the muscle.

*Determination of the* p*H of Muscle* in situ.—Recent improvements in glass electrodes have enabled the changes in the *p*H of muscle to be followed. In rabbits' muscle the steady fall in *p*H from the moment of death is invariably interrupted for a short time at *p*H 6·3, after which the course again become linear. From *p*H 6·0 onwards the rate of acidification is comparatively slow, but still linear; the final *p*H is reached abruptly.

Red Colour in Bacon.—As a result of two years' research on the mechanism of the formation of nitrosohaemoglobin (Brooks, *Proc. Roy. Soc.*, 1937, B, **123**, 368) it is now possible to estimate both the concentration of nitrite in the pickle and the period of curing that will give a satisfactory colour to bacon.

Experiments have shown that pickle may be stored for at least 10 days without appreciable change in its nitrite-content. It is proposed, therefore, to transport samples of pickle in thermos flasks at temperatures below 10° C., without the addition of antiseptic.

Bacterial Infection of Eggs.—The black rot of eggs is usually caused by species of *Proteus* and the red rot by species of *Pseudomonas*. The latter are always the cause of green rot, and occasionally of blackening. Typical coliform organisms, often found in black rots, apparently do not contribute to the rotting, but produce a strong "fishy" odour, and some species of *Pseudomonas* produce a distinctive "cabbage-water" odour. All these organisms may be found on the shell of eggs. The untreated shell of eggs of good quality is extraordinarily resistant to infection, but washing renders it susceptible to invasion.

CHANGES IN THE EGG DUE TO CHANGES IN THE PRESSURE OF CARBON DIOXIDE.—The reversible changes caused by changing the pressure of carbon dioxide in the atmosphere have been investigated. By controlling the pressure of the gas the *p*H of the white can be adjusted from 9·5 to 6·5. In the range 6·5 to 8 bicarbonate ion is the important buffer, but in the range 8 to 9·5 the bicarbonate is dissociated, giving carbonate ion, so that the buffering system is a complex one of bicarbonate, carbonate and dissociated protein. The yolk differs from the white in that the system of carbon dioxide and bicarbonate plays little part as a buffer, and that, owing to the large amount of fat present, it has a greater solvent action than the white on carbon dioxide. Experiments have shown that, in spite of its highly porous nature, the shell does not adsorb carbon dioxide to any significant extent.

ROLE OF ACETALDEHYDE IN THE CATABOLISM OF CARBOHYDRATES.—Experiments in which apples and oranges were fed with acetaldehyde vapour have confirmed the theory that the acetaldehyde taken up by the fruits is oxidised to carbon dioxide after having catalysed the conversion of triose into ethyl alcohol. The conclusion was drawn that the capacity of the fruit to utilise atmospheric oxygen is not impaired by the treatment.

STARCH-LIQUEFYING ENZYME.—By treatment with dilute iodine under controlled conditions it has been found possible to destroy completely the saccharogenic (or $\beta$) amylase present in aqueous extracts of barley or wheat and to leave in an active state a thermolabile catalyst which may be described as a dis-aggregating enzyme. Its action results in a progressive decrease in the viscosity of starch paste without any increase in reducing power.

TRANSFORMATIONS IN IODINE COLORATION DURING AMYLOLYSIS.—By means of a technique (to be published elsewhere) Hanes and Cattle have found that it is now possible to follow quantitatively the alteration in iodine coloration under the action of different amylases, and so to delineate throughout the various degradation processes the relation between iodine coloration and reducing power. This provides a highly diagnostic criterion for the recognition of features of similarity and difference in the action of starch-splitting enzymes.

OTHER INVESTIGATIONS.—Other matters discussed in the report include the changes in the sugar-content of potatoes, the storage of broccoli, the preservation of peas and asparagus by freezing, the effect of various methods of manuring, the storage properties of apples, the storage of hothouse grapes and the ripening of imported pears and plums.

The final section deals with engineering investigations.

# Straits Settlements

## ANNUAL REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1937

IN his Annual Report, Mr. M. Jamieson gives the usual account of the chemical work done for the various government departments at Singapore and Penang. The work done during the year showed a great increase on 1936, owing to the increase in the amount of chandu dross returned on sale to Government, and to direction of public interest to the quality of imported food.

CONTAMINATION OF SPIRITS WITH LEAD.—The application of the prohibition of lead-contaminated liquors led to a considerable number of lead determinations being made on samsoo spirits imported into the Colony. The practice adopted has been to warn the importer if the lead-content exceeds 0·75 part per million and to refuse admission to liquors containing more than 1·5 part of lead per million. Of the 78 locally distilled samsoos, 50 per cent. contained no lead or only a trace,

and 92 per cent. contained less than 0·75 part per million. Of the 83 imported samsoos, 42 contained no lead, and 95 per cent. contained less than 0·75 p.p.m.; the highest figure was 1·3 p.p.m.

TOXICOLOGICAL.—Caustic soda continued to be used as a means of suicide. It was detected in 51 specimens of vomit and in 12 specimens of viscera. Other exhibits contained tar acids from disinfectants, arsenic (4), alcohol (7), morphine, prussic acid, clove oil, copper, zinc and corrosive sublimate. Five specimens in which no poison was found were connected with cases of poisoning by eating boxtal fish eggs. This fish belongs to the notoriously dangerous *Tedrodon* species.

*Ammonia Gas in the Hold of a Vessel.*—Free ammonia was detected at the bottom of a large deep hold, in sufficient concentration to cause tears and discomfort. It was found to originate from the reaction in the hot moist atmosphere between spillings of cement and of ammonium sulphate from damaged bags in consignments that had been landed before the ship reached Singapore.

AN ABNORMAL INK.—A letter, which it was supposed might have been written in jail, was characterised by the unusual greenish blue colour of the ink and by the occurrence of a large number of black discrete particles visible under low magnification. Comparative experiments with different materials were made to test the theory that the letter had been written with an old dried ink residue wetted with urine. These showed that although such a mixture did in fact give results similar to the exhibit, rather more similar results could be obtained by writing with the inky corroded matter scraped off old pen nibs and stirred up with water. It is of interest perhaps to record that other methods which proved unsuccessful in producing the appearance described above included treatment of ordinary ink with urine (fresh or stale) or, with saliva or with both, treatment of artificially evaporated ink with water and treatment of the dried residue in an old ink well with water.

BLINDING POWDER.—Two persons suspected of having been concerned in a robbery in which blinding powder was used were brought to the laboratory. On one person, under the fingernails and between the fingers, pepper was found. A coat connected with the case was found to bear deposits of pepper. The other accused person had no pepper stains on his body, but a trace of pepper was found on trousers belonging to him although these had been soaked in water, presumably for cleansing.

HOME-MADE BOMBS.—A Chinese was arrested with a live home-made bomb in his possession. The bomb was found to consist of a tobacco tin containing pieces of rough iron, small stones and about 3½ ounces of a mixture of potassium chlorate, sulphur, camphor, red phosphorus and arsenic sulphide. This mixture was extremely finely divided and exploded readily and violently when struck or rubbed between two hard rough surfaces. There can be little doubt that the bomb would have exploded on impact had it been thrown or dropped. Other exhibits in the same case included small quantities of the explosive or its ingredients present in a pocket of the accused and elsewhere and pieces of broken iron from which the smaller fragments in the bomb could have been derived.

In another case in which a bomb had been thrown into an amusement park, killing one Chinese and injuring others, examination of the residue indicated that the bomb had probably been improvised from a flash-light powder which consisted essentially of magnesium powder and ammonium perchlorate.

# Fruit and Vegetable Preservation Research Station, Campden

## ANNUAL REPORT FOR THE YEAR 1936–1937

In the Introduction the Director (Mr. F. Hirst) makes it clear that the Station is at present concerned chiefly with improvement of the general quality of canned produce and the study of nutritional values. The Station has again kept in close touch with the Rowett Research Institute, where feeding experiments with "fresh" foods and canned foods are nearing completion. Arrangements have been made that in all cases of alleged food poisoning a thorough scientific study of all the facts shall be made at the Station and the problem referred to the leading medical authority on the subject.

During the year reviewed 705 technical enquiries were answered, and 293 samples of canned products were examined and reported upon.

A few cases of illness attributed to eating canned vegetables were investigated, but in every instance it was found that canned products were not responsible for the illness.

The following subjects *inter alia* were studied in the course of the experimental and research work at the Station.

Fruit Gumming on Plums.—Certain tentative conclusions were drawn from the investigation carried out by the Director with W. B. Adam:—(1) Gumming appears to be a varietal characteristic and is influenced by seasonal conditions. (2) Gumming is mainly due to physiological causes, but some is due to external injury or insect damage. (3) Rootstock and the age of the trees appear to have little influence on the extent of gumming. (4) Grading by removal of fruit showing external gum does not separate the plums in accordance with their content of internal gum, although possibly grading according to the position of the external gum may give a better separation.

Tin in Canned Vegetables and Fruits.—Figures given in this communication (by W. B. Adam and G. Horner) show the value of improvements in lacquering that have been made or are being undertaken. The effect of factors such as temperature, time of storage, type of lacquer used, state of ripeness, and hydrogen ion concentration is given, and it is shown that fruits and vegetables in lacquered cans seldom contain more than 40 mg. of tin per kg. even after long storage. These figures refer to the total tin in the canned product; the soluble tin is unlikely to exceed half these amounts. It may be possible to reduce the tin-content almost to zero if the developments at present in the experimental stage can be put into commercial practice.

Losses of Soluble Solids in the Blanching of Vegetables.—In this investigation (by G. Horner) the effect of the blanching process on the sugar-content and nitrogen-content of three vegetables is described, and the losses of these constituents are tabulated. Steam blanching causes much smaller losses of sugar and protein, while the amount of shrinkage is also less than with water blanching.

Composition and Texture of Dried Peas.—This investigation has been continued by W. B. Adam (*cf.* Analyst, 1937, **62**, 738). It was again found that the texture of harvested peas appears to be virtually unaffected by the application of artificial fertilisers. The application of fertilisers alters the mineral composition of the peas, the direction and extent of the alteration depending on whether the land has been previously treated with lime. The addition of common salt has a pronounced effect on the mineral composition. Early cutting and rapid drying tend to give peas a firm texture and low swelling properties.

MINERAL CONTENT OF CANNED VEGETABLES.—The losses of the various mineral constituents of certain fresh vegetables during cooking and canning operations are shown in a series of tables (G. Horner). Considerable loss of potassium and some loss of phosphate occur during blanching, and further losses of all constituents result when the covering liquid is discarded.

MEASUREMENT OF THE INTERNAL CAPACITY OF CANS.—W. Adam and J. Stanworth describe a flotation balance for measuring the internal capacities of cans. The variations in the capacities of commercially packed cans are recorded; all the cans of each size were identical before filling and seaming.

STUDIES ON THE MOULD *Byssochlamys fulva*.—This heat-resistant mould, which is responsible for the disintegration of canned fruits, has been known for about five years, and has been studied for most of this time at Campden. Recent work is discussed in a communication by T. G. Gillespy. Many practical canning and bottling trials have been conducted with the aim of preventing the effects of this mould, for which purpose the sources of infection and the heat resistances of the ascospores have been studied. The maximum heat resistance is at about *p*H 5. At *p*H 3 the resistance is considerably greater than at neutrality. The protective effect of sucrose was greater at 90° C. than at 85° C. The survival times for 10 per cent. of the asci, when heated in fruit syrups, exceeded 45 minutes at 85° C. and 15 minutes at 90° C. Normally the enzyme that is the cause of the break-down of canned and bottled fruit infected with *B. fulva* is not rapidly deactivated after the death of the mould. Filtered, poisoned or chloroformed juices from infected bottles have been kept for many months in glass and have not lost their enzymic activity. Experiments indicated that the enzyme becomes deactivated after a time in plain cans, and probably also in lacquered cans, but less rapidly.

# British Standards Institution

THE following Standard Specifications have been issued*:

No. 283—1938. PRUSSIAN BLUE FOR PAINTS.
Nos. 303, 318—1938. GREEN PIGMENTS FOR PAINTS.
No. 314—1938. ULTRAMARINE BLUE FOR PAINTS.
Nos. 320, 333—1938. VERMILION AND RED PIGMENT FOR PAINTS.

No. 515—1938. CARBOLIC ACIDS 60's.
No. 517—1938. CRESYLIC ACID OF HIGH ORTHOCRESOL CONTENT.
No. 521—1938. CRESYLIC ACID (50/55 PER CENT. METACRESOL).
No. 522—1938. ORTHOCRESOL, METACRESOL AND PARACRESOL.
No. 523—1938. PHENOL.

The foregoing five Specifications were revised in September, 1938. The methods of testing and apparatus referred to in the Appendixes have been recommended by the Standardisation of Tar Products Test Committee, and issued in its publication, *Standard Methods for Testing Tar and its Products*, 2nd Ed., 1938.

No. 808—1938. MODIFIED TECHNIQUE OF THE CHICK–MARTIN TEST FOR DISINFECTANTS.

The Chick–Martin Test, devised at the Lister Institute (*J. Hygiene*, 1908, **8**, 655), was designed primarily to estimate the value of disinfectants to be used outside the body.

The possibility of the substitution of yeast for faeces (Garrod, *J. Hygiene*, 1934, **34**, 322; 1935, **35**, 219) has been studied by a panel consisting of representatives of the Lister Institute,

* These Specifications can be obtained from the Publications Department, British Standards Institution, 28, Victoria Street, London, S.W.1.

the British Medical Association, the Government Laboratory, and the British Disinfectant Manufacturers' Association. The Panel found that yeast is free from the objections alleged against faeces. It has also recommended further modifications of the original technique, with the result that the test now gives more consistent results than hitherto. Experiments have shown that the coefficients obtained are substantially the same as those obtained with the original method.

DRAFT OF SPECIFICATION IN COURSE OF PREPARATION.

A limited number of copies of the following Draft Specification, issued for technical comment only, are available to specially interested members of this Society, who should apply (mentioning their membership of the Society), and send their comments, direct to the British Standards Institution, 28, Victoria Street, London, S.W.1.

CE(C) 9509. SALT FOR DAIRY PURPOSES.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

# Food and Drugs

**Colorimetric Estimation of the Colouring Matter of Bran in Flour. R. Müller.** (*Z. Unters. Lebensm.*, 1938, **76**, 225–228.)—The husk of cereal fruits contains an indicator dye related to the flavone series. Before this dye can be isolated and measured colorimetrically it is necessary to remove carotene by a preliminary extraction with ether, in which the indicator dye is insoluble. The dye is then extracted with 75 per cent. alcohol, in which it dissolves, forming a colourless, slightly turbid solution containing suspended albuminous and gummy matter. Upon the addition of ammonia the yellow colour is developed and, after the addition of kieselguhr, the mixture is filtered, yielding a clear solution, the colour of which is measured by comparison with standard potassium dichromate solutions in a Krumholz microphotometer (*Mikrochem.*, 1936, **20**, 227), fitted with a 13 × 13 mm. cell and a blue filter transmitting at 400 to 500$m\mu$. A series of twenty comparison solutions is prepared from an aqueous potassium dichromate solution. The first solution contains 0·030 g. of potassium dichromate per 100 ml. and the concentration of any other member of the series may be calculated by regarding the series as a geometrical progression with a common ratio 5/4 (*e.g.* solution No. 20 contains 2·082 g. per 100 ml.). With these solutions a calibration curve is constructed. The air-dried meal (5 g.) is extracted with 10-ml. portions of ether in a sintered glass crucible (Schott No. I, G3) until the carotene is completely removed. The residue is sucked dry by means of the water-pump and then centrifuged with 10 ml. of 75 per cent. alcohol. The supernatant liquid is mixed with 1 ml. of 25 per cent. ammonia solution and 0·2 g. of kieselguhr and filtered. Measurement of the yellow colour is made within four hours. The serial number of the comparison solution is recorded as the "yellow value" of the flour, intermediate values being denoted by, *e.g.* 6+ or −7, according to which comparison solution the flour solution approximates. The investigation of about 200 samples of wheat flour showed that they could be arranged in 9 classes according to their yellow value, which varied from an average of 3 for the first class to 14 or 15 for the ninth class. The yellow value increased with the degree of "grinding out" of the wheat grain. (*Cf.* Abst., ANALYST, 1936, **61**, 851.) A. O. J.

**Detection of an Unusual Artificial Dye in Egg-Pastry. J. Grossfeld and K. Schwarz.** (*Z. Unters. Lebensm.*, 1938, **76**, 232–233.)—A yellow colouring matter for use in egg-pastry, and for which the claim is made that it is not detectable by chemical means, is sold in solid and liquid form. When treated with sodium hydroxide solution the solid swells and dissolves, forming a reddish-yellow solution which resembles the liquid preparation. When heated with sodium hydroxide solution a peculiar odour, resembling that of meat-broth, is observed. The dye is insoluble in the solvents used for the extraction of artificial dyes, *viz.* water, alcohol, ether, chloroform, benzene, trichloroethylene, glacial acetic acid and acetone, but is partly or completely soluble in aqueous alkaline solutions, *e.g.* sodium hydroxide, sodium carbonate, ammonia and disodium phosphate. Wool fibres are dyed by a solution of the dye in aqueous alkali, which has been acidified with tartaric acid. The colouring matter may be detected in pastry by the following method:—The pastry (20 g.) is finely ground, passed through a 0·3-mm. mesh sieve, mixed with water to a thin paste and treated with 2 ml. of ammonia solution (sp.gr. 0·96), and the mixture is ground in a mortar for 10 minutes. Alcohol (150 ml.) is added gradually, with stirring, and the liquid is filtered. The deep yellow filtrate is diluted with 100 ml. of water, acidified with tartaric acid and, after the introduction of an unmordanted wool fibre, is distilled without a fractionating column until 150 ml. of distillate have been collected. The mixture is then heated on the water-bath for one hour. Under these conditions the wool fibre is dyed a fine yellow colour and from it the dye may be extracted with ammonia solution. If this solution is acidified with tartaric acid it may be used to dye another wool fibre. A. O. J.

**Analytical Classification of Fish-liver Oils. VI. Iodine Value of the Unsaponifiable Matter. D. C. M. Adamson, N. Evers and W. Smith.** (*Pharm. J.*, 1938, **141**, 271–272.)—The chief constituents of the unsaponifiable matter of fish-liver oils are: Cholesterol (iodine value 67); hydrocarbons (*e.g.* squalene or spinacene, $C_{30}H_{50}$, iodine value 371); dihydric alcohols—batyl alcohol (iodine value nil), selachyl alcohol (iodine value nil), chimyl alcohol (iodine value nil); vitamin A (iodine value 356), which is present in the unsaponifiable matter of some fish-liver oils, such as halibut-liver oil, in proportions up to 50 per cent.; vitamin D, which is present in such small proportion as to have no appreciable effect on the iodine value. If the following precautions are observed in the determination concordant values are obtained:—The S.P.A. method for the determination of unsaponifiable matter must be carried through in its entirety with careful attention to every detail; the residue must be dried in a current of oxygen-free nitrogen; the flask containing the residue must be weighed, a similar flask being used as tare, and a blank test must be made on the alcoholic potassium hydroxide used, the ethereal extract from which is evaporated and dried in the tare. The residue of unsaponifiable matter should be dissolved in 5 ml. of carbon tetrachloride, 10 ml. of pyridine sulphate dibromide reagent pipetted in, and the flask stoppered and left to stand for 3 to 5 minutes; 5 ml. of 10 per cent. potassium iodide solution are then added, followed by 40 ml. of water, and the solution is titrated with N/10 sodium thiosulphate solution. The

blank titration on the pyridine sulphate dibromide reagent should be made after contact with the residue obtained from the evaporation of the ether-soluble material in the potassium hydroxide. The following table shows results obtained with medicinal cod-liver oils.

| | Unsaponifiable matter | | |
|---|---|---|---|
| | Per Cent. | Iodine value | Blue value |
| Norwegian oils .. | 0·88 | 120 | 11·0 |
| | 1·15 | 122 | 11·6 |
| | 1·02 | 125 | 24·0 |
| | 1·19 | 120 | 13·0 |
| British oils .. .. | 1·15 | 128 | 10·6 |
| | 1·20 | 124 | 10·6 |
| | 0·80 | 110 | 6·8 |
| | 1·10 | 105 | 19·0 |
| | 1·40 | 130 | 40·0 |
| | 1·03 | 106 | 14·0 |
| | 1·23 | 110 | 5·2 |
| | 1·24 | 117 | 8·8 |
| | 1·08 | 130 | 8·9 |
| | 1·29 | 120 | 13·0 |
| Newfoundland oils .. | 1·31 | 123 | 23·0 |
| | 1·38 | 119 | 17·0 |
| | 1·42 | 100 | 15·5 |
| | 1·32 | 119 | 18·0 |
| | 1·22 | 120 | 10·0 |

Oils from individual species of the family *Gadidae* gave values lying in a similar range, and a number of miscellaneous fish-liver oils other than those from *Gadidae* and *Elasmobranchii* gave a wide range of iodine values from 72 to 313. Oils from various fish of the Order *Elasmobranchii* (which includes the sharks, rays, skates and dog fish) generally gave lower values, except such shark-liver oils as contain large quantities of squalene, which have much higher values. The determination of iodine value of the unsaponifiable matter is probably of little use in detecting shark-liver oil in cod-liver oil, but may be useful as a supplementary analytical characteristic. Shark-liver oil always contains a high percentage of unsaponifiable matter, and this is no doubt a more reliable indication of adulteration. E. M. P.

**Action of Strychnine on Bordeaux B. D. B. Dott.** (*Pharm. J.*, 1938, **141**, 261–262.)—When a weighed quantity of strychnine is dissolved in water containing its equivalent of *N* sulphuric acid, the corresponding weight of azorubrum is dissolved in cold water, and the two solutions are mixed, there separates immediately a compound of strychnine with α-naphthalene-azo-β-naphthol-3:6-disulphonic acid (the acid of Bordeaux B). The precipitate is filtered from the sodium sulphate solution, washed with a little water, and dried at about 105° C. When powdered it is nearly black, sparingly soluble in water, and more soluble in alcohol. Analysis shows that the azorubrate contains 48·77 per cent. of strychnine; accepting the formula given in the British Pharmaceutical Codex, the di-strychnine compound should give 50·41 per cent. In separating strychnine and caffeine from compound syrup of glycerophosphates the precipitate obtained

with potassium ferrocyanide was yellow even after re-precipitation. When it was shaken with chloroform a red colour resulted, and a yellow colour was imparted to benzene. The precipitate weighed 0·0125 g. instead of 0·0100 g., a difference of 25 per cent., which may be unimportant from the medicinal point of view but is important analytically. The colouring matter could be removed by extracting with benzene and shaking out the alkaloid with acidified water.

E. M. P.

**Methods of Assaying the Content of Morphine in Opium and of Cocaine in Raw Cocaine and in Coca Leaves.** (*Bulletin of the Health Organisation of the League of Nations*, 1938, **7**, *Extract No.* 6.)—*Determination of the Morphine Content of Opium.*—The opium cakes are sampled in such a manner, from the outside to the centre, that the various parts of the cake are included in the same proportions as in the cake itself, in order to allow for possible loss of moisture from the outer layers. The sample should be 2·5 per cent. of the weight of the cake if this is spherical or cylindrical, 12·5 per cent. if brick-shaped, and at least 10 per cent. if the cake is irregularly-shaped or was not made homogeneous at the time of preparation or has since dried unevenly. The sample if soft is then kneaded until homogeneous, or, if hard, it is powdered, passed completely through a 0·3-mm. sieve and then mixed. A portion of the sample is dried at 103°–105° C. and the percentage of moisture is computed. Another portion, weighing 4 g., is triturated in a mortar with 1 g. of calcium hydroxide (at least 85 per cent. pure) and 10 ml. of water. The paste is stirred up with a further 10 ml. of water and allowed to stand for 15 minutes with frequent stirring. The suspension is then diluted with water to 45 g., shaken continuously for 30 minutes, and filtered through a Schott und Gen. No. 3G3 filter. For the determination of the extractives, 3 g. of the filtrate are evaporated and dried at 103°–105° C. The percentage of extractives is calculated from the formula

$$E = \frac{(1000 + F)M}{3 - M}$$

Where M = weight of the residue from 3 g. of filtrate

F = percentage of moisture in the opium.

For the determination of the morphine content, 25 g. of the filtrate are treated with 2·5 ml. of 90 per cent. alcohol and 12·5 ml. of ether followed by 1 g. of ammonium chloride. The mixture is then shaken vigorously for 5 minutes, and after occasional shaking for a further 30 minutes, it is allowed to stand overnight, and the precipitated morphine is transferred to a sintered glass filter (Schott und Gen. No. 3G4), and any precipitate in the flask is washed on to the filter first with 3 ml. of ether and then with 3 ml. of saturated aqueous morphine solution until the filtrate ceases to give the chloride reaction. The flask, in which some traces of morphine may be left, and the filter are both dried for half-an-hour at 103°–105° C. Ten ml. of methyl alcohol are warmed in the flask to dissolve the traces of morphine remaining, and the warm solution is poured on to the filter. This treatment is repeated with further 10-ml. portions until all the morphine is dissolved. Any impurities in the morphine remain on the filter. The filtrate, which should be quite clear, is titrated with 0·1 *N* hydrochloric or sulphuric acid,

5 to 10 drops of 0·05 per cent. methyl red solution being used as indicator. When a faint orange colour is formed, the solution is diluted with 120 ml. of freshly-boiled water, which turns the colour back to yellow. The titration is then continued until the colour begins to change to red.

The percentage of morphine in the dried opium is given by the formula:

$$\frac{(1000 + E + F)(A + 1)\,0{\cdot}114}{100 - F}$$

The percentage of morphine in the original opium is given by the formula:

$$\frac{(1000 + E + F)(A + 1)\,0{\cdot}114}{100}$$

where E = percentage of extractives in the original opium, F = percentage of moisture in the original opium, and A = number of ml. of 0·1 *N* acid required in the titration.

In these formulae a correction of +1 ml. of 0·1 *N* acid (equivalent to +0·0285 g. of morphine) is introduced to allow for the morphine that has not separated.

*Determination of the Ecgonine-alkaloids Content of Raw Cocaine and of Coca Leaves.*—The *raw cocaine* is carefully sampled and the moisture-content of the sample is determined by drying 1 g. in a vacuum desiccator containing sulphuric acid until the loss in weight is not more than 1 mg. in 24 hours. To determine the ecgonine-content, about 0·5 g. of the sample is dissolved in 15 ml. of 2 *N* hydrochloric acid, and the solution is boiled under reflux for 5 hours. After cooling slowly over a period of at least 2 hours, the solution is filtered through a plug of cotton-wool, which is washed with small quantities of 2 *N* acid until the filtrate measures 25 ml. at 20° C. The solution is well mixed and its optical rotation for sodium light is measured at 20° C. in a 2-dm. tube. The percentage of ecgonine in the raw cocaine is given by the formula:

$$\frac{a \times 25}{2 \times 57} \times \frac{100}{w} \text{ or practically } \frac{22a}{w}$$

Where $a$ = optical rotation and $w$ = weight of cocaine ; +57° = specific rotatory power of ecgonine.

$$\text{Cocaine} = \text{Ecgonine} \times 1{\cdot}64.$$

As a check on the content of ecgonine, the combined acids (benzoic, cinnamic, truxillic) may be determined. About 0·5 g. of the raw cocaine is dissolved in 5 ml. of acetone and 5 ml. of 2 *N* sodium hydroxide solution, and the solution is boiled under reflux for 15 minutes. The acetone is then evaporated, and the aqueous solution is transferred to a separator with about 15 ml. of water, acidified with 10 ml. of 2 *N* hydrochloric acid and extracted with three 30-ml. portions of a 2:1 mixture of ether and petroleum spirit. The combined extracts are dried with sodium sulphate, and the solvent is removed under slightly reduced pressure, the temperature being kept below 30° C. The residue is dissolved in 5 ml. of neutral 95 per cent. alcohol and titrated with 0·1 *N* sodium hydroxide solution, 2 drops of 1 per cent. phenolphthalein solution being used as indicator.

1 ml. of 0·1 *N* alkali ≡ 0·0185 g. of ecgonine
≡ 0·0303 g. of cocaine.

The *coca leaves* are sampled and the samples ground to pass through a 2-mm. sieve. About 2 g. of the sample are dried at 103°–105° C. to determine the moisture-content. Another portion of the sample, weighing 20 g., is triturated in a mortar with 20 ml. of 2 *N* sodium carbonate solution and allowed to stand for half-an-hour with occasional stirring. The mixture is then extracted continuously with ether for a few hours, allowed to stand overnight in contact with ether, and again extracted until a total extraction of 8 hours has been given. The ethereal solution is then extracted with 20, 15 and 10 ml. of 0·1 *N* hydrochloric acid, each extract being filtered through cotton-wool. To the combined acid extracts are added 30 ml. of a 2:1 mixture of ether and petroleum spirit, followed by 1 g. of sodium bicarbonate in small amounts at a time. After shaking, the ethereal layer is removed, and the aqueous solution is re-extracted with three further quantities of solvent mixture (30 ml.). The combined extracts are dried, filtered and evaporated. The residue is dissolved in 5 ml. of neutral 95 per cent. alcohol and titrated with 0·1 *N* acid, with methyl red as indicator. When a faint orange colour is produced 50 ml. of water are added and the titration is completed.

$$1 \text{ ml. of } 0{\cdot}1\ N \text{ acid} \equiv 0{\cdot}0185 \text{ g. of ecgonine}$$
$$\equiv 0{\cdot}0303 \text{ g. of cocaine.}$$

As a check, the combined acids may be determined. Five ml. of 2 *N* sodium hydroxide solution are added to the solution from the titration of the alkaloids, and the mixture is boiled until the volume is reduced to about 10 ml. The boiling is continued under reflux for a further 5 minutes, after which the solution is acidified and extracted, and the extract is titrated with alkali as described above.

F. A. R.

**Volatility of Ephedrine and of Pseudoephedrine in Relationship to the Codex (1937) Method of Determination. R. Monnet and P. Durand.** (*J. Pharm. Chim.*, 1938, **28**, 145–151.)—According to the French Codex (1937) ephedrine hydrochloride is determined by extraction in ether from an ammoniacal solution, the residue after evaporation of the solvent being dried at 100° C. (for an unspecified period) and weighed. This method is now questioned on account of the volatility of the ephedrine (*cf.* Warren, Watkins and Keenan, *J. Amer. Pharm. Assoc.*, 1931, 128), and in the experiments described 0·4-g. portions of ephedrine were heated in capsules (diameter, 7 cm.) for various times at various temperatures, and the losses in weight were determined. Solutions of the sample in ether, which were subsequently evaporated, were used in order to ensure uniform distribution of the sample in the capsule, and in this connection it is pointed out that if ether containing peroxide compounds is used, decomposition of the sample into benzaldehyde may result; this is detectable by its odour. The results for 4 samples are plotted and they show that volatilisation is complete after 4 to 5 hours at 100° C., the loss being 10 to 12 per cent. in 10 minutes; the walls of the oven were covered with a white sublimate, and alkaline white vapours having an odour similar to that of methylamine, and producing white fumes with the vapour of hydrochloric acid, were observed. Pseudoephedrine (2 samples) behaved similarly, although volatilisation was slower, 7 to 10 hours being required for its completion, whilst mixtures

of the two alkaloids (in proportions ranging from 25 to 75 per cent.) behaved additively, except that the initial volatilisation was greater than that of ephedrine alone. Similar tests at temperatures ranging from 12° to 60° C. showed losses that were progressively smaller as the temperature decreased, being appreciable (*e.g.* 4 to 5 per cent. after 6 hours, for each alkaloid alone or for mixtures) at 30° C. At 12° to 19° C., however, there was no loss in air (after 8 days) or in a desiccator containing calcium chloride, whilst in a vacuum the loss was 8 to 10 per cent. in 12 days. It is therefore recommended that for the purpose of the determination the alkaloid should be dried over calcium chloride until the weight is constant.

J. G.

**Detection and Determination of Pyridine-β-Carbonic Acid Diethylamide. H. J. Van Giffen.** (*Pharm. Weekblad*, 1938, **75**, 1040–1041.)—Pyridine-β-carbonic acid diethylamide or nicotinic acid diethylamide, $NC_5H_4.CON(C_2H_5)_2$, is a cardiac stimulant used in practice as a 25 per cent. solution in water, known as coramine (*cf.* Thom, *Handbuch der Praktischen und Wissenschaftlichen Pharmazie*, Vol. VI, Part 1, p. 541). It is a yellow, oily liquid with little or no odour or taste, and can be distilled at 150° C. (at 2 mm.). It is readily soluble in ether and other organic solvents, and is miscible with water in all proportions, the 25 per cent. solution being clear and colourless and giving a flocculent precipitate with a solution of tannic acid, but no reaction with potassium mercuric iodide. A potassium permanganate solution is decolorised only after a considerable period, and a saturated solution of sodium carbonate or a conc. solution of sodium hydroxide precipitates the pyridine-β-carbonic acid diethylamide from it as a light yellow oil. This may be determined by hydrolysis with alkali to the sodium salt of nicotinic acid and diethylamine, the latter being then separated by distillation and titrated with acid. A solution of 400 mg. of sample in 100 ml. of water and 100 ml. of 10 *N* sodium hydroxide solution is heated gently with pumice in a 1-litre flask, so that it boils in 20 minutes. The solution is then distilled rapidly into 25 ml. of 0·1 *N* hydrochloric acid (the end of the condenser being below the surface of the acid), until the distillate amounts to 100 ml., when 25 ml. of water are added, and 25 ml. more of distillate are collected. If the excess of acid is titrated with 0·1 *N* sodium hydroxide solution, allowance being made for the titration obtained in a blank experiment, each 1 ml. of acid used is equivalent to 17·813 mg. of pyridine-β-carbonic acid diethylamide.

J. G.

# Biochemical

**Calcium and Sodium Contents of Blood-plasma and Blood-serum. H. Waelsch and S. Kittel.** (*Z. physiol. Chem.*, 1938, **255**, 36–52.)—Samples of blood were taken from a large number of subjects, some in normal health and others suffering from various disorders. One half of the blood was treated with heparin immediately after being drawn, and portions of this heparinised blood were removed at regular intervals and centrifuged, and the amount of calcium present in the plasma was estimated. The other half of the blood was treated

with pyramidone instead of heparin, and estimations of the calcium-content of the plasma isolated from it were made from time to time in a similar manner. Slight but significant variations in the calcium content of plasma after the heparinised blood had been standing for different lengths of time were observed, but plasma from the blood treated with pyramidone showed much smaller variations in calcium-content. This is taken as evidence in favour of the migration of calcium between corpuscles and plasma in normal blood. The calcium-content of the plasma was in general higher than that of the serum, but there were a few exceptions. Estimations of sodium in the plasma of heparinised blood also gave evidence of the migration of sodium between the blood corpuscles and the plasma when the heparinised blood was allowed to stand for some time. F. A. R.

**Colorimetric Determination of Sodium as Uranyl Manganese Sodium Acetate. W. C. Woelfel.** (*J. Biol. Chem.*, 1938, **125**, 219–227.)—Exactly 1 ml. of the sample, containing 0·1 to 0·5 mg. of sodium is transferred to a 15-ml. centrifuge tube, and sodium chloride solutions (1 ml.) containing respectively 0·12, 0·20 and 0·36 mg. of sodium are transferred to three similar tubes. To each tube are added 8 ml. of freshly-filtered uranyl manganese acetate solution. This is prepared by mixing a hot solution of 80 g. of uranyl acetate (dihydrate) and 46 ml. of 30 per cent. acetic acid in water to make 520 g., with a hot solution of 245 g. of manganic acetate (tetrahydrate) and 23 ml. of 30 per cent. acetic acid in water to make 520 g., adding one-third the volume of 95 per cent. alcohol to the cooled mixture, and saturating if necessary with uranyl manganese sodium acetate by adding 0·2 g. of the solid. The contents of each tube are stirred until precipitation begins and for 1 minute after, and the tubes are allowed to stand overnight. They are then centrifuged, the supernatant liquid is removed, and the tubes are allowed to drain. The precipitates are washed with 5 ml. of a freshly-filtered saturated solution of uranyl manganese sodium acetate in glacial acetic acid, centrifuged and drained as before. The precipitates are then dissolved in 10 ml. of potassium periodate reagent, prepared by dissolving 5 g. of potassium periodate in 800 ml. of water and 100 ml. of 85 per cent. phosphoric acid (the solution keeps well in the refrigerator). The solutions are heated in a boiling water-bath to complete the conversion of the manganous ion into permanganate. The colour of the unknown sample is then compared with those of the three standards.

When estimating the sodium-content of urine, 1 ml. (normally) of the sample is diluted to 10 ml. This solution and the three standard sodium chloride solutions (10 ml.) are each treated with 0·2 g. of a mixture of powdered calcium hydroxide (100 g.) and phenolphthalein (0·25 g.), and allowed to stand for 30 minutes with occasional shaking. The solutions are then filtered, and exactly 1 ml. of each of the filtrates is transferred to 15-ml. centrifuge tubes; the subsequent procedure is as described above. When working with urines containing less than 10 mg. of sodium per 100 ml., the urine filtrate must be concentrated and freed from potassium. Ten ml. of (undiluted) urine is treated with the calcium hydroxide-phenolphthalein mixture as described above, and three 10 ml. portions of water are treated in the same manner. Conc. hydrochloric acid is added, drop by drop,

to 5 ml. of each of the filtrates until the solutions are just acid. To the solutions not containing urine, 1 ml. of each standard sodium chloride solution is added and the samples are concentrated to about 1 ml. To each solution is added 0·75 ml. of saturated ammonium perchlorate solution and, after standing for half-an-hour, the precipitate is filtered off and washed with 95 per cent. alcohol. The filtrates are evaporated to 1 ml. and transferred to 15-ml. centrifuge tubes, with the aid of 8 ml. of uranyl manganese acetate reagent. The subsequent procedure is carried out as above.

To estimate sodium in blood serum, 0·1 ml. is ashed by the method of Ball and Sadusk (*J. Biol. Chem.*, **113**, 661), using 0·2 ml. of 4 *N* sulphuric acid. To the ash 1 drop of 2 *N* sulphuric acid is added, and the solution is transferred to a 15-ml. centrifuge tube by means of two 0·5-ml portions of water and the 8 ml. of uranyl manganese acetate reagent. The general procedure is thenceforward adopted.

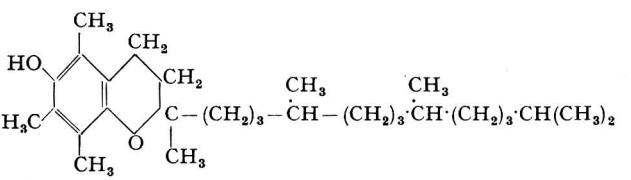
F. A. R.

**Determination of Nicotinic Acid, Nicotinamide and possibly other Pyridine-like Substances in Human Urine. S. P. Vitter, T. D. Spies and A. P. Mathews.** (*J. Biol. Chem.*, 1938, **125**, 85–98.)—This method, like that of Karrer and Keller (*cf.* ANALYST, 1938, **63**, 669), is based on the formation of a red colour when certain pyridine derivatives are fused with 2:4-dinitrochlorobenzene. The solution, containing 0·1 to 0·5 mg. of nicotinic acid or nicotinamide, is evaporated just to dryness at 80° to 100° C., and 1 ml. of a 1 per cent. solution of 2:4-dinitrochlorobenzene in 95 per cent. alcohol is added to the dry residue. An excess of the reagent should be avoided. After being allowed to stand for 1 hour, the mixture is evaporated to dryness at 100° to 105° C. and heated for 10 minutes between these temperature limits to bring about fusion. The fused mass is allowed to cool, dissolved in 10 ml. of cold 0·1 per cent. alcoholic sodium hydroxide solution and filtered at once. The solution is made up to 15 ml. with the alcoholic sodium hydroxide solution, and the colour is measured in a suitable instrument. The authors used a photelometer and constructed calibration curves from the results given by known amounts of nicotinic acid and nicotinamide. The two curves were different. In estimating the nicotinic acid or nicotinamide-content of urine, 15 ml. of the sample are decolorised by boiling with 0·1 to 0·3 g. of vegetable carbon (Darco). Three ml. of the filtered solution are evaporated just to dryness, and 1 ml. of alcoholic 0·1 per cent. 2:4-dinitrochlorobenzene is added. The ensuing procedure is as described above. Known amounts of nicotinic acid and nicotinamide were added to urine, which was treated according to this procedure. Results within 10 per cent. of the theoretical were obtained. The urine of people who have ingested nicotinic acid or nicotinamide may contain either or both of these substances together with trigonellin and nicotinic acid—glycine conjugate. Trigonellin gives no colour with dinitrochlorobenzene. Nicotinic acid gives a purple colour, and the amide, and probably also the glycine conjugate, a red. It has been found that the colour obtained after ingestion of not more than 100 mg. of nicotinic acid is red, but with larger doses (100 to 500 mg.) a purple colour is obtained. Normally, therefore, the calibration curve for nicotinamide is used and the results are recorded in terms of nicotinamide. Pellagrins in relapse and

normal persons who had been fed for some time on a pellagra-producing diet did not excrete nicotinic acid or amide. Persons on a "normal" diet excreted 20 to 50 mg. of the acid daily. F. A. R.

**Quantitative Determination of Nicotinic Acid Amide in Animal Tissue. P. Karrer and H. Keller.** (*Helv. Chim. Acta*, 1938, **21**, 1170–1171.)—The method of estimating nicotinamide by measuring the intensity of the colour produced by treating with 2:4-dinitro-1-chlorobenzene (*cf.* ANALYST, 1938, **63**, 669) has now been applied to animal tissue. The finely-minced material is digested with twice its volume of water for 1 hour and filtered, and the extraction is repeated twice more. The combined filtrates are concentrated to 300 ml., and the concentrate is heated on the water-bath for 1 to 1½ hours with 30 to 40 ml. of *N* potassium hydroxide solution. The solution is then neutralised with acetic acid and evaporated to dryness, and the residue is dehydrated in a vacuum desiccator with phosphorus pentoxide. The dry powder is refluxed three or four times with benzene for 2-hour periods. The combined benzene extracts are distilled, and the residue is fused with four times its weight of dinitrochlorobenzene. The melt is dissolved in ether, and the ethereal solution is exhaustively extracted with water. The colour formed on the addition of 1 to 2 drops of 20 per cent. potassium hydroxide solution is measured in a Leifostufenphotometer, and the quantity of nicotinamide present is calculated from a calibration curve. The nicotinamide contents expressed as mg. per kg. of material of various tissues were found to be:—horse muscle tissue, 0·225; ox muscle tissue, 0·212; sheep muscle tissue, 0·193; pig muscle tissue, 0·206; codfish muscle tissue, 0·175; horse liver, 1·05 to 1·45; horse kidney, 1·98 to 2·10. F. A. R.

**Constitution and Estimation of α-Tocopherol and Similar Compounds. P. Karrer, R. Escher, H. Fritzsche, H. Keller, B. H. Ringier, and H. Salomon.** (*Helv. Chim. Acta*, 1938, **21**, 939–953.)—In order to decide whether α-tocopherol was derived from coumaran or from chroman, a simpler substance, namely the product obtained by condensing crotyl bromide with trimethyl hydroquinone, was used as a model. This substance was rapidly and quantitatively oxidised by ferric chloride in alcoholic solution, and titration with ferric chloride solution, either by using starch-iodide paper as indicator or potentiometrically, indicated that 1 mol. of the substance was equivalent to 2 mol. of ferric chloride. The properties of the oxidation product indicated that the parent substance possessed the chroman structure. α-Tocopherol was found to behave in exactly the same way, so that it, too, must be a chroman derivative and is therefore represented by the formula:—



The potentiometric titration of α-tocopherol can be carried out not only with ferric chloride solution, but with alcoholic silver nitrate solution, ammoniacal silver nitrate solution, and best of all with auric chloride solution. This last-mentioned method is proposed for the determination of α-tocopherol. F. A. R.

**Quantitative Determination of the Tocopherols in Different Raw Materials. P. Karrer and H. Keller.** (*Helv. Chim. Acta*, 1938, **21**, 1161–1169.)—The determination of α-tocopherol and also of β-tocopherol can be carried out by potentiometric titration with gold chloride solution (*cf.* preceding abstract). About 10 mg. of the tocopherol are dissolved in 250 to 400 ml. of 80 per cent. ethyl alcohol at 50° C. An aqueous solution of gold chloride (approximately 0·015 *N*) is added in small amounts at a time to the solution, the potential of which is measured after each addition with the aid of a platinum electrode and a normal calomel electrode. Since the redox-potential only slowly adjusts itself, the titration may occupy up to 3 hours. The end-point of the titration is indicated by a sudden rise in the potential. One ml. of 0·01 *N* gold chloride solution was equivalent to 2·16 mg. of either α- or β-tocopherol; that is 1 mol. of tocopherol required two equivalents of gold chloride. The tocopherol contents of wheat germ, maize germ and lettuce, were determined by extracting the material with benzene, saponifying the extract, extracting the unsaponifiable matter from the soap solution, and titrating a weighed amount of this unsaponifiable matter as described for tocopherol itself. Similarly the unsaponifiable matter of linseed oil, olive oil, sesame oil and refined coconut oil was titrated. The following results expressed as per cent. of α + β-tocopherol were obtained:—Unsaponifiable matter of wheat germ oil, 13·4; wheat germ oil, 0·52; wheat germ, 0·03; unsaponifiable matter of maize germ oil, 10·2; maize germ, 0·16; unsaponifiable matter of lettuce oil, 4·3; lettuce (dried), 0·055; unsaponifiable matter of linseed oil, 2·34; linseed oil, 0·023; unsaponifiable matter of olive oil, 0·935; olive oil, 0·008; unsaponifiable matter of sesame oil, 0·63; sesame oil, 0·005; unsaponifiable matter of coconut oil, 0·55; coconut oil, 0·0027.

The tocopherols are not oxidised by gold chloride solution unless present in the unesterified state. It should also be noted that since α-tocopherol possesses two or three times the vitamin E activity of β-tocopherol, the potentiometric titration gives only a rough indication of the biological activity. Carotene and other carotenoids reduce gold chloride and so interfere with the titration. One ml. of 0·01 *N* gold chloride solution was equivalent to 0·7 mg. of β-carotene; that is, 1 mol. of β-carotene required 8 equivalents of gold chloride. The carotene contents of the materials assayed above were so small that the error introduced by its presence could be neglected. Glutathione, ascorbic acid, dihydrocodehydrase and similar substances occurring in living tissue also reduce gold chloride, but they are soluble in water and insoluble in benzene, petroleum spirit or ether, and so are readily removed. F. A. R.

**Vitamin $A_1$ and $A_2$ Contents of Mammalian and other Animal Livers. A. E. Gillam.** (*Biochem. J.*, 1938, **32**, 1496–1500.)—The amounts of vitamins $A_1$ and $A_2$ in the livers of a number of animals and birds have been determined spectrophotometrically. The livers of the lion, gibbon, alligator, rabbit, rat, mouse, guinea-pig, dog, cat, ox, cow, lamb, pig, frog, white whale, hen, duck, turkey, pigeon and kingfisher, contained vitamin $A_1$ (1 to 32 mg. per 100 g. of liver) but no vitamin $A_2$. The liver of a very old python contained 86 mg. of vitamin $A_1$ per 100 g. but no vitamin $A_2$. The liver of a giant monitor (*Varanus salvator*)

contained 250 mg. of vitamin $A_1$ per 100 g. and traces of vitamin $A_2$. Appreciable amounts of vitamin $A_2$ were found only in the livers of animals that habitually feed on fish, namely, in the seal and in the otter; and in that of a rat that had been given a concentrate of freshwater fish liver. The otter's liver contained 47 mg. of vitamin $A_1$ per 100 g. It is concluded that mammals and birds cannot metabolise vitamin $A_2$, but can store this vitamin in the liver when it is ingested in the food. F. A. R.

**Formaldehyde Azo Reaction for Vitamin $B_1$. H. W. Kinnersley and R. A. Peters.** (*Biochem. J.*, 1938, **32**, 1516–1520.)—The original formaldehyde azo test (*cf.* ANALYST, 1934, **59**, 563) has been modified as follows. A mixture of 1·25 ml. of the special reagent and 0·5 ml. of diazotised sulphanilic acid is added to 0·1 to 0·3 ml. of a solution containing 10 to 20$\gamma$ of vitamin $B_1$, having a $p$H of 6. After standing for 2 hours, the solution is extracted twice with 2 ml. of butyl alcohol, and the combined butyl alcohol extracts are extracted with 2 ml. of 0·005 $N$ hydrochloric acid followed by two extractions with 1 ml. An equal volume of ethyl alcohol is added, and the resulting pink solution is compared with standards of vitamin $B_1$ similarly treated. The accuracy of the method is $\pm 5$ per cent. for amounts of aneurin hydrochloride of approximately 20$\gamma$. Reducing agents, such as cysteine or sodium hydrosulphite, delayed the appearance of the colour, and traces of copper, mercury and silver interfered, but apparently not other metals.

To estimate the amount of vitamin $B_1$ in foodstuffs, a convenient amount of the finely-ground material is treated with 1 litre of boiling water, and the $p$H is adjusted to 3·5. The mixture is cooled and filtered, and the filtrate is concentrated to 150 ml. The precipitate that forms is centrifuged, and a slight excess of 10 per cent. sodium phosphotungstate solution at $p$H 6 is added. The solution is then acidified to $p$H 1 with 20 per cent. sulphuric acid and after standing for 12 hours is centrifuged. The precipitate is collected and ground with baryta three times, and the excess of baryta is removed with sulphuric acid. The solution is concentrated to a suitable volume and a portion of it is taken for test as described above. The results obtained with a variety of foodstuffs by this method are compared in the following table with the results obtained by the pigeon test:

| Food material | Moisture content Per Cent. | Vitamin $B_1$ ($\gamma$) estimated by | |
|---|---|---|---|
| | | Colour test $\gamma$ | Bird test $\gamma$ |
| Yeast concentrate .. .. | — | 120 | 145 |
| Wheat germ .. .. | 7 | 20 | 13·5 |
| Peas .. .. .. | 11·65 | 3·5 | 2·9 |
| Breakfast oats .. .. | 10·0 | 3·0 | 2·5 |
| Haricot beans .. .. | 12·7 | 2·2 | 2·15 |
| Oatmeal .. .. .. | 10·3 | 2·0 | 1·9 |
| Maize .. .. .. | 9·7 | 2·0 | 1·5 |
| Lentils .. .. .. | 12·7 | 1·75 | 1·6 |
| Barley .. .. .. | 12·0 | 1·2 | 0·7 |

By the use of taka-phosphatase (taka-diastase contains 0·5 per cent.) at approximately $p$H 4, over 90 per cent. of the phosphoric esters of vitamin $B_1$

(co-carboxylase) were converted into the free vitamin. Application of this treatment to yeast showed that this contained combined vitamin $B_1$, but no evidence was found for the presence of combined vitamin $B_1$ in the foodstuffs tested.

F. A. R.

**Extraction and Determination of Vitamin C in Plant Tissue. N. C. Thornton.** (*Contrib. Boyce Thompson Inst.*, 1938, **9**, 273–281.)—A rapid procedure for estimating the amount of vitamin C in bananas was required. Bananas contain enzymes that inactivate vitamin C even when 8 or 18 per cent. acetic acid or 8 per cent. trichloroacetic acid are used for the extraction. The technique adopted to overcome this difficulty was to transfer about 20 g. of the sample to a porcelain mortar containing 50 ml. of a mixture of equal parts of 1 *N*, 2 *N* or 3 *N* sulphuric acid and 0·25 *N* metaphosphoric acid, and grind the tissue with a porcelain pestle by the aid of 3 to 8 g. of acid-washed quartz sand to an extremely fine consistence. The mixture was transferred to a 200-ml. volumetric flask and made up to volume with the acid mixture. After being thoroughly mixed, the suspension was centrifuged, and aliquot portions (10 ml.) of the clear solution were used for titration with 2:6-dichlorophenolindophenol. The indicator was standardised against a solution of ascorbic acid in the particular acid mixture used. In titrating the banana extract, the end-point is reached at the point where the pink colour of the extract persists for approximately 10 seconds with constant agitation of the solution. It was shown that reduction with hydrogen sulphide was unnecessary, for apparently the strong acid used completely inhibited the oxidation of the vitamin. It was very necessary, however, to add the acid before grinding, for when the acid was added 1 minute after grinding, as much as 50 per cent. of the vitamin was destroyed. The vitamin C content of bananas was found to be 15·0 to 16·6 and 11·5 to 13·9 mg. per 100 g. of fresh tissue for green and yellow fruit, respectively.

F. A. R.

**Photochemical Decomposition of *l*-Ascorbic Acid. A. E. Kellie and S. S. Zilva.** (*Biochem. J.*, 1938, **32**, 1561–1565.)—*l*-Ascorbic acid suffered photochemical decomposition when a solution at *p*H 7 was exposed to ultra-violet light in the absence of oxygen. In the presence of oxygen *l*-ascorbic acid was oxidised, the rate of oxidation being considerably increased by the ultra-violet light; dehydroascorbic acid was formed as an intermediate product of the oxidation. Lactoflavin was without effect on the anaerobic photochemical decomposition, but it increased the rate of oxidation when oxygen was present. Both lactoflavin and methylene blue brought about photochemical decomposition of *l*-ascorbic acid by visible light in the presence of air, a change which did not occur in the absence of these sensitisers. A solution of *l*-ascorbic acid of *p*H 3 was more stable to ultra-violet light than a solution of *p*H 7, whether oxygen was present or not.

F. A. R.

**Responses of *Daphnia magna* to Vitamin E. A. Viehoever and I. Cohen.** (*Amer. J. Pharm.*, 1938, **110**, 297–315.)—The transparent crustacean, *Daphnia magna*, is a suitable subject for fertility tests on account of the ease with which the growth of the ovaries and of the parthenogenic embryos in the brood sac can

be observed. The organisms were normally propagated on cultures made from Bovung (dried, shredded cow manure), Wizard sheep manure (dried, shredded) and Cellu soya bean flour with added urea. Starvation of the experimental animals, resulting from an excessive number of young in a limited volume of Wizard culture medium retarded or destroyed the reproductive cycle; on the addition of a few drops of wheat germ concentrate, however, the ovaries developed normally. In stagnant Bovung cultures, where the supplies of nutritive material were almost exhausted, actual resorption of the oocytes was observed to take place. The addition of wheat germ concentrate to such a culture was followed by the development of embryos. *Daphniae* were also grown on a supposed vitamin E-free medium composed of corn starch, casein, ether-extracted yeast, cod-liver oil, olive oil and salt mixture; growth was slow and only one animal out of several dozens released an embryo into the brood sac. The organisms were also grown on Bovung and Wizard media that had been extracted with petroleum spirit. Whilst the former allowed of full growth and reproduction, the latter resulted in impaired growth and fertility. When the sexually-retarded animals were transferred to a culture of petroleum spirit-extracted Wizard sheep manure to which had been added a few drops of wheat germ concentrate, the animals grew rapidly and developed embryos freely; controls without vitamin E produced very few embryos. The addition of vitamin E to a presumed adequate culture medium, as, for example, Bovung, increased the number of young produced. These results show that the reproduction of *Daphnia magna* is dependent upon the presence of vitamin E in the culture medium, and suggest the possible use of *Daphnia magna* as a test animal for the quantitative estimation of the vitamin. The effect of other vitamins on the organisms is being examined. F. A. R.

# Bacteriological

**Bacteriological Examination of Harz Cheese. F. W. Gutsche.** (*Z. Unters. Lebensm.*, **1938**, **76**, 209–225.)—A number of samples of Harz cheese which had been stored under varying hygienic conditions were examined microscopically to ascertain whether any relation exists between the bacterial content and the conditions of storage. It was found that unhygienic storage was not associated with an increase of the bacterial content, and in some instances high bacterial counts were given by cheese of excellent quality which had been adequately stored. Apparently there is no limiting value for the number of bacteria present that will indicate the conditions of storage or the dietary value of the cheese. From a study of the bacterial content of cheese during the process of ripening, it is concluded that the excretion products of the bacteria play some part in the ripening process. Temperature was found to have a profound influence on the rate of ripening. Cheese kept at 7° C. remained fresh for 14 days, showing no change in firmness, appearance or taste. At room temperature ripening proceeded more rapidly, and in 8 to 10 days the cheese had lost its firmness and its taste had become sharper and more ammoniacal. Cheese kept in an incubator at 37° C. lost its firmness in 4 hours. After 10 hours it had a pasty, dark, and unappetising appearance and a highly disagreeable taste and odour. Similar tests with cheese

wrapped in cellophane showed that the wrapping hinders the ripening process by restricting the surface increase of bacteria and, possibly, by the exclusion of air. Determination of the number of bacteria present in ripened and unripened cheese led to the conclusion that during ripening the number of short rods and micrococci diminished, the number of long rods increased, and that of yeasts and moulds diminished. In the interior of the cheese the average bacterial count had diminished. It was observed that *Streptococcus lactis* tends to form pure colonies, and that in the neighbourhood of these colonies the bright colour of the cheese indicated rapid ripening. Cheese kept under a bell-jar for 3 weeks at room temperature showed a diminution of the bacterial count to approximately one-half. Possibly, with this method of storage, saturation with the excretal products produces a condition unfavourable to multiplication. Inoculation of fresh Harz cheese with *B. proteus* and *B. coli* showed that, whilst both organisms increase the rate of ripening, *B. proteus* has the greater influence, probably because of its greater power of decomposing proteins. Such inoculated cheese was toxic for mice. Among mice fed with cheese inoculated with *B. proteus* there was a mortality of 50 per cent., and among mice fed with cheese inoculated with *B. coli* the mortality was 30 per cent. Details are given of an embedding process for the preparation of cheese for microscopical examination. A. O. J.

**Diphtheria Outbreak Associated with Ice-Cream. E. Bloch.** (*Lancet*, 1938, I, 837–8.)—The outbreak occurred in Glasgow in the latter half of August, 1937. It involved 13 persons, including one secondary case, 6 of whom died; 9 were infected in one day and the majority sickened two days after consuming the ice-cream. The source of the outbreak was traced to one of thirteen ice-cream shops in the district. Three persons found in the shop in the course of the investigation were swabbed and one, a daughter of the shop-keeper, aged 12, was found to be carrying virulent diphtheria bacilli in her throat and nose. Later the shop-keeper himself was found to be a virulent nasal carrier, and of his family, whose home was away from the shop, another daughter (aged 2), a son aged 5, and the mother were all found to have diphtheria bacilli in their throats or noses, the virulence of which was proved in the case of the mother. It subsequently transpired that both children had been sent to fever hospitals in February and July, 1937, respectively, for treatment for diphtheria, but this information was at first withheld. The shop assistant, aged 19, also showed diphtheria bacilli in her throat, the virulence of which was not tested. The ice-cream was of the custard-ice type and made by the hot-mix method. The shop sold 8 gallons per week-day and 12 gallons on Sunday, mostly to children. It was estimated that about 70 persons would partake of 1 gallon of ice-cream. During the week the proprietor would make "mix" and freeze it daily, but towards the end of the week he would make a large quantity and store it for the week-end in uncovered pails on open shelves in the scullery at the rear of the shop. In several instances the organism found was typed and found to belong to type IV. D. R. W.

**Action of Sodium Polyanethole Sulphonate ("Liquoid") on Blood Cultures. T. von Haebler and A. A. Miles.** (*J. Path. and Bact.*, 1938, **46**, 245–252.)—The anti-coagulant "Liquoid," manufactured by Hoffman-La Roche, is

a synthetic polymer of sodium anethole sulphonate, and has been used on the Continent for the past five years as an adjuvant to blood culture. It is reported to be a stable compound, unchanged by heating or by the action of dilute acids and alkalis; it is at least as effective as hirudin as an anti-coagulant, and is anti-complementary and destructive of the bactericidal power of human, rabbit or guinea-pig blood. By its action fresh blood is converted into a favourable culture medium for a large number of micro-organisms. "Liquoid" may be added directly to withdrawn blood or incorporated into liquid culture media in concentrations of 0·1 to 0·2 per cent. This paper reports an investigation of the action of "Liquoid" in modifying the capacity of defibrinated and also whole normal human blood to support the growth of pathogenic bacteria. The investigation showed that "Liquoid" and trypsinised broth were equally good in inhibiting the bactericidal action of blood with defibrinated blood; that "Liquoid" can be used successfully with whole blood, a rather larger percentage being required to maintain the uncoagulated state; that it is unimpaired by autoclaving at 115° C. for 30 minutes or by storing at 2° C. for two months. The concentration recommended is 0·03 to 0·05 per cent. In this strength it is effectively anti-coagulant and destroys the bactericidal property of normal human blood and provides a suitable medium for the cultivation of a large number of pathogenic micro-organisms including *B. influenzae*, *Br. melitensis*, the *Meningococcus*, the *Gonococcus*, *Streptococcus pyogenes*, *Streptococcus viridans*, the *Pneumococcus*, *B. Friedländer* and *B. typhosus*. D. R. W.

**Researches on the Somatic Antigens of the Typhoid Bacillus. The Chemical Nature of the "O" and "Vi" Antigens. A. Boivin and Mesrobeanu.** (*C. R. Soc. Biol.*, 1938, **128**, 5–8; *Bull. Hyg.*, 1938, **13**, 748–9.)—By the extraction of "O" and "Vi" strains of *B. typhosum* with trichloroacetic acid a gluco-lipoid is obtained as a white powder forming an opalescent colloidal solution in water. The "O" fraction is precipitated by an "O" antiserum and produces "O" agglutinins in rabbits; the "Vi" fraction is precipitated by "Vi" antiserum and produces "Vi" agglutinins. By heating the "O" fraction with strong hydrochloric acid 46·1 per cent. of reducing sugar and 27·2 per cent. of fatty acids are produced; the "Vi" antigen yields 21 per cent. of reducing sugar and 26·5 per cent. of fatty acids by the same treatment. Heating with weak acetic acid splits the "O" fraction into insoluble lipoids and the soluble polysaccharide haptane, but the "Vi" antigen is unchanged by this treatment even after 12 hours at 100° C. A solution of the "O" fraction gives no precipitate with alum, uranium or lanthanum salts and only does so with large quantities of phosphotungstic acid in the presence of a large quantity of sulphuric acid. The "Vi" fraction is precipitated by all these reagents and a method of separating the "Vi" fraction has thus been evolved. The trichloroacetic acid extract is purified by dialysis, uranyl acetate is added, and the precipitate separated by centrifuging. The supernatant liquid, which is freed from uranium salts by dialysis, contains the "O" antigen; the precipitate containing the "Vi" antigen is dissolved in citric acid solution and purified by dialysis. D. R. W.

# Agricultural

**Preservation of Cyanogenetic Plants for Chemical Analysis. R. R. Briese and J. F. Couch.** (*J. Agric. Res.*, 1938, **57**, 81–107.)—Owing to the rapidity with which the hydrocyanic acid content changes after plants are gathered, resulting in a variable loss, an efficient preservative of the hydrocyanic acid in the plants is essential if any length of time has to pass before analysis. Hydrocyanic acid is formed in plants from the interaction of an enzyme, emulsin, and a glucoside containing a CN group capable of hydrolysis to hydrocyanic acid, and the rate of formation once it begins is rapid at first, diminishing after some hours (8–12), and going on slowly for an indeterminate time. The plants used in the investigation, generally both fresh and dry, included some 12 varieties of sorghum, wild cherry (*Prunus serotina* and *P. melanocarpa*); arrowgrass and Sudan grass. It was found that the fresh plants stored at ordinary temperature without preservatives lost 13 to 83 per cent. of their hydrocyanic acid in 1 to 6 days. At refrigerator temperature for 1 to 5 days, fresh spur feterita* yielded as much hydrocyanic acid as before storage, but this was one-third to one-half less than was obtained from 24 hour maceration of non-refrigerated plants. With fresh plants in water to which chloroform was added, with or without alcoholic potassium hydroxide, in all but 2 samples losses occurred up to 62 per cent. Chloroform was effective as a preservative for 4 days for one sample of hegari.* With acid solutions rapid losses occurred (least with salicylic acid); with alkaline solutions losses of from 32 to 96 per cent. were recorded; organic bases gave better results. Alcohol in concentrations of 10, 15, 20 and 25 per cent. preserved for 3 to 7 days with losses up to 8 per cent., but after a week losses were greater, until after 7 to 8 weeks 20 per cent. of the hydrocyanic acid had been lost. In concentrations of 50 and 95 per cent. alcohol inhibited cyanogenesis. An effective preservative was found in mercuric chloride in the proportion of 1 per cent. by weight for fresh plants, and when stored in this solution there was no loss of hydrocyanic acid after 6 months. For dried plants a 2 per cent. solution was a reasonably good preservative for 3 months. The above concentrations of mercuric chloride retarded but did not stop enzymic action in cyanogenetic plants, and buffering did not counteract this effect; the addition of chloroform did not accelerate enzymolysis. The addition of enzyme, however, greatly accelerated the rate of formation of hydrocyanic acid in the presence of mercuric chloride. The optimum temperature for storage of samples preserved with mercuric chloride (of 9° C., 25° C. and 37° C.) appeared to be room temperature (25° C.). The proportion of hydrocyanic acid in plants, determined after maceration with water or dilute alcohol, depends on the balance between cyanogenesis and conversion of the free hydrocyanic acid into other compounds, so that the figure obtained may be considerably below the true figure. The recovery of hydrocyanic acid from samples preserved with mercuric chloride depends on the liberation of hydrocyanic acid from mercuric cyanide with stannous chloride (only in one test potassium iodide was more satisfactory). Mercurous iodide appears to be volatile in steam and it deposits in the condensers where it reacts with water to form mercuric iodide and metallic mercury, and the mercuric iodide may render distillates turbid and interfere with the end-point in titration.

* Varieties of sorghum.

D. G. H.

# Water

**Determination of Manganese in Water containing Chlorides and Silicates. V. Mühlenbach.** (*Z. Unters. Lebensm.*, 1938, **76**, 254–258.)—The application of Marshall's method (Abst., ANALYST, 1901, **26**, 195) to the determination of manganese in saline water derived from Devonian strata with intermediary clay and marl beds in Latvia has been investigated. The water had a high chloride-content (>3·3 g. Cl per litre) and, owing to the presence of much calcium (>0·4 g. per litre), in the residue left after evaporation, it was found preferable to remove the chloride by heating with nitric acid, thus preventing the formation of the sparingly soluble calcium sulphate which results from the customary method of treatment with sulphuric acid. The greater part of the silica (27 mg. per litre) was removed simultaneously, and this method was preferable to treatment of the dry solid matter with hydrofluoric acid. The determination was made colorimetrically in a Proskauer colorimeter. A slight modification of the method of Hartwig and Schellbach (*Z. Unters. Nahr. Genussm.*, 1913, **26**, 439; Abst., ANALYST, 1914, **39**, 54) is recommended for the preparation of the standard comparison solutions. To 2 litres of water are added 40 g. of ammonium persulphate, 12 ml. of dilute nitric acid and 12 ml. of 1 per cent. silver nitrate solution, and the solution is boiled for 20 minutes. This solution is used to dilute a stock solution of 2·8769 g. of potassium permanganate in 1 litre of water, 10 ml. being diluted to 100 ml. and 10 ml. of this being diluted, in turn, to 100 ml. to produce a solution containing 0·01 mg. of manganese per ml. The procedure is as follows:—The water (100 ml. to 2 litres according to the amount of manganese present) is acidified with 3 ml. of nitric acid and evaporated to dryness on the water-bath in a glazed porcelain vessel. The solid residue is heated with 10 ml. of conc. nitric acid until all the acid is driven off, and this treatment is repeated twice if the chloride-content is high. The residue is treated with a little water and nitric acid and transferred by means of 75 to 80 ml. of water acidified with 3 ml. of nitric acid to a filter crucible attached to a water-pump. The residue in the crucible is washed with 10 ml. of water, and the combined filtrate and washings are mixed with 0·5 ml. of silver nitrate solution (2 g. of Ag per litre) and boiled, after which 10 ml. of 10 per cent. ammonium persulphate solution are added, and boiling is continued for 3 minutes. The solution is rapidly cooled and washed into the colorimeter tube with ammonium persulphate solution, which is also used for diluting to the mark. The comparison solution, prepared in the manner described, is introduced into the comparison tube from a narrow burette in amounts increasing from 0·5 to 10 ml., ammonium persulphate solution again being used to dilute the solution to the mark. With solutions of known manganese-content this method was found to be sufficiently accurate even when the high manganese-content necessitated much dilution. A. O. J.

# Organic

**Determination of Water in Alcohol. H. G. Botset.** (*Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 517–518.)—Methods are known in which the water-content of alcohol is determined in terms of the temperature at which cloudiness (due to

phase separation) of a mixture of the sample and a suitable organic solvent first occurs (*cf.* Rising and Hicks, ANALYST, 1926, **51**, 472). The present method is analogous to these; thus, carbon tetrachloride is miscible with ethyl alcohol but not with water, so that if a mixture of 10 ml. of absolute alcohol and 10 ml. of carbon tetrachloride is titrated with water, a certain quantity (actually, 2·03 ml. at 25° C.) must be added before cloudiness appears. Then the difference between this quantity and the quantity of water required by a mixture of 10 ml. each of carbon tetrachloride and the sample to be tested, corresponds with the amount of water already present in the alcohol. Actually, the true figure is slightly less than this difference value, because 10 ml. of the sample will contain less than 10 ml. of alcohol. Empirical calibration curves were therefore established by titrating 10-ml. portions of alcohol solutions containing known quantities of water as described above, at 20°, 25° and 30° C., and these are reproduced in the original paper. The relationship is linear for the range covered (*i.e.* 0 to 15 ml. of water in 10 ml. of alcohol solution), and the method is accurate to within ±2 per cent. if a 10-ml. burette graduated in 0·05 ml. is used for the titration. J. G.

**Determination of Ethylene Dibromide. M. W. Brenner and G. L. Poland.** (*Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 528–529.)—The sample is added to a mixture of 10 ml. of a 20 to 30 per cent. solution of potassium iodide and 50 ml. of alcohol in a 250-ml. flask, which is then fitted to a reflux condenser by means of a ground-glass joint, and the contents are boiled gently for 3 hours. When the mixture has cooled, the condenser-tube is rinsed with several 10-ml. portions of water, and sufficient water is added to bring the total volume to approx. 200 ml. (to minimise the effect of the alcohol on the end-point); the iodine liberated is then titrated with 0·01 or 0·1 *N* sodium thiosulphate solution, with starch as indicator. The function of the alcohol is to minimise losses of iodine and dibromide during the boiling operation. The method is intended particularly for the determination of the amount of ethylene evolved by certain plant tissues during ripening, this gas being first converted into the dibromide, and tests on standard solutions of purified ethylene dibromide in 95 per cent. alcohol showed that the values obtained varied from 65·8 to 96·9 per cent. of the amount taken, for 1·000 to 136·95 mg. of sample. The most accurate results were obtained with the greatest quantities of ethylene dibromide; thus, the figures exceeded 94 per cent. of the theoretical values for 27·39 mg. or more of sample. A curve relating the logarithms of the numbers of mg. of ethylene dibromide present and found by the method is given, and enables a correction to be applied when small quantities are involved. It is also desirable to make a blank experiment on the reagents, although in the present instance there was no evidence of an error from this source. Other substances (*e.g.* organic iodides, α, β-dibromides, and certain oxidising and reducing agents) interfere, but are not likely to be present in the gases evolved by plant tissues.
J. G.

**Sandal Seed Oil (*Santalum album* Linn.). M. K. Madhuranath and B. L. Manjunath.** (*J. Indian Chem. Soc.*, 1938, **15**, 389–392; *cf.* Rao, *Quarterly J. Mysore Forest Dept.*, 1934, 7; Iyer, ANALYST, 1935, **60**, 319; Sreenivasaya and Narayana, *J. Indian Inst. Sci.*, 1936, 19A, 1.)—Sandal oil was obtained by (*a*) ex-

traction and (*b*) expression of seed yielding to petroleum spirit 44 per cent. of oil. The extracted oil was golden yellow and the expressed oil dark brown in colour, and the two oils had the following characteristics:—Sp.gr. (*a*) at 25/25° C., 0·9356, (*b*) at 27/27° C., 0·9346; $n_D^{30}$, (*a*) 1·4891, (*b*) 1·4884; saponification value, (*a*) 176, (*b*) 176; iodine value, Hanus, (*a*) 153, (*b*) 153; Reichert–Meissl value, (*a*) 0·9, (*b*) 0·8; thiocyanogen value, (*a*) 151; acetyl value, (*a*) 22, (*b*) 25; acid value, (*a*) 29, (*b*) 44; unsaponifiable matter, (*a*) 8·8, (*b*) 13·0 per cent.; viscosity, (*a*) 243 at 50/50° C. The maximum absorption of oxygen by the extracted oil was 13 per cent., and the oil on drying was converted into an elastic film. The total fatty acids (87 per cent.) consisted of 51 per cent. of solid acids and 49 per cent. of liquid acids, having respectively mean molecular weights of 288·6 and 301·6, and iodine values (Hanus) of 119 and 108. The methyl esters of the liquid acids were oxidised and brominated and found to consist mainly of oleic acids with a small amount of linolenic acid. The acids obtained from the insoluble lead salts in the Twitchell separation were esterified with methyl alcohol and the esters were separated by distillation at 1 mm. pressure into 5 fractions and a residue. The first fraction yielded a small amount of pure palmitic acid. The other fractions were refractionated at a pressure of 0·6 mm., and most of the material distilled over between 172 and 175° C. No pure substance could be isolated from the highly resinous residue. The mean molecular weights and iodine values of the fractions indicated that the solid acids consist of one component with traces of palmitic acid. Analysis showed the acid to be a triolefinic compound of the formula $C_{18}H_{30}O_2$. Quantitative catalytic hydrogenation resulted in the production of stearic acid. The new pure acid had a thiocyanogen value of 130 and a maleic anhydride number of 8·4 (ANALYST, 1936, **61**, 812). No crystallisable material was given on bromination or with maleic anhydride. The acid differed from the puricic acid of Toyama and Tsuchiya (ANALYST, 1935, **60**, 570), since it could not be polymerised to elaeostearic acid; it has been given the name "santalbic acid." D. G. H.

**Determination of Age of Inks by the Chloride Method. J. Finn, Jr., and R. E. Cornish.** (*Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 524–525.)—According to Turkel's method (*cf.* Mezger, Rall and Hess, *Arch. Kriminol.*, **1933**, **92**, 108) the extent of the migration of the chloride ions in ink writing into the surrounding paper is a measure of the age of the writing (see also Heess, ANALYST, 1935, **60**, 338). The present authors, however, had reason to doubt whether the validity of the test might not be upset by the fact that inks of different compositions might behave differently in this respect, and the experiments now described confirm this suspicion. Comparative tests on a number of commercial inks were therefore made by the authors' method (*cf.* Cornish, Finn and McLaughlin, *Ind. Eng. Chem., News Ed.*, 1934, **12**, 315), in which the document is immersed in a 2 per cent. solution of silver nitrate, washed 3 times with distilled water, and finally developed with a photographic developer ("D72"); this destroys the ink, but, unlike the permanganate solution usually recommended, does not blacken the paper. Since different inks were found to have different migration rates, the experiments were continued with batches of the ink having the slowest migration rate of those examined, to which were added various inorganic chemicals. Tests made approx.

1 and 2·5 months after the date of writing showed that salts containing chloride ions migrate progressively from ink without evaporation at a rate which is greatly increased by the addition of sodium chloride (*e.g.* 0·5 per cent.), and to a less extent by hydrochloric acid (*e.g.* 1 per cent. of 12 *N* acid); with the acid, however, the image fades rapidly, and this is attributed to evaporation. Calcium, zinc, sodium and cupric chlorides produced the same rate of chloride migration, indicating that the deliquescence of the added salt is not an important factor, in spite of the fact that it has been shown that paper stored in a warm place shows no detectable chloride migration even after 6 months. Stannous chloride, however, reduced the rate of migration to considerably less than that of the untreated inks and, in fact, inhibited it almost completely. This is attributed to the formation of a basic stannic chloride which binds the chloride so firmly as to prevent its diffusion, although it allows a silver image to be developed. Alternatively, the tin remains in the paper as non-diffusible stannous compounds, which reduce silver nitrate directly and so give a spurious chloride image. It is suggested that if a known amount of sodium chloride could be added to inks used by banks and federal offices, this would not only serve as an aid in the identification of the ink, but would also facilitate the estimation of the age of writing made with it. J. G.

**Chemistry of Lignin. XI. Lignin from Wheat Straw. M. Phillips and M. J. Goss.** (*J. Biol. Chem.*, **125**, 241–246.)—Wheat straw was extracted with alcohol-benzene and then repeatedly with 2 per cent. alcoholic sodium hydroxide solution at room temperature. The lignin fraction so obtained was purified by dissolving it in a mixture of acetone (2 vols.) and alcohol (1 vol.), filtering off the insoluble matter and evaporating the filtrate to dryness. Analysis of the powder so obtained, which constituted 5 per cent. of the original material, gave data that agreed with the formula $C_{38}H_{31}O_7\begin{cases}(OCH_3)_4\\(OH)_5\end{cases}$. Of the five hydroxyl groups, three could be methylated with diazomethane; these may therefore be phenolic or enolic in character. When fused with potassium hydroxide, this lignin fraction gave protocatechuic acid. The straw remaining from the first extraction was extracted under reflux with 4 per cent. aqueous sodium hydroxide solution. The lignin fraction so obtained constituted 1 per cent. of the straw and had a composition represented by the formula $C_{40}H_{42}O_{16}$. Four methoxyl and four hydroxyl groups were present. A third lignin fraction was obtained from the residual straw by treatment with cold fuming hydrochloric acid. It formed 2·5 per cent. of the straw and had a higher carbon-content than the other two lignin fractions. F. A. R.

**Solubility of Casein in Salts of Certain Organic Acids and its Fractionation by means of these Acids. R. H. McKee and S. P. Gould.** (*J. Agric. Res.*, 1938, **57**, 125–143.)—To determine the solubility of casein in the solutions of salts of organic acids the salt was dissolved in water, enough of the acid or alkali corresponding to the salt used was added to bring the *p*H to 4·6, and water was added to bring the mixture to 55 ml. Solubility was determined on the

empirical basis of 5 g. of casein to 50 ml. of solution. Stoppered flasks were placed in incubators at the various temperatures and progress towards solubility equilibrium was followed by taking the $n_D$ reading at suitable intervals. The solution was filtered as soon as equilibrium was reached, and the casein precipitated at 35–40° C. with acetic acid, filtered, washed, dried and weighed. Solubilities at 45° C. (as g. of casein dissolved in 100 g. of the solvent) were as follows:—Potassium thiocyanate, 3·4201; sodium cymenesulphonate, 9·6753; sodium benzenesulphonate, 3·0480 g. Solubilities increased with temperature as a straight line function, but with 10 per cent. solutions slightly curved lines resulted. The possibility of selective solubility or fractionation was considered in the course of the solubility determination. Primary evidence was afforded if the refractometer showed an increased index on addition of further amounts of casein after equilibrium had already been attained with the amount already present. This result, if present, was checked by further tests, such as potassium and nitrogen determinations, formaldehyde absorption, and solubility in pyridine solution. Sodium benzenesulphonate and sodium cymenesulphonate solutions at a $p$H of 4·6 were found to separate casein into at least two components, but potassium thiocyanate was not a fractionating agent. By using salts of nearly neutral action such as the sulphonates the possibility of hydrolysing proteins is reduced to a minimum, and qualitative but not quantitative fractionation appears to be effected. The theory that casein is a mixture or composite of loosely bound aggregates rather than a compound, is strongly supported by the present work, and the fact that fractionation equilibrium was not dependant on time (as shown by the refractometer) supports Linderstrøm–Lang's casein mixture theory, rather than Sørensen's aggregate hypothesis. D. G. H.

**Preparation of Ammonium Aurintricarboxylate (Reagent for Aluminium). J. A. Scherrer and W. H. Smith.** (*Bureau of Stds. J. Research*, 1938, **21**, 113–115.)—When satisfactory the reagent shows a definite pink colour with 0·02 mg. of aluminium in 50 ml., and only a slight straw colour if aluminium is absent. In view of great difficulties in obtaining a satisfactory reagent from commercial sources or by existing methods, the following method of preparation was worked out: Four g. of sodium nitrite are added in small portions at a time, and with vigorous stirring, to 44 ml. of conc. sulphuric acid. The solution is cooled to 10° C., and 12 g. of salicylic acid are added in portions, with stirring, during 5 to 10 minutes. The liquid being cooled to 3° C. to 5° C. by standing the vessel in crushed ice, 3·5 ml. of an approximately 37 per cent. solution of formaldehyde are added, drop by drop, with stirring. The liquid is kept in an ice bath for 20 hours, with occasional stirring during the first hour, and afterwards is slowly poured into 2 l. of distilled water, with stirring. After 1 hour the precipitate of aurintricarboxylic acid is filtered off and washed three times with water. It is then purified by extracting three times by boiling for 2 to 3 minutes with 1 litre of water containing 50 ml. of conc. hydrochloric acid, the residue being washed with water after each extraction. The residual mass is dissolved in an excess of ammonia, and the solution is evaporated to dryness, yielding a residue of the ammonium salt, which should finally be ground and bottled. S. G. C.

# Inorganic

**Separation of Cadmium from Zinc. C. Zöllner.** (*Z. anal. Chem.*, 1938, **114**, 8–15.)—The separation of cadmium from zinc by precipitation as cadmium sulphide at a controlled degree of acidity can be quantitatively effected in one operation as follows: The sulphate solution, free from chloride, and containing 15 ml. of strong sulphuric acid in a total volume of 100 ml., is heated to boiling, and treated with hydrogen sulphide until cold. The dense, reddish-yellow to orange-red precipitate is left to settle for a short time, collected, and washed with acidulated hydrogen-sulphide water; it is dissolved in hot 25 per cent. hydrochloric acid, the solution is evaporated with 25 ml. of 15 per cent. sulphuric acid, and the cadmium is weighed as sulphate as usual. The results of the published test separations of the two metals in any proportions are excellent. In working out this procedure, the author reverted to the directions of Follenius (*Z. anal. Chem.*, 1874, **13**, 428), who found that the maximum acidity at which cadmium sulphide is quantitatively precipitated is 17·6 ml. of strong sulphuric acid in 100 ml. of solution. W. R. S.

**Gravimetric Determination of Bismuth by means of Picric Acid. H. Etienne.** (*Bull. Soc. Chim. Belg.*, 1938, **47**, 287–303.)—Bismuth may be quantitatively precipitated as basic picrate. To the solution of bismuth nitrate (100 ml.) rendered slightly acid with nitric acid, is added an excess of a cold saturated aqueous solution of picric acid (strength about 1 per cent.); 100 ml. of picric acid solution are required with more than about 0·05 g. of bismuth, and less with smaller amounts. One ml. of a 1 per cent. solution of methyl orange is added, and ammonium carbonate solution (10 per cent.) is introduced, drop by drop, until the colour changes to yellow. The precipitate of basic bismuth picrate settles out rapidly; it may be filtered off shortly after precipitation and washed with cold water. Ashing of the precipitate is not advised owing to its explosive nature. Instead, it is converted by boiling with ammonium carbonate solution into basic carbonate which is filtered off and washed with hot water until free from ammonium picrate. The precipitate is dissolved in nitric acid, the solution is evaporated to dryness, and the residue is ignited to give bismuth oxide ($Bi_2O_3$), which is weighed. With small quantities of bismuth (less than 0·01 g.) conversion into basic carbonate can safely be dispensed with, the picrate precipitate being dissolved directly in dilute nitric acid, the solution evaporated to dryness, and the residue ignited to give bismuth oxide. Precipitation was found to be complete down to the smallest amount of bismuth taken (0·002 g.). The method effects a good separation of bismuth from lead, but the precipitate must be well washed with a solution of picric acid (0·5 g. per litre); with a largely preponderating amount of lead present, double precipitation is necessary. Good results were obtained in tests, substantially complete recovery of as little as 3 mg. of bismuth from 20 g. of lead having been effected. Copper and cadmium are without effect, but iron and phosphoric and arsenic acids interfere when present in more than small amounts. S. G. C.

**New Procedure for the Analysis of Dental Gold Alloys. R. Gilchrist.** (*Bureau of Stds. J. Research*, 1938, **20**, 745–771.)—The procedure provides for the separation and gravimetric determination of silver, iridium, tin, gold, indium, copper, zinc, nickel, palladium, rhodium and platinum. Many of the steps employed in the previous method of Swanger (*Analysis of Dental Gold Alloys, Bureau of Stds. Sci. Paper*, 1926) have been retained, and the new features introduced are (1) the collective separation of indium, copper, zinc and nickel from platinum, palladium, and rhodium by precipitation with sodium nitrite in alkaline solution at the colour-change point of thymolphthalein, (2) the precipitation of gold by means of sodium nitrite, (3) an improved method for the hydrolytic precipitation of tin, (4) provision for determining indium, a recently proposed alloying constituent. The alloy sample is dissolved as far as possible in *aqua regia*, and the insoluble residue of silver chloride and metallic iridium is separated as in Swanger's method. *Separation of Tin.*—Tin is then separated from the solution containing the remaining constituents by boiling the liquid for 5 minutes after the addition of sufficient sodium hydroxide to bring the *p*H to about 1·5 [thymol blue (thymolsulphonephthalein) colour change from red to orange]. The stannic hydroxide is allowed to settle out by heating the liquid on a steam-bath for 20 minutes, filtered off and washed with hot water acidified to between *p*H 1·5 and 4. For reprecipitation of the stannic hydroxide to free it from contamination by other metals, the precipitate is dissolved in 5 ml. of hot conc. sulphuric acid in the presence of the filter-paper, which is destroyed by the addition of nitric acid and further heating to remove the nitric acid. The sulphuric acid residue is diluted with 50 ml. of water and cooled rapidly to prevent hydrolysis of the stannic sulphate, and the small amount of gold present as metallic residue is filtered off, washed with dilute sulphuric acid and retained. The stannic sulphate solution is diluted to 200 ml. and heated at 85° C., for 1 hour. The stannic hydroxide precipitate is filtered off, washed as previously directed, and finally ignited and weighed as stannic oxide. [It is noted that in this precipitation a small quantity of stannic hydroxide tends to adhere obstinately to the glass of the beaker, and it is advised that it be finally dissolved off by treatment with hot conc. sulphuric acid, and the solution, after dilution, boiled with addition of ammonium acetate, the small precipitate being filtered off and added to the main precipitate of stannic hydroxide, and the filtrate rejected.] The filter containing the small amount of gold recovered, as described above, is added to the solution obtained after reprecipitation of the stannic hydroxide; the whole is evaporated, the organic matter is destroyed by heating with sulphuric and nitric acids, the bulk of the sulphuric acid is removed by evaporation and the residue is dissolved in diluted *aqua regia* and added to the main solution. *Collective precipitation of gold, indium and base metals.*—The solution is concentrated to 150 ml. and partially neutralised with sodium hydroxide to *p*H 1·5 (as shown by thymol blue indicator). It is heated to boiling and the gold is precipitated by the addition of 10 ml. of 10 per cent. sodium nitrite solution. The liquid is again brought to *p*H 1·5. A further 20 ml. of the nitrite solution are then added and the solution is rendered slightly alkaline (blue colour with thymol blue). The liquid is boiled for 5 minutes, more sodium hydroxide being added from time to time in order to restore the

alkalinity. Under these conditions gold, indium, copper, nickel and zinc are quantitatively precipitated, leaving the platinum metals in solution. The precipitate is filtered off and washed first with dilute sodium nitrite solution rendered faintly alkaline to the blue change point of thymol blue, and then with water to remove sodium nitrite. The base-metal hydroxides may be dissolved from the precipitate by washing it with dilute hydrochloric acid, leaving the gold as residue. To ensure freedom from traces of platinum and rhodium, as well as to recover a small quantity of gold, the "base-metal" solution should be "collectively precipitated" a second time on the lines described above. *Separation of indium.*—It is first necessary to remove and recover a small but significant amount of palladium which is present in the solution containing the base metals. For this purpose the excess of acid in the solution is eliminated by evaporating it to a moist residue; 5 ml. of conc. hydrochloric acid are added, and the solution is diluted to 200 ml. The palladium is precipitated by the addition of alcoholic dimethylglyoxime solution, the precipitate being filtered off and washed with dilute hydrochloric acid and then with water. Dimethylglyoxime is removed from the filtrate by evaporation with nitric acid, after which the residue is evaporated twice with hydrochloric acid, and 1 g. of ammonium chloride and 200 ml. of water are added. Indium is precipitated as hydroxide by means of ammonia. A double precipitation is required to free it from copper and zinc. The indium hydroxide is finally dissolved in dilute sulphuric acid, the acidity of the solution is adjusted to 0·02 *N*, the indium is precipitated as the yellow sulphide by means of hydrogen sulphide, and the precipitate is filtered off, washed with 0·01 *N* sulphuric acid and finally ignited and weighed as $In_2O_3$. Other constituents are determined on lines similar to those of Swanger's methods. S. G. C.

**Determination of Ferric Oxide and Sulphide Sulphur in Basic Slags. E. Maurer and F. Haderer.** (*Iron and Steel Inst.*, Preprint No. 8, May, 1938.)—The slags considered contained ferric oxide, ferrous oxide, sulphide, a small amount of sulphate, together with silica, lime and magnesia. When such a slag is dissolved in hydrochloric acid, the hydrogen sulphide, as it is formed, reacts with the ferric iron going into solution, with the result that elemental sulphur separates out and reduction of ferric to ferrous iron takes place. Hence not only is the determination of sulphide sulphur invalidated, but also the directly titrated ferrous iron in the solution appears too high, and consequently the ferric iron, calculated by difference between the ferrous and the total iron, is too low. *Sulphide sulphur.*—This may be determined from the difference between the total sulphur content and the sulphate sulphur. It may also be determined by the evolution method of Treadwell (*Analytische Chemie*, 1927, p. 315), in which metallic tin powder is added to the hydrochloric acid used for dissolving the sample, the tin rapidly dissolving and forming stannous chloride which reduces the tervalent iron going into solution. (*Cf.* also H. A. Bright, *Bureau of Stds. J. Res.*, 1937, **18**, 137; Abst., ANALYST, 1937, **62**, 497.) *Ferric iron.*—Faber's method (*Z. chem. Mineralogie*, 1935, **10**, 67), in which mercuric chloride is introduced to precipitate hydrogen sulphide formed during the dissolution of the sample in acid and so to prevent reduction of ferric iron, was found to yield inaccurate results as only a portion of

the hydrogen sulphide was removed by precipitation. Quadrat's method (*J. Iron and Steel Inst.*, 1930, (2), 175) proved satisfactory. This method involves the following steps:—The total iron is determined by the Reinhardt–Zimmerman method. The total sulphur content is found, after oxidation by bromine, as barium sulphate, and the sulphate sulphur is determined in a solution of the slag in hydrochloric acid, the difference between these two sulphur results giving the amount of sulphide sulphur present. A sample is then dissolved in a hot mixture of 100 ml. of dilute sulphuric acid (1 : 5) and 5 ml. of conc. hydrochloric acid in a current of carbon dioxide; the hydrogen sulphide evolved—termed "remaining sulphide sulphur" because some hydrogen sulphide becomes oxidised to sulphur by ferric iron in the dissolving flask—is absorbed in cadmium acetate solution and determined as usual; ferrous iron is titrated in the solution of the sample. The difference between the total sulphide sulphur and the "remaining sulphide sulphur" gives a measure of the amount of sulphur, which, in the form of hydrogen sulphide, reduced ferric iron in the solution to ferrous iron; the amount of ferric iron thus reduced is calculated from the equation: $2FeCl_3 + H_2S = 2HCl + 2FeCl_2 + S$, giving a correction value as in the following example: (1) Total sulphur, 0·3530; (2) sulphate sulphur, 0·0270; (3) total sulphide sulphur, by difference, 0·3260; (4) "remaining sulphide sulphur," from cadmium sulphide, 0·1035; (5) sulphide sulphur consumed in reduction of iron (by difference between (3) and (4)), 0·2225; (6) total iron, 8·92; (7) directly titrated ferrous iron, 7·355; (8) ferric iron, by difference, 1·565; (9) ferric iron reduced by hydrogen sulphide, calc. from (5), 0·775; true values: ferrous iron, 6·58; ferric iron, 2·34 per cent. In the course of tests, the elemental sulphur liberated by oxidation of hydrogen sulphide by ferric iron during solution of the sample in acid in an inert atmosphere, was recovered by Soxhlet extraction, and was found to be equal to (5) above. S. G. C.

**Determination of Boron in Steel and Cast Iron. J. L. Hague and H. A. Bright.** (*Bureau of Stds., J. Research*, 1938, **21**, 125–131.)—The method, which is applicable to iron and steel containing 0·005 to 0·1 per cent. of boron with a degree of accuracy of ±0·002 per cent., involves the application of Chapin's methyl borate distillation process followed by acidimetric titration of boric acid in presence of mannitol. A 5-g. sample is dissolved as far as possible in 25 ml. of conc. hydrochloric acid by heating in a flask fitted with a reflux condenser. The solution is cooled, oxidised by the addition of 10 ml. of hydrogen peroxide (30 per cent.), and heated under reflux to decompose excess of peroxide. After again cooling, 20 g. of anhydrous calcium chloride are added, and the flask is fitted with a stopper carrying (*a*) an entry-tube leading from another flask containing methyl alcohol, (*b*) a leading tube to a water condenser, which, in turn, leads into a stoppered receiving flask provided with a guard tube containing water. About 25 ml. of methyl alcohol are first slowly distilled into the sample-flask, which is then heated on a water-bath to prevent further condensation of methyl alcohol. The methyl alcohol distillation is continued until about 150 ml. of condensate have been collected. The receiver is changed, the distillation is continued, and, meanwhile the first condensate is made alkaline with sodium hydroxide and the methyl alcohol is removed by evaporation in a platinum dish.

A second 150 ml. of condensate, together with the contents of the guard tube, rendered alkaline as before, are added to the platinum dish, and the whole is evaporated to dryness. The residue is dissolved in a little water, and the solution is transferred to a flask; the final volume of solution should not exceed 25 ml. The solution is acidified to methyl red indicator with hydrochloric acid, not more than 1 drop of the dilute acid (1:1) being added in excess; the solution is boiled for 15 seconds, cooled, and exactly neutralised to methyl red with sodium hydroxide solution. One g. of mannitol, or more, if necessary, is added and the boric acid mannitol complex is titrated with $N/10$ sodium hydroxide solution, phenolphthalein being used as indicator. The authors deduct a "blank" obtained by carrying out the determination on a sample of boron-free steel. The acid-insoluble residue from the steel may be examined for boron by fusion with sodium peroxide and subsequent distillation of methyl borate from the acidified solution as above. In tests of the method on various samples, however, no boron was found in the acid-insoluble residue. Selenium and tellurium do not interfere, but germanium, a minor constituent very rarely present in ferrous alloys, causes a small positive error. S. G. C.

**Colorimetric Titration of Small Amounts of Fluoride with Thorium Nitrate. D. Dahle, R. U. Bonnar and H. J. Wichmann.** (*J. Assoc. Off. Agr. Chem.*, 1938, **21**, 459–474.)—The sample fluoride solution (40 ml.) is placed in a 50-ml. Nessler tube, and 40 ml. of distilled water is placed in another similar Nessler tube (titration tube). One ml. of sodium alizarine sulphonate indicator solution (0·01 per cent. in water) is added to each tube. Sodium hydroxide solution (0·2 per cent.) is added to the sample solution until the colour matches that in the titration tube (faintly pink). To each tube exactly 2 ml. of 0·05 $N$ hydrochloric acid are added. Thorium nitrate solution (0·25 g. per litre, exact strength not important) is added from a burette to the sample tube until, after mixing, the colour "barely changes to a faint pink." The same volume of thorium nitrate solution is added to the titration tube, and the contents of this are then titrated with standard sodium fluoride solution (1 ml. = 0·01 mg. of fluorine) until the colour matches that of the sample solution, the two solutions being diluted to the same volume before the final comparison is made. The fluorine-content of the sample solution is then equal to that of the titration solution. The method is suitable for the titration of quantities of fluorine up to 50$\gamma$. The method is intended to be applicable to distillates obtained by distilling fluorine-containing materials with perchloric acid. It was found, however, that if considerable amounts of the acid were allowed to pass over into the distillate, errors occurred in the titration owing to the large amount of salt formed on neutralisation of the acid. In order to avoid this, it is desirable to distil below 140° C. and to shield the flask to avoid over-heating. S. G. C.

**Determination of Ortho-, Pyro- and Meta-Phosphoric Acids by Colorimetric pH Titrations. A. B. Gerber and F. T. Miles.** (*Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 519–524.)—For the present purpose the meta-, pyro- and ortho-phosphoric acids are considered, respectively, as mono-, di- and tri-basic acids which

may be neutralised in successive 3 steps. Thus, about 10 g. of the anhydrous sample (weighed out by difference) are poured slowly, so as to avoid local overheating, into 200 ml. of ice-water in a 400-ml. beaker provided with a stirrer. The mixture is then diluted to 500 ml. in a graduated flask, and 25 ml. are pipetted out into each of 2 Almquist* titrating flasks. Titrations as follows are made without delay:—(1) To one portion is added 0·5 ml. of a solution prepared by diluting to 100 ml. a solution of 0·4 g. of bromo-cresol green in 4·1 ml. of 0·1408 *N* sodium hydroxide solution and a few ml. of alcohol. This is titrated with a 0·1408 *N* solution of sodium hydroxide, which must be free from carbonate, until the *p*H is 4·2, and the volume is then diluted to 100 ml.; titration is then continued, and the volumes required to bring the solution to *p*H 4·2, 4·4 and 4·6 (when compared against colour standards) are recorded. (2) To the solution at *p*H 4·6 are added 25 ml. of 0·85 *N* silver nitrate solution (*i.e.* an excess of at least 5 ml.), the mixture being shaken well to coagulate the precipitate; 0·5 ml. of a 0·2 per cent. solution of methyl red in a mixture of 60 ml. of alcohol and 40 ml. of water is then added. Titration is continued until the pink colour is just discharged, and the end-point is clearer if the solution is shaken well after each addition and the precipitate allowed to settle. (3) To the other 25-ml. portion are added 20 g. of neutral sodium nitrate and 0·5 ml. of a solution prepared by dissolving 0·4 g. of thymol blue in 6·1 ml. of 0·1408 *N* sodium hydroxide solution and 10 ml. of water and diluting to 100 ml. If pyro phosphoric acid predominates, the use of 14 g. of sodium chloride and 0·5 ml. of a mixture of 40 ml. of Oleo red-B (La Motte's strong solution) and 20 ml. of 60 per cent. alcohol is preferable. Titration with the alkali to *p*H 8·4 is carried out, the volume is diluted to 100 ml., and the quantities of alkali required to produce *p*H 8·4, 8·6, 8·8, 9·0, and 9·2 are recorded. From each volume is subtracted the blank found by titrating to *p*H 8·8 a solution of 20 g. of the salt used in 100 ml. of water. Titrations to a range of *p*H values are made because the required end-point depends on the proportions of the 3 acids present; a preliminary titration, or the nomogram given in the original paper, enables the exact *p*H of the colour-change to be ascertained, the corresponding volumes of alkali being used for the purpose of calculating the results. These are obtained from the following equations:

(*a*) 0·01 BCG = g. of total $P_2O_5$ (ortho + pyro + meta),
(*b*) 0·01 (TB − BCG) = g. of $P_2O_5$ (ortho + pyro),
(*c*) 0·01 [MR − (TB − BCG)] = g. of $P_2O_5$ (ortho),

where BCG, TB and MR are the respective values from the titrations in which the indicators bromo-cresol green, thymol blue and methyl red were used as described above. The corresponding chemical equations and a nomogram for the rapid calculation of results are given in the original paper. The titrations are made on 2 aliquot portions of the solution in order to avoid confusion arising from the colour of the mixture of indicators. Advantages claimed for the method are rapidity and simplicity; elimination of the difficulty of producing a phosphate of constant

* An Almquist flask is a 250-ml. conical flask marked to indicate 100 ml., near the base of which is fitted a vertical side-arm having the same dimensions as the test tubes used in the *p*H colorimeter; any coloured liquid in the flask therefore, rises in this tube and may be matched by the usual colorimetric method.—ABSTRACTOR.

composition as in a gravimetric determination; the method does not depend on the selective precipitation of pyro-phosphoric acid (*e.g.* as the zinc or magnesium compound) which has been shown to be unreliable. The titration may also be made potentiometrically (with a low-resistance glass electrode), in which case still less time is required, as there are only 2 instead of 3 operations, there is no colour-matching, and only changes in *e.m.f.* (and not *p*H values) need be recorded. Limitations of the method are that the end-point values cannot be accurately applied to concentrations of phosphorus pentoxide differing widely from 0·4 per cent. unless the appropriate equivalence points are calculated, and that inaccurate results are obtained at temperatures exceeding 25° C. Weak acids or bases that have a buffer action interfere, and although strong bases or mineral acids do not affect the ortho- and pyro-values, the bromocresol green titration then no longer represents the total $P_2O_5$. Under such conditions an aliquot portion of the sample must first be converted into the ortho-phosphoric acid by diluting it to 100 ml. and boiling it with 7 ml. of conc. nitric acid for 15 minutes. The solution is nearly neutralised with 50 per cent. sodium hydroxide solution, then cooled, and accurately adjusted to *p*H 4·6 to the bromocresol green indicator. If the solution is then titrated with the standard alkali, with methyl red as indicator, 1 ml. $\equiv$ 0·005 g. of $P_2O_5$; the ortho- and pyro-values are calculated in the usual way, the meta-value being obtained by difference. Basic and metallic elements which give insoluble precipitates during the thymol blue titration interfere to extents that depend on the amount present, the results being high for the pyro-acid and low for the ortho- and meta-acids. Iron and aluminium are without influence if the concentration of each does not exceed 0·1 per cent. Tests on mixtures of pure phosphates acidified with sulphuric acid showed that, in general, the error of the determination is $\pm$0·4 per cent., and is greatest for the meta-acid, as this involves several titrations. Since the work of Lum, Malowan and Durgin (*Chem. & Met. Eng.*, 1937, **44**, 721) suggests that polymers of the phosphoric acids may exist, the possibility of interference with the titration by these must be considered. As such compounds cannot be identified by known methods, a series of 13 "anhydrous" acids covering the range 74 to 85 per cent. of $P_2O_5$ was analysed, and the results are shown on a trilinear chart which relates the proportions of the 3 acids concerned. The results suggest that an equilibrium exists of the type $2P_2O_5,2H_2O \rightleftharpoons P_2O_5,3H_2O + P_2O_5,H_2O$, and having an equilibrium constant of 0·06; compositions calculated from this value agree well with those found by colorimetric titration. Although this does not preclude other reactions involving polyphosphoric acids, it is concluded that any polymers present behave on titration similarly to the corresponding simple acids; thus, *e.g.* polymers of meta-phosphoric acid behave as monobasic acids. Although therefore the method gives no information regarding the molecular structure of the acids present, it does show the amounts of $P_2O_5$ associated with 1, 2 or 3 mol. of water, which are designated as meta-, pyro- or ortho-phosphoric acid, respectively, and are represented for convenience by the simple formulae.

J. G.

# Microchemical

**Micro-determination of Bivalent Iron in Minerals and Rocks. M. Shioiri and S. Mitui.** (*Mikrochimica Acta*, 1938, **3**, 291–299.)—Bivalent iron is determined on 3 to 30-mg. samples of rocks and silicate minerals. The sample is decomposed by heating with 0·2 ml. of dilute sulphuric acid (1 : 3), and 10 drops (0·3 ml.) of hydrofluoric acid in a stream of water vapour and carbon dioxide. Subsequently the solution is titrated with 1/50 *N* permanganate solution in the presence of boric acid, as in the macro method of J. B. Cooke. Reliable results are obtained agreeing well with those given by macro determinations. J. W. M.

**Microgravimetric Separation of Nickel and Uranium. E. Kroupa.** (*Mikrochimica Acta*, 1938, **3**, 306–312.)—The solution of the nitrates of nickel and uranium is evaporated to dryness in a Berlin porcelain crucible (C 1) with glazed base. The residue is taken up in 1 drop of 1:1 nitric acid and 5 to 10 ml. of water. The solution is heated on the water-bath, and the nickel is precipitated as the dimethylglyoxime, about a sixfold excess of a 1 per cent. alcoholic solution of dimethylglyoxime being used (1 ml. to precipitate 1·6 to 1·7 mg. of nickel), followed by 1 ml. of a 50 per cent. ammonium acetate solution that has been nearly neutralised with ammonia. The precipitate is filtered off at once by means of a Berlin porcelain filter-stick, and washed three times with 0·5 ml. of hot water and 3 times with 0·5 ml. of warm 25 per cent. alcohol. The crucible, together with the filter-stick and precipitate, are finally dried at 120° C. and weighed. The filtrate, containing the uranium, is evaporated in a platinum crucible, and the residue is ignited. The ignition residue is evaporated to dryness with 0·5 ml. of 1:1 nitric acid, dissolved in 1 drop of glacial acetic acid and 0·5 ml. of hot water and quantitatively transferred to a 20-ml. platinum crucible (weighing 10 to 11 g.). The uranium is precipitated as uranyl oxyquinolate, three times the calculated amount of oxine reagent being used, and the excess of acetic acid is neutralised by the addition of 2 to 4 drops of ammonia solution (1:1). After being heated for 10 minutes over the water-bath and standing for 30 minutes the mixture is filtered through a platinum filter-stick, which has previously been weighed together with the crucible. The precipitate is then washed with hot water, dried, cautiously ignited to $U_3O_8$ in an electric oven, and finally weighed together with the platinum filter-stick and crucible. Excellent results are obtained on amounts of nickel varying from 0·5 to 7·5 mg. and of uranium varying from 0·8 to 4·5 mg.

J. W. M.

**Collected References. Behaviour of extremely Thin Surfaces of Metals in Different Conditions. M. Kindinger and K. Koller.** (*Mikrochimica Acta*, 1938, **3**, 317–325.)—The behaviour of thin layers of metals is described under the following headings:—(1) Optical constants (1 reference), (2) Electrical conductivity (1 reference), (3) Ageing phenomena, (4) Structure (2 references), (5) Density (2 references), (6) Resistance to α-rays, (7) Effects of temperature (1 reference), (8) Determination of thickness, (9) Theories, including electronic structure and uptake of gases. A further list of references is given at the end (40).

J. W. M.

# Physical Methods, Apparatus, etc.

**Spectrophotometric Determination of the Orthophosphoric Ion and of the Sodium Ion. A. Leclère.** (*J. Pharm. Chim.*, 1938, **28**, 152–158.)—*Orthophosphoric acid.*—Acetic acid is added to the solution (which should contain the equivalent of not more than 0·5 mg. of $P_2O_5$), until the *p*H is approx. 5 (to methyl orange). This is followed by 2 ml., per 10 ml. of solution, of a reagent containing 50 g. of crystallisable acetic acid and 100 g. of crystalline sodium acetate in sufficient water to make a volume of 1 litre; then by 2 ml. of a 4 per cent. solution of uranium acetate, per 10 ml. These conditions ensure the minimum solubility of the uranyl phosphate and tendency for uranium oxide to be precipitated, as well as the most reproducible results, and for these reasons also, uranium nitrate should not be used. The mixture is then heated for 5 minutes on the water-bath, the flocculent precipitate being separated by means of the centrifuge while the solution is still warm, and washed twice with a warm solution of the acetic acid and sodium acetate reagent diluted 100-fold; the last wash should give only a faint brown colour when acidified, diluted 10-fold, and tested with a solution of potassium ferrocyanide. The precipitate is dissolved (in the centrifuge-tube) in 0·5 ml. of warm 10 per cent. hydrochloric acid, and the solution is transferred to a 10-ml. graduated flask with the aid of a 50 per cent. (by vol.) solution of glycerin, and 0·5 ml. of a 20 per cent. solution of potassium ferrocyanide is added to the mixture. The flask is filled to the mark and the contents well mixed, and between 20 and 30 minutes later the extinction-coefficient is measured in a Pulfrich spectrophotometer with the aid of Wratten filters Nos. 53 and 57 (*i.e.* corresponding with mean wave-lengths of 530 and 570$m\mu$, respectively). If the depth of liquid used is 10 mm., the sensitiveness is 4 or 10 mg. of $P_2O_5$ per l., according to the screen used, respectively. *Sodium.*—If the solution to be tested contains a phosphate, this must first be removed by precipitation as described above, except that the dilute acetic acid and sodium acetate solution should be replaced by a 0·5 per cent. solution of glacial acetic acid, the amount of acid required to produce a *p*H of 5 being determined beforehand on a separate portion of the sample, in order to avoid the error involved by the addition of methyl orange, which contains the sodium ion. The higher acidity during precipitation is then without influence. The reagent (after Blanchetière and Kahane) is a solution containing 32 g. of crystalline uranyl acetate, 100 g. of magnesium acetate, 20 ml. of acetic acid, 500 ml. of 90 per cent. alcohol, and sufficient water to bring the total volume to 1 litre. The precipitation, which should be carried out in the centrifuge tube, is aided by scratching the walls of the tube, and after 15 minutes of contact the precipitate is separated at room temperature with the aid of the centrifuge, and then washed 3 times with 5 ml. of 95 per cent. alcohol. The remainder of the procedure is similar to that already described, the sensitiveness being of the order of 1 or 2 mg. per litre. J. G.

**Investigation of the Falsification of Writing with the Aid of a Stereo-Microcamera having a very High Efficiency for the Reproduction of Depth-effects. C. J. Tirion.** (*Arch. Kriminol.*, 1938, **103**, 35–41.)—There are

3 distinct methods by means of which stereo-photomicrographs may be taken, *viz.*:— (1) The "crossed focus" method, in which 2 photographs are taken with the camera in 2 positions which are such that their optical axes converge and cross at the object at an angle of 15°; alternatively the position of the camera may be fixed, the object being moved, so that the planes (*e.g.* of the paper) in the 2 positions meet at an angle of 15°. (2) The 2 positions of the plate in the camera are plane-parallel in relationship to one another, the objective or object being displaced in a plane-parallel direction. (3) The object and the camera remain fixed in position for both exposures, but the direction of the incident light is altered. It is considered that the first two methods only are suitable for the examination of writing on paper, because the natural stereoscopic effect of an ink-line is small and must be magnified. Existing apparatus (*e.g.* the Brewster prism-stereoscope) is not considered suitable, and any stereoscopic instrument used for visual (as distinct from photographic) observations should provide for adjustment of the distance between the two eyepieces. Other limitations of existing apparatus and the criteria of the ideal instrument are discussed. The latter include the production of an image that is sharp all over the field, so that irregularities on the borders of the ink-strokes and "feathering" of the ink are rendered visible, provision for an adjustable angle of convergence (see [1], above) and provision for obtaining a degree of magnification that is critical for the object in question, *i.e.* it should not be too great or too little. This is important, because on it depends the extent of the stereoscopic effect obtained, and a linear magnification ranging between 15 and 45 (100, in special cases) is considered adequate for most purposes. It is stated that it is possible to obtain a 75- to 125-fold increase in the stereoscopic effect with a linear magnification of 25, without loss of sharpness. Other desiderata are provision for adequate space for manipulation (*e.g.* when making chemical tests on the ink) between the objective and the specimen, and for the production of a final photograph large and clear enough to be examined without the aid of a lens; 12 × 16·5 or 13 × 18 cm. are suitable dimensions. Small photographs are unsuitable for the reproduction of colour-effects. The author claims that his apparatus satisfies all these requirements, but he does not describe it. Six pairs of stereo-photomicrographs are, however, reproduced in the original paper, and they illustrate specimens such as the feathering of ink lines on a fold of paper, the superposition of typescript on print on a grey paper, the crossing of ink strokes, and broken lines in printed matter. In one case a comparison is provided between the results obtained with the author's apparatus and an instrument of the type referred to in (3) above (see also, Osborn, *Questioned Documents*). J. G.

**Erratum.**—October issue, p. 728, l. 1, for "*Nitragyne Speciosa*" read "*Mitragyne Speciosa.*"

# Reviews

FOOD TABLES. By V. H. MOTTRAM and ELLEN M. RADLOFF. Pp. 63. London: Edward Arnold & Co. 1937. Price 5s.

These tables should be of wide use, not only to the dietitians or "nutritionists" for whom they appear to be primarily intended by the authors, but also to all those who have, from time to time, to concern themselves with the composition of individual foods or of meals and dietary regimes.

The authors have, very wisely in my opinion, ignored certain classical and established figures, and pinned their faith to results obtained by some of the more recent methods of analysis. It is refreshing, for example, to find that in several places they have called attention to, and given figures for, the difference between phytin and non-phytin phosphorus; since the phytin phosphorus of many foods is possibly not utilised at all in the human gastro-intestinal tract, the amount present in a food may be of botanical rather than of dietetic interest.

A distinguished member of this Society, reviewing these Tables elsewhere, has criticised the authors for giving values to a number of "significant figures" that they themselves admit to have no significance. The authors explain in their Preface that they have given the figures as determined, frequently by calculation from other figures, although they are fully aware that practical nutritionists should, and in most instances will, use rounded off figures. Why, asked that reviewer, give these meaningless numbers at all?

There is no simple answer to this question, which seems to me to overlook what is really the guiding principle. There have always been among analysts two schools of thought on this matter, the one school insisting that only those figures should be given that have statistical significance, allowing for the precision of the methods used, and the other insisting that the figures to be reported should be based upon the figures found, whether or not the last one or two of the reported figures are indeed significant. Surely the answer is that the latter figures should in fact be published, in such places as the columns of THE ANALYST, when the reader is himself an analyst, or at least a chemist, and is capable of judging for himself of the significance of the figures. On the other hand, where the information is intended for those less analytically expert, who are to some extent likely to be misled by them, rounding off is not only justified, but desirable. I am inclined, therefore, in this particular instance, to agree with my fellow-member reviewer, for it is certain that these valuable Tables will find their greatest use outside the circle of professional analysts.

Besides protein, fat and carbohydrate contents, for most of the large number of foods included in the 54 pages of the tables the calorific values and the contents of calcium, phosphorus and iron are also given. Moreover, all of these have been calculated not only for 100-gram portions of food, but also for 1 oz., 4 oz. and 1 lb., thereby saving the practical dietitian a deal of arithmetic.

The distinction recognised by the authors between phytin and non-phytin phosphorus has an interesting analogy in the difference between easily available and poorly available iron, on which matter the publications of McCance and Widdowson may be consulted. It is hoped that in subsequent editions Professor

Mottram and Dr. Radloff may be able to call attention to the proportions of "dipyridyl" iron in many of the more important foods.

There is one other matter that might receive attention in subsequent editions. At several places in the tables figures appear in italics. On page 9 it is made quite clear what the italicising indicates, but the authors seem to have become less alert as they went on. The next set of italic figures appears on page 22, and their meaning can possibly be found by analogy with the earlier. The same applies to those on page 25. Those on pages 28, 30, 37 and later have presumably also the same meaning, but it would be very much better if they were all covered by a note in the general introduction. Incidentally, if the figures were rounded off in the manner already suggested, there might be room for a further column, in which could be inserted figures for, say, magnesium or some other element, when it is of particular importance in a particular food. It would be pleasant to see the barium content of Brazil nuts recognised here, if only as a tribute to Mr. Seaber's pertinacity.

A. L. BACHARACH

QUALITATIVE ANALYSIS FOR STUDENTS OF PHARMACY AND MEDICINE. By CHARLES B. JORDAN, Ph.C., M.S., D.Sc., and HENRY GEORGE DEKAY, Ph.D. Second edition. Pp. xii + 178. New York: McGraw-Hill Book Co., Inc.; London: McGraw-Hill Publishing Co., Ltd. 1938. Price 15s.

The advent of a new text-book on elementary qualitative analysis or even the revision of an old edition requires some justification, especially when comparison with a standard text-book published in 1909 indicates how little the scheme of analysis has changed since that time. In fact, one of the few reasons that would justify the publication of such a book is a definite need existing among a particular group of students. This need is presumably felt to exist among students of pharmacy and medicine, and the book may quite properly be regarded as meeting their requirements, and should make an appeal to them that the merely chemical text-book does not; for the importance in pharmacy of many of the reactions used in the conventional scheme of qualitative analysis is emphasised throughout. Thus, several of the incompatibilities met with in dispensing are explained, as are the reasons for the use of certain chemicals as antidotes to poisons. Moreover, constant reference is made to the contents of the United States Pharmacopoeia and the National Formulary, so that the student can see the practical application of many of the things he is asked to do. The theoretical background of the book is sound and it may be said to be written around the concepts of ionisation and solubility product. Unfortunately, two criticisms may be levelled against it: first, the price is too high for a book of this type; secondly, more attention might have been paid to the newer "spot" reactions. It may be difficult to modernise a subject that has hardly changed in thirty years, but the only "spot" reagents that find mention are aurin tricarboxylic acid, $\alpha$-nitroso-$\beta$-naphthol and dimethylglyoxime. So many are in common use to-day that it seems a pity to have missed the opportunity of referring to them. Incidentally, chemical historians should note the final departure from the analytical scene of blowpipe analysis; even Thenard's blue is now made on burnt filter-paper instead of on the once-popular charcoal block!

F. A. ROBINSON

AN INTRODUCTION TO MICROCHEMICAL METHODS. CECIL WILSON, B.Sc. Pp. xi + 196, with 93 diagrams. London: Methuen. 1938. Price 7s. 6d.

This book, as its title states, is intended as an introduction to microchemical methods, for senior students of chemistry, and as such fulfils its purpose admirably. It is clearly and simply written and the beginner obtains the impression that the micro methods are as easy to learn as those of the normal laboratory scale, which indeed they are. As the book is intended for student beginners, the author devotes half the space to the use of the microscope for observing physical properties and the results of crystal tests. A short chapter is devoted to a few typical spot tests, without, however, any reference to the precautions necessary in the presence of interfering ions. A useful chapter on inorganic qualitative analysis includes complete separation tables for most of the commoner ions. The filter-stick procedure for inorganic quantitative analysis, and a few examples of its application in the estimation of barium, phosphorus, nickel and copper and two examples involving a separation are given. The uses of the tintometer, colorimeter, spectrograph and nephelometer and the practice of photomicrography are briefly described. The chapter on organic preparative methods is well written and illustrated. As the book is intended to be introductory, only two pages are devoted to organic quantitative analysis. Within its scope the book is excellent and may be found of use to teachers of chemistry. J. W. MATTHEWS

THE SOYBEAN INDUSTRY. By A. A. HORVATH, D.Sc. Pp. vi + 221. London: E. & F. N. Spon, Ltd. 1938. Price 16s.

The Foreword to this book describes the author as a research worker and practical technologist in the soybean industry, and he has provided perhaps the most comprehensive treatment of the subject in our language. Practical details of many of the manufacturing operations are included. The soya bean has now become the source not only of a valuable oil and a food for cattle, but of numerous products of industrial importance. After dealing with the edible oil and flour, and the technical uses of the oil, the author describes the commercial extraction of phosphatides (mainly lecithin and cephalin) from the beans and the employment of these substances as emulsifiers in foodstuffs and for a variety of purposes in the manufacture of soaps, cosmetics and paints. Then follows an account of the production of soy protein and its use in the manufacture of plastic products, and of the commercial preparation of detergents, sizings and adhesives from the solvent-extracted meal.

When manufactured from shelled beans, whole soya bean flour contains on the average about 40 per cent. of protein, 20 per cent. of oil and 3 per cent. of phosphatides. By treatment with steam, the resultant flour is freed from the "bean" flavour (mainly due to methyl-*n*-nonyl ketone), and since the enzymes are thereby rendered inactive a flour of good keeping quality is obtained.

Soybean oil is obtained by pressure and by solvent extraction, the latter method having been greatly developed in recent years in order to obtain the maximum yield of oil. The extracted meal is less liable than the "press meal" to develop rancidity, and is a more satisfactory stock food. Moreover, the meal can be more advantageously used for industrial purposes. The various extraction

methods are described, the most commonly-used solvent being a petroleum spirit of boiling range about 52° to 93° C. Ethylene dichloride is a suitable solvent, as are mixtures of benzene, or petroleum fractions, with trichloroethylene. The last-named solvent, however, has pronounced toxic properties.

This book is commendably free from typographical errors, but its price seems rather excessive. ARNOLD R. TANKARD

TABLES OF REAGENTS FOR INORGANIC ANALYSIS. First Report of the "International Committee on new Analytical Reactions and Reagents" of the "Union Internationale de Chimie" (in English, German and French). Pp. xxiv + 409. Leipzig: Akademische Verlagsgesellschaft m.b.H. 1938. Price RM.36, bound; RM.34, unbound.

To describe this book is to commend it. It is the work of an International Committee whose members are acknowledged authorities on the newer analytical chemistry. It is slightly annoying, though quite unimportant, that the only bit of consecutive writing in the book, the Preface, however good it may be in German and French, is in the English version a precise and painful translation. This book, however, is not literature but a piece of apparatus; and, judged as such, it is well made and useful. It is, essentially, a trilingual tabular summary of spot reactions, drop reactions and other highly sensitive qualitative tests for cations and anions, including all such tests discovered between 1910 and 1936, and a selection of those known earlier.

As the Committee remark in their Preface, the newer drop reactions and spot tests are much less known and used than they deserve to be, largely because their number is so great that it is very difficult for the average chemist, who can devote but a limited time to the study of technique, even to look up, let alone to evaluate critically the tests which might best serve his purpose. The Committee therefore decided, very wisely in the reviewer's opinion, that the interests of chemists generally would best be served by publishing their compilation without delay, even though they recognise that a longer period of preparation would have increased the critical value of their work.

The Tables list the known tests for each ion, giving for each test (*a*) the reagent and an indication of its mode of use, (*b*) the type of reaction (whether a precipitation or a colour test, etc.), (*c*) the phenomena of the test, (*d*) its sensitivity, (*e*) remarks (which indicate, *inter alia*, interfering ions and the Committee's opinion of the test), and (*f*) references to the original papers or books where the test is more fully described.

The comprehensive character of the list may be inferred from the fact that some 1600 separate tests are given for the 58 cations and about 350 tests for the 38 anions. In order to keep this mass of material down to a manageable size and make it intelligible in three languages, much use has been made of symbols, and as a result the Tables look rather formidable at first glance. The system of symbols, however, is so simple and sensible that it is very quickly grasped, and its use certainly makes it easy to see at once the essential features of each test.

To the busy chemist who occasionally needs to find quickly a convenient and sensitive test for some unusual substance, this Report is invaluable, because in it

he can learn in two or three minutes what would take him as many hours to hunt up in the Abstracts. The Committee is authoritative and one need not hesitate to rely upon the tests they remark as "recommended," or to pass over those marked "not recommended." On the other hand, for those who are specially interested in the critical study and development of microchemical tests, it is very useful to have at hand so complete and compact a survey of their field. The utility of the Tables is considerably enhanced by an appended "Register of Reagents," which is really an index whereby one can trace easily all the reactions given by each.

The thanks of all chemists are due to the Committee for their arduous and unselfish labour for the common good, and it is to be hoped that the success of these Tables will be such as to encourage them in the continuance of their valuable critical work.

H. V. A. BRISCOE

SCIENCE AND NUTRITION. By A. L. BACHARACH, M.A., F.I.C. Pp. 149. London: Watts & Co. 1938. Price 2s. 6d.

The aim of this book (one of The Changing World Library Series) is to put before the reader "the kind of methods used in the laboratory study of nutritional problems and the kind of knowledge obtained thereby." The treatment of the subject is intended to have a chemical rather than physiological tendency and the reader is expected to be, in the main, a "layman" or a "man in the street."

The book opens with a chapter on the use of animal experiment—not the ethical considerations but the ability of such experiments to give quantitative evaluations and an examination of the extent to which the animal experience may be translated into the human. There follows a section on "classical" nutritional science, this being dealt with in chapters—carbohydrate chemistry, carbohydrates in the body, fat chemistry, fats in the body, protein chemistry, amino acids in the body, pure chemistry being followed by the physiological chemistry in each group. These chapters are, the reviewer suggests, made more difficult than they need be; they are certainly stiff reading. They might perhaps have been made more easy of understanding if a number of side issues had been avoided and a simpler, more strictly connected account of the chemistry been given.

As a test of the suitability of the treatment of a difficult subject for the man in the street, the reviewer tried it on his wife—a course permitted by the Preface. In view of the maxim that none should conduct experiments save those who have done many, it would be wrong to record precisely the result of this single trial; suffice it to say that it did disclose some fault either in the book or in the particular man in the street chosen. But indeed it is ungenerous to be critical of a gallant attempt to write organic chemistry for the general reader.

There follow short chapters on the major mineral elements, hormones and trace elements, and then the dive into the vitamins takes place. Vitamins, as one would expect, are most interestingly and instructively dealt with; the methods of vitamin research are expounded and there is related how through the misty regions of biological assay there have emerged the several pure chemical entities which are vitamins or pro-vitamins.

Finally comes the consideration of diets, not in detail. This part is short but deals with big issues, and economics raises its baleful head. The author scorns a

minimum, a safe, a normal, and even perhaps a good diet: he goes all out for the optimum diet, which should be secured by a reduction in retail prices or an increase in wages, or both. "The fact that such a diet is immeasurably beyond the reach of the average, or even the best paid, urban industrial worker is not germane to the purely dietary aspect of the problem." Germane or not, it is depressing to think that it is "immeasurably" beyond reach.

And even an average sort of optimum diet, though it may be too much for some, is not apparently sufficient when the population is viewed as a whole. Statistically, it is argued, it can be shown that what is optimal for many must be sub-optimal for from a third to a half of the population; those on the lower side of the mode will be receiving their optimum, or more, while their less fortunate brothers on the upper side may come perilously near starvation. "It is surely better . . . that a dozen men shall be overfed than that one should starve to death." The prescription of over-feeding is regarded by the author, it is true, as a temporary measure, and it is to be hoped that some more economical way of feeding the population may ultimately be devised.

Professor J. C. Drummond, too, in the Preface which he contributes, when referring to the very recent tracing of the cause of pellagra to a deficiency of nicotinic acid, and contrasting the use made of this knowledge in two quarters of the globe, says, "It is not a question of nicotinic acid: it is simply one of £ s. d."

Not all dietary reforms, however, are expensive. The annual cost of a generous daily prophylactic dose of calciferol for the child population of these islands (5 millions) is given as £100,000. Infantile rickets could therewith be rapidly, completely and finally eradicated. This cost the author can "leave to the reader to evaluate in terms of bombers and tanks." But the reader could make another evaluation; he could find that the annual cost per child is two-fifths of a shilling—the price of a modest pint. Either evaluation is startling.

However, it is for the future, in which the present should play its part, to decide how to answer social questions arising from the work of the scientists in recent years. Mr. Bacharach describes how this work has been done. In spite of the manifest difficulties which this book must present to the general reader it is to be hoped that it will be widely read; and there must be many, disdaining to be called general readers in this sphere, who could read it with great profit. It should cause all to think, for Professor Drummond warns us in his Preface: "The survival of democracy or its annihilation during the next few years may easily be determined by the measure of attention given in the various countries to what have come to be called the problems of human nutrition." E. HINKS