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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

DEATH

WE record with regret the death of

William Robert Dracass

MICROCHEMISTRY GROUP

A JOINT Meeting of the Microchemistry Group with the Cardiff and District Section of the Royal Institute of Chemistry and the Local Section of the Society of Chemical Industry was held at Cardiff on Friday, May 17th, 1946. By permission of the Directors of Spillers Ltd., the Roath Dock Flour Mills were visited in the afternoon. After tea at the Park Hotel, a meeting was held at 6.30 p.m. in the Physics Lecture Room of University College, Cathays Park, and the following papers were read: "Determination of Cyanide by the Picrate Method," "A Water-bath Rack for Heating Simultaneously Many Tubes of Reactants," by J. G. A. Griffiths and J. K. Whitehead; "Some Observations on the Kjeldahl Method for the Determination of Nitrogen," by A. E. Beet; "Methods for the Construction of Microchemical Apparatus," by R. Belcher.

Analytical Methods Committee

SUB-COMMITTEE ON DETERMINATION OF METALLIC IMPURITIES IN FOODSTUFFS

THE Sub-Committee on Determination of Arsenic, Lead, etc., in Food-colouring Materials has been reconstituted with wider terms of reference to include Determination of Metallic Impurities in Foodstuffs. The Sub-Committee consists of Messrs. A. D. Mitchell, D.Sc., F.R.I.C. (*Chairman*), C. L. Hinton, F.R.I.C., E. G. Kellett, M.A., D.Phil., R. W. Sutton, B.Sc., F.R.I.C., N. D. Sylvester, M.Sc., F.R.I.C., P. F. Wyatt, B.Sc. and N. L. Allport, F.R.I.C. (*Hon. Secretary*).

REPORT ON THE MICROBIOLOGICAL ASSAY OF RIBOFLAVINE AND NICOTINIC ACID

THE Analytical Methods Committee has received from its Sub-Committee on Vitamin Estimations the following Report based on the work of its Microbiological Panel. The Report has been approved by the Analytical Methods Committee and its publication authorised by the Council.

The members of the Panel were D. W. Kent-Jones (*Chairman*), E. C. Barton-Wright (*Hon. Secretary*), D. H. F. Clayson, E. R. Dawson, W. B. Emery,* F. W. Norris, S. A. Price, Mrs. R. P. Whitfield,* and E. C. Wood. The Report deals with the microbiological assay of riboflavine and of nicotinic acid or its amide in certain specified classes of foodstuffs, namely, cereals and cereal products; malt and malted products; yeast and yeast products; meat and meat extracts.

GENERAL INTRODUCTORY REMARKS

1. The following, amongst others, are references to the literature which were found specially helpful by the Panel in compiling their Report: Snell and Strong,[1] Barton-Wright and Booth,[2] Barton-Wright,[3,4] Krehl, Strong and Elvehjem,[5] Strong and Carpenter,[6] Kent-Jones and Meiklejohn,[7] and Wood.[8]

2. At the end of this Report will be found two tables showing the results obtained by the laboratories which collaborated in this investigation. In the riboflavine assays both of the basal media recommended in this Report (see pages 3–4) were used. The inherent precision of the methods used to obtain these results has not been investigated statistically.

* Mr. Emery and Mrs. Whitfield were unable to continue to serve on the Panel up to the completion of this Report and were replaced by their colleagues Mr. D. R. Badham and Miss J. L. Ward respectively.

It will be seen, however, that the figures obtained by the collaborating laboratories on any one sample are in reasonable agreement. The accuracy, as distinct from the precision, of the methods described cannot be checked until estimations by alternative methods become available. From these points of view this Report must be regarded as tentative.

3. Any laboratory wishing to try the methods described is invited to apply to the Hon. Secretary of the Vitamin Estimations Sub-Committee (Mr. Eric C. Wood, Virol Ltd., Hanger Lane, London, W.5) for samples of the reference materials used by the Panel. The Sub-Committee would be interested to receive from any such laboratories reports on their experience with the methods.

4. It must be emphasised that, in carrying out these microbiological assays, normal bacteriological procedures and precautions must be adopted in the preparation of cultures, sterilisation of media and glassware, etc. It is essential that glass-distilled water be used in the preparation of the media and their different constituents, and in the extraction of samples. References in this Report to sterilisation at 10 or 15 lb. pressure imply the use, in an autoclave, of saturated steam at the stated pressure in excess of atmospheric pressure, whereby a temperature of 115° C. or 121° C., as the case may be, is obtained.

5. The organisms to be used are specified on pages 398 and 401. It is important to employ for assay purposes a strain known to be suitable in respect of its nutritional requirements. The members of the Panel all possess cultures of strains which are satisfactory in this respect, and would be glad to assist other workers who may have difficulty in obtaining suitable cultures.

6. The Panel believes that, given a suitable extraction procedure, the methods described would give equally reliable results with extracts prepared from other classes of foods or from biological materials. The method of extraction to be used will depend upon the kind of material to be assayed.

MICROBIOLOGICAL ASSAY OF RIBOFLAVINE

ORGANISM—

The Organism used for the assay of riboflavine is:

Lactobacillus helveticus (*L. casei* ϵ).

PREPARATION OF MEDIUM FOR CULTURE—

Stab cultures of *Lactobacillus helveticus* are carried on a yeast-water - glucose - agar medium.

Prepare the medium as follows—Steam fresh starch-free baker's yeast with 10 times its weight of water for 2 hours, or, alternatively, take 1 g. of Difco Yeast Extract* for every 100 ml. of intended volume. Autoclave for 45 minutes at 15 lb. pressure, cool and, if necessary, allow to settle and decant or centrifuge off. Add 0·5 g. of glucose, 0·6 g. of hydrated sodium acetate and 1·5 g. of agar per 100 ml. of final volume. Steam or autoclave to dissolve the agar, transfer to tubes and sterilise for 15 minutes at 15 lb. pressure. Store the tubes of medium preferably in a refrigerator; it will keep indefinitely. Sub-culture at least once a month, and more frequently if found desirable.

INGREDIENTS AND REAGENTS—

All ingredients and reagents should be of Analytical Reagent quality wherever possible.

Photolysed Peptone (*Sodium Hydroxide Treated*) *Solution*—Dissolve 40 g. of Difco Bacto-peptone* in 250 ml. of glass-distilled water and add 20 g. of sodium hydroxide dissolved in 250 ml. of water. Allow the mixture to stand for 24 hours in the light at room temperature, exposing it to strong light (*e.g.*, a 100-watt tungsten filament lamp at 18 in.) for at least 12 hours of this period. At the end of this time, neutralise the solution with glacial acetic acid (about 28 ml.) and add 11·6 g. of hydrated sodium acetate. Make up the volume to 800 ml. and preserve the solution under toluene† in a refrigerator. This solution will normally keep for a fortnight; discard if a precipitate forms before this time.

Yeast Supplement—Dissolve 100 g. of Difco Yeast Extract* in 500 ml. of water and add to it 150 g. of basic lead acetate also dissolved in 500 ml. of water. Adjust the *p*H of the mixture to approximately 10 with ammonia, using B.D.H. universal indicator externally and filter off the precipitate. Make the filtrate distinctly acid to litmus paper with glacial acetic

* Baird & Tatlock (London), Ltd.

† Toluene used for preserving the various solutions must be S-free. Alternatively, chloroform can be used.

acid and pass in hydrogen sulphide until all the lead is precipitated. Filter off the lead sulphide and adjust the volume of the supplement to 1,000 ml. There is no need to remove hydrogen sulphide by aeration, although some workers prefer to do so. Preserved under toluene, the extract will keep for three months in a refrigerator.

Cystine Solution—Boil 2 g. of *l*-cystine in 50 ml. of water, adding gradually to the boiling liquid 5 ml. of concentrated hydrochloric acid. With this amount of hydrochloric acid there will be no precipitation of cystine after long standing. Make up the solution to 500 ml. with water and preserve under toluene in a refrigerator. This solution keeps indefinitely.

Inorganic Salt Solution A—Dissolve 25 g. of KH_2PO_4 and 25 g. of K_2HPO_4 in 250 ml. of water. The solution keeps indefinitely in presence of toluene.

Inorganic Salt Solution B—Dissolve 10 g. of $MgSO_4.7H_2O$, 0·5 g. of $MnSO_4.4H_2O$ and 0·1 g. of $FeCl_3$ (anhydrous) in 250 ml. of water and add 5 drops of concentrated hydrochloric acid to prevent any precipitation. This solution keeps indefinitely.

Stock Solution of Riboflavine—Weigh out accurately 25 mg. of riboflavine, add 1 ml. of glacial acetic acid, mix with a little water and make up to 1 litre with water. This solution, preserved under toluene in a refrigerator, will keep for 14 days in the dark.

Standard Solution of Riboflavine—Dilute 4 ml. of the stock solution to 1 litre. This solution, which contains 0·1 μg. of riboflavine per ml., must be prepared afresh each day and must not be exposed to strong light.

Bromothymol Blue Indicator—Dissolve 0·1 g. of bromothymol blue in 5 ml. of 95% alcohol, add 1·6 ml. of 0·1*N* sodium hydroxide and make up to 250 ml. with 20% alcohol.

Bromocresol Green Indicator—Dissolve 0·1 g. of bromocresol green in 5 ml. of 95% alcohol, add 1·45 ml. of 0·1*N* sodium hydroxide and make up to 250 ml. with 20% alcohol.

Basal Media—

The Panel examined two basal media, which are here termed basal media X and Y. Basal medium X has a simpler composition than basal medium Y; it has proved reasonably satisfactory in the hands of most operators and can be employed with confidence. The fuller basal medium Y, however, was preferred by the Panel, because with it the slope of the standard curve was steeper and the range of assay rather greater.

Basal Medium X—

This simple riboflavine-free basal medium contains, when diluted in the test, photolysed (sodium-hydroxide-treated) peptone 0·5%, glucose 1%, sodium acetate 0·6%,* xylose 0·1%, cystine 0·01%, riboflavine-free yeast supplement 0·2% and inorganic salts. If 100 assay tubes (see page 401) are to be set up from the stock solutions of the above concentrations the following amounts of the different constituents should be taken:

Table I

Basal medium X†

Photolysed (sodium-hydroxide-treated) peptone solution	100 ml.
l-Cystine solution	25 ml.
Glucose	10 g.
Xylose	1 g.
Yeast supplement	20 ml.
Sodium chloride	5 g.
Inorganic salt solution A	5 ml.
Inorganic salt solution B	5 ml.
Glass-distilled water to	500 ml.

Adjust to *p*H 6·8 with sodium hydroxide, using bromothymol blue as external indicator. The medium may be stored for not longer than 48 hours in a refrigerator.

---

* Contained in the photolysed peptone solution.

† All the basal media in this paper are what has often been described, in others concerned with the microbiological assay of vitamins, as double strength. In use the media are diluted to half their original strength, since 5 ml. are always made up to 10 ml. by the addition of water, food extracts, etc., in the tubes. Since some confusion has occurred between double strength and single strength media the Committee has not used these terms but has described the procedure exactly as it is carried out.

BASAL MEDIUM Y—

For the preparation of the fuller basal medium Y, the following extra stock solutions will be required:

(1) Xanthine solution.
(2) Adenine + guanine + uracil solution.
(3) *dl*-Tryptophan solution.
(4) Nicotinic acid solution.
(5) Pyridoxine solution.
(6) *p*-Aminobenzoic acid solution.
(7) Ca-*d*-pantothenate solution.

For the preparation of these stock solutions, see under assay of nicotinic acid.

TABLE II

BASAL MEDIUM Y†

(Sufficient for 100 assay tubes.)

Photolysed (sodium-hydroxide-treated) peptone solution	100 ml.
l-Cystine solution	25 ml.
dl-Tryptophan solution	25 ml.
Glucose	20 g.
Xylose	1 g.
Yeast supplement	20 ml.
Sodium chloride	5 g.
Ammonium sulphate	3 g.
Xanthine solution	10 ml.
Adenine + guanine + uracil solution	10 ml.
p-Aminobenzoic acid solution	4 ml.
Pyridoxine solution	1 ml.
Ca-*d*-pantothenate solution	1 ml.
Nicotinic acid solution	1 ml.
Inorganic salt solution A	5 ml.
Inorganic salt solution B	5 ml.
Glass-distilled water to	500 ml.

Adjust the *p*H to 6·8 with sodium hydroxide, using bromothymol blue as external indicator. The medium may be stored for not longer than 48 hours in a refrigerator.

PREPARATION OF EXTRACTS—

A successful assay depends upon the choice of a suitable extraction procedure. Appropriate procedures for the foods specified are given below, with some observations on their adaptation to other foods. Any starch in a sample must be destroyed and all free fatty acids removed. Different materials need slightly different treatments depending on their starch and fat contents.

For the extraction of all cereals, except oats and maize, adopt the following procedure:

Hydrolyse a sample of suitable weight with 50 ml. of 0·1 *N* hydrochloric acid for 15 minutes at 15 lb. pressure in an autoclave. Cool and add 2 ml. of a 2·5 *M* sodium acetate solution, adjust the *p*H to 4·5 with sodium hydroxide, using bromocresol green as external indicator, and make up the extract to a suitable volume. Filter, adjust an aliquot to *p*H 6·8 with sodium hydroxide solution, using bromothymol blue as external indicator. Filter off any precipitate which may form and make up to suitable volume. Dilute the final solution so that each ml. of extract contains as nearly as possible 0·05 μg. of riboflavine.

Materials with a high fat content, *e.g.*, wheat germ, soya bean, oats, maize and mixed diets, should be given a preliminary extraction for 16–18 hours with light petroleum (b.pt. 40° to 60° C.) in a Soxhlet extractor to remove free fat and free fatty acids. Hydrolyse with 0·1 *N* hydrochloric acid as described for cereals. Cool the extract, add 2 ml. of 2·5 *M* sodium acetate and adjust the *p*H to 4·5 with sodium hydroxide. Make up to volume, filter, take an aliquot and extract with ether in a separating funnel. After removal of the ether readjust the *p*H of the extract to 6·8, filter if necessary as above and make the solution up to a suitable volume.

† See footnote on p. 399.

All foods that have been subjected to heat treatment, *e.g.*, bread as well as malted cereals, must be given an ether extraction after hydrolysis. The extracts are preserved under sulphur-free toluene.

SUB-CULTURE FOR INOCULUM—

Take 5 ml. of basal medium X or Y, add 2·5 ml. of standard solution of riboflavine and 2·5 ml. of distilled water, and sterilise at 10 lbs. pressure for 10 minutes. After cooling, inoculate the tube with a portion of the stab culture (page 398) by means of a sterile needle, and incubate for 18 hours at 37° or 40° C.

INOCULUM—

Centrifuge the sub-culture aseptically, pouring off the supernatant liquid and adding 10 ml. of 0·9% sterile saline solution. Add 1 ml. of this suspension to 10 or 20 ml. of 0·9% sterile saline solution, shake, and use this dilute inoculum for inoculating the assay tubes. Make up fresh inoculum from the stab culture for each new assay. In no circumstances must the previously prepared inoculum be added to a fresh tube to prepare the inoculum for a new assay. If this be done, the blanks may show unrestricted growth. For the inoculation of the tubes, add 1 drop of the diluted suspension to each tube with a sterile pipette.

ASSAY PROCEDURE—

Carry out the assay in ordinary chemical or bacteriological test-tubes (18–20 mm. × 150–160 mm.). Pipette 5 ml. portions of the basal medium to be used into each of a series of tubes. Add graduated doses (see below) either of the standard solution of riboflavine or of a test extract *and make up to a total volume of* 10 *ml. with distilled water.* Plug the tubes with cotton-wool, or cap with inverted specimen tubes, and sterilise at 10 lb. pressure for 10 minutes. Allow to cool and inoculate when convenient.

All operations of preparing and adding the standard riboflavine solution and extracts to the medium, cooling the medium after sterilisation, and inoculation must be carried out in a dim light.

The standard curve may be established with the following dose-levels of standard solution of riboflavine—Blank (nil), 0·5 ml. (0·05 μg.), 1·0 ml. (0·10 μg.), 1·5 ml. (0·15 μg.), 2·0 ml. (0·20 μg.). The test curve should be established with such quantities of the test extract as are expected to contain corresponding amounts of riboflavine. All dose-levels should be set up at least in triplicate.

Incubate the tubes at 37° C. or 40° C.* for 72 or 96 hours. Then titrate them with 0·1 *N* sodium hydroxide, using bromothymol blue indicator. A comparator is used as follows:

Prepare a buffer solution of *p*H 6·8 by mixing 50 ml. of 0·2 *M* monopotassium phosphate with 23·65 ml. of 0·2 *N* sodium hydroxide and dilute to 200 ml. Arrange in the comparator four tubes as shown in the following diagram, in which the line of sight of the operator is towards the top of the page—

A	B
C	D

Tube A—distilled water.
Tube B—20 ml. of buffer solution + 20 drops of indicator.
Tube C—assay tube + 10 drops of indicator.
Tube D—assay tube at same dose-level, no indicator.

Titrate tube C with 0·1 *N* sodium hydroxide and for every ml. or part of a ml. of standard alkali used add an extra drop of indicator. Add to tube D slightly less than the same amount of standard alkali. The titration is completed when the colours seen through C and D exactly match. The remaining tubes are titrated by direct matching against tube C.

For the computation of results see page 403.

MICROBIOLOGICAL ASSAY OF NICOTINIC ACID

ORGANISM—

The organism used for the assay of nicotinic acid is:

Lactobacillus arabinosus 17–5.

* The exact temperature is relatively unimportant, but conditions should be such that all tubes in an assay are maintained at the same mean temperature during the incubation period.

CULTURE—

Stab cultures are carried in the same yeast-water - glucose - agar medium as described for *L. helveticus* (page 398).

INGREDIENTS AND REAGENTS—

Many of the ingredients and reagents required are described under this heading in the assay method for riboflavine (page 399). Additional ingredients are as follows:

Vitamin-free Casein Hydrolysate—Use solid spray-dried casein hydrolysate.*

dl-*Tryptophan Solution*—Boil 2 g. of *dl*-tryptophan* with a little water and add concentrated hydrochloric acid drop by drop until it is dissolved. Make up to 500 ml. and store in a refrigerator (it keeps more or less indefinitely). This solution is equivalent to 2 mg. of *l*-tryptophan per ml.

Adenine + Guanine + Uracil Solution—Dissolve 0·1 g. of each material* by heating in water with concentrated hydrochloric acid added drop by drop. Make up to 100 ml. Store in a refrigerator (it keeps a fortnight).

Xanthine Solution—Dissolve 0·1 g. of xanthine* in a little concentrated ammonia solution and make up to 100 ml. Store in a refrigerator (it keeps a fortnight).

Aneurine Solution—Dissolve 0·1 g. of aneurine hydrochloride† in 2% hydrochloric acid and make up to 100 ml. Dilute so that the final solution contains 100 μg. per ml. (it keeps more or less indefinitely).

Calcium d-*Pantothenate Solution*—Dissolve 0·1 g. of calcium *d*-pantothenate* or 0·2 g. of *Ca*-dl-*pantothenate* in 100 ml. of water. Dilute so that the solution contains 100 μg. of calcium *d*-pantothenate per ml. Store in a refrigerator (it keeps a week).

Pyridoxine Solution—Dissolve 0·1 g. of pyridoxine hydrochloride* in 100 ml. of water. Dilute the solution so that it contains 100 μg. per ml. Store in a refrigerator (it keeps a week).

p-*Aminobenzoic Acid Solution*—Dissolve 0·1 g. of *p*-aminobenzoic acid* in 2 ml. of glacial acetic acid and make up to 100 ml. with water. Dilute so that the solution contains 100 μg. per ml. Store in a refrigerator (it keeps a week).

Biotin Solution—Dissolve 25 μg. of biotin* in 250 ml. of water after addition of 1 ml. of inorganic salt solution A. This solution contains 0·1 μg. of biotin per ml. (it keeps for three months).

Stock Solution of Nicotinic Acid—Dissolve 0·1 g. of nicotinic acid in 100 ml. of water (the solution keeps a week).

Standard Solution of Nicotinic Acid—Dilute the stock solution to contain 0·1 μg. per ml. This solution should be prepared afresh each day.

BASAL MEDIUM—

If 100 assay tubes are to be set up, the following amounts of the different constituents should be taken.

TABLE III

Vitamin-free casein hydrolysate	10 g.
Cystine solution (page 399)	50 ml.
dl-Tryptophan solution	50 ml.
Glucose	20 g.
Sodium acetate (hydrated)	33 g.
Xylose	1 g.
Ammonium sulphate	3 g.
Sodium chloride	5 g.
Adenine + guanine + uracil solution	10 ml.
Xanthine solution	10 ml.
Aneurine solution	1 ml.
Ca *d*-pantothenate solution	1 ml.
Riboflavine stock solution (page 399)	8 ml.
Pyridoxine solution	1 ml.
p-Aminobenzoic acid solution	1 ml.
Biotin solution	4 ml.
Inorganic salt solution A	5 ml.
Inorganic salt solution B	5 ml.
Glass-distilled water to	500 ml.

* Obtainable at present from Ashe Laboratories, Ltd.
† Obtainable at present from Roche Products, Ltd.

After mixing, adjust the medium to *p*H 6·8 with approximately 0·5*N* sodium hydroxide, using bromothymol blue as external indicator.

PREPARATION OF EXTRACTS—

Hydrolyse 5-g. samples of material, which should be finely ground, with 50 ml. of *N* hydrochloric acid (*not* 0·1*N*) for 15–20 minutes at 15 lb. pressure in an autoclave, cool, add 2 ml. of 2·5*M* sodium acetate, adjust the *p*H to 4·5 with strong sodium hydroxide solution, using bromocresol green as external indicator, and make up to 500 ml. with water. Filter, take an aliquot, readjust *p*H to 6·8 with 0·5 *N* sodium hydroxide, using bromothymol blue as external indicator, and make up to such a volume that each ml. of the diluted extract contains approximately 0·05 μg. of nicotinic acid.

In the case of samples with a high nicotinic acid content, *e.g.*, wheat bran, yeast, meat extracts, etc., only 1 g. should be weighed out to prepare the initial extract. Samples with a high fat content, *e.g.*, meat, germ, soya bean, maize, oats, etc., should be given a short preliminary extraction with light petroleum (b.pt. 40° to 60° C.).

SUB-CULTURE FOR INOCULUM—

Take 5 ml. of the riboflavine basal medium X or Y and add 2·5 ml. of Standard riboflavine solution and 2·5 ml. of distilled water (or take 5 ml. of the nicotinic acid basal medium + 2 μg. of nicotinic acid and make up to 10 ml. with water). Sterilise at 10 lb. pressure for 10 minutes. After cooling, inoculate the tube with a portion of the stab culture (page 402) by means of a sterile needle and incubate for 18 hours at 37° C.

INOCULUM AND ASSAY PROCEDURE—

As described for the assay of riboflavine (see page 401) with the following modifications:

(1) It is particularly important that the tubes be sterilised at only 10 lbs. pressure for only 10 minutes.
(2) It is not necessary to work in a dim light, since nicotinic acid is not photo-sensitive.
(3) The standard curve may be established with the following dose-levels of standard solution of nicotinic acid—Blank (nil), 0·5 ml. (0·05 μg.), 1·0 ml. (0·10 μg.), 1·5 ml. (0·15 μg.), 2·0 ml. (0·20 μg.), 2·5 ml. (0·25 μg.), 3·0 ml. (0·30 μg.). The test curve should be established as with the riboflavine assay.

For the computation of results see below.

METHOD OF COMPUTATION OF RESULTS

Originally the method of computing the results was to draw a graph for the standard curve and to read from this the amount of the vitamins found in the various amounts of the food extracts. Provided there was general agreement at the various levels and no evidence of a tendency to give regularly high or low results as the quantity of extract increased, an average figure was accepted. This method is illustrated in the paper by Kent-Jones and Meiklejohn.

It is now preferred to use the slope-ratio method, which has been discussed by Wood[8] and may be conveniently and advantageously employed in all assays in which the points produced by plotting "dose of Std. Prep." against "response" (or magnitude of the effect produced) lie on, or close to, a straight line. This method has been adopted in this Report. The successive steps necessary are as follows:

(1) Plot on squared paper the mean response (mean titration in ml. of standard sodium hydroxide solution) at each dose-level of Std. Prep. against the dose in μg. The "blank" or zero-dose response should be included. It is usual to measure the response vertically and the dose horizontally. Any convenient scale may be used.

(2) Draw the straight line which lies most evenly between the plotted points. In most cases this may be done with negligible error by inspection, but the equation of the "best" line can be exactly calculated, if necessary, by the method of least squares, details of which will be found in textbooks on statistics (*e.g.*, Fisher's "*Statistical Methods for Research Workers*," Section 26). If the point corresponding to the "blank," or that corresponding to the maximum dose, is obviously not on the "best" line through the other points, ignore it.

(3) On the same graph, plot the points for the various doses of the Test Prep. Any scale may be used provided that the zero corresponds with that of the Std. Prep. graph.

Draw the "best" line through these points (including the "blank" point if it has been used in drawing the Std. line) as before.

(4) Determine the slopes of the two lines, *i.e.*, for each line in turn (*a*) read off from the graph the values of dose and of response corresponding to any two convenient points on, and at opposite ends of, the line,* (*b*) subtract the lower dose from the higher dose and the lower response from the higher response to obtain the increase in response for a certain increase in dose, (*c*) calculate therefrom the slope, *i.e.*, the increase in response per unit of Std. Prep. or of Test Prep. as the case may be (convenient units are 1 μg. of Std. Prep. and 1 g. of Test Prep.).

(5) Divide the slope of the test line, in ml. per g., by the slope of the standard line in ml. per μg. to obtain the vitamin content of the Test Prep. in μg. per g.

(6) If the assay is perfectly valid, the two lines should in theory intersect exactly on the vertical axis of response. In practice, a small divergence may occur; but if the distance between the intercepts of the two lines on the vertical axis is more than a few tenths of a ml., the assay is of doubtful value.

The following numerical examples, one of a valid and the other of an invalid assay, may make the procedure clearer.†

The second example is given to illustrate how invalidity is detected, and no significance should be attached to the fact that it happens to be an assay of riboflavine. If the methods of this Report are followed precisely, assays of riboflavine are in general no more likely to be invalid than are assays of nicotinic acid. It should also be noted that although the result of an assay which is moderately invalid (as in the example) will be unreliable, it will not be widely different from the truth; it will almost certainly be of the correct order of magnitude.

Example 1—The dose-levels used and the mean titrations obtained in an assay of a meat extract for nicotinic acid were as follows:

TABLE IV

Volume of Std. Soln. of nicotinic acid, ml.	0·0	0·5	1·0	1·5	2·0	2·5
Corresponding wt. of nicotinic acid, μg.	0·0	0·05	0·10	0·15	0·20	0·25
Mean titration, ml.	1·45	3·35	4·85	6·15	7·85	9·45
Volume of test extract, ml.	1·0		1·5		2·0	
Corresponding wt. of meat extract, mg.	0·2		0·3		0·4	
Mean titration, ml.	4·85		6·40		7·70	

The Standard points, including the "blank," when plotted (see graph) all lie very close to a straight line, while the three Test points and the "blank" also are fitted well by another straight line. The values of response, as read off by eye from the Std. line, which correspond to zero-dose and to 0·25 μg. of nicotinic acid, are respectively 1·60 ml. and 9·43 ml., giving an increment of 7·83 ml. for 0·25 μg. or a slope of $7{\cdot}83 \times 4 = 31{\cdot}32$ ml. per μg. Similarly, the values of response read off from the Test line which correspond to zero dose and to 0·4 mg. of meat extract are 1·55 ml. and 7·85 ml. respectively, giving an increment of 6·30 ml. for 0·4 mg. or a slope of 15,750 ml. per g. The mean nicotinic acid content of the meat extract is given by the ratio of these two slopes, *i.e.*, $15{,}750/31{\cdot}32 = 503$ μg. per g. This result appears very reliable, since not only do all points lie quite near to the appropriate line, but the two lines are separated at their intersections with the "response"(vertical) axis by a distance corresponding to only 0·05 ml. approximately.

The estimates of the nicotinic acid content of the extract given by direct reading at the three dose-levels used are 500, 513, 490, with a mean of 501 μg. per g., in very good agreement with the slope-ratio estimate.

Example 2—In an assay for riboflavine the experimental data in Table V were obtained.

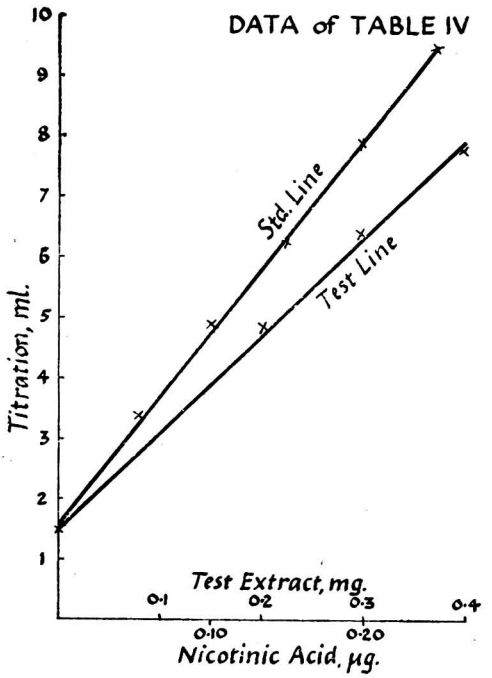
TABLE V

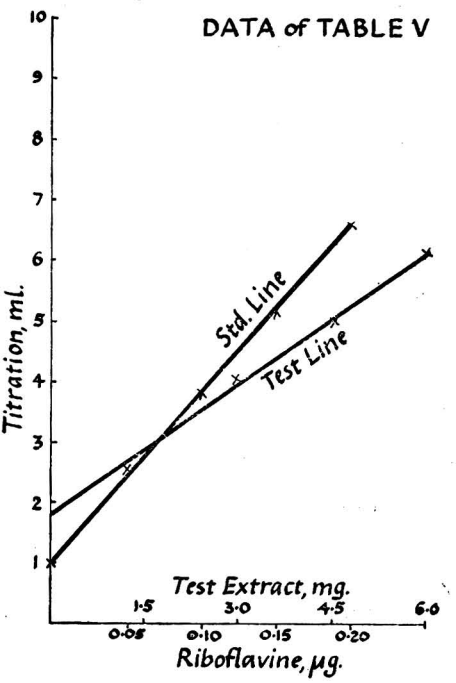
Riboflavine, μg.	0·0	0·05	0·10	0·15	0·20
Mean titration, ml.	1·06	2·50	3·80	5·12	6·59
Test extract, mg.	3·0		4·5	6·0	
Mean titration, ml.	4·02		4·95	6·11	

* These values of response, as read off from the line, must not be confused with the *experimental* mean responses at the same two dosage-levels, which will differ slightly unless they happen by chance to be exactly on the line.

† The experimental data have been previously published, in the first case by Kent-Jones and Meiklejohn[7] and in the second case by Wood.[8]

The plotted points will again be seen to be well fitted by two straight lines, but this time the Test line does not pass through or even near the "blank." The slopes of the Std. and Test lines are 27·65 ml. per μg. and 696·7 ml. per g. respectively, and their ratio is 25·2 μg. per g. The direct-reading method of estimating the result gives 35·7, 31·6 and 30·7, with a mean of 32·7 μg. per g., which is 30% higher than the "slope-ratio" estimate. But the regular downward trend of the individual direct-reading estimate would indicate to an experienced user of this method of calculation that the assay was unreliable. Using the slope-ratio method, it will be noticed at once that the distance between the two lines where they intersect the vertical axis is about 0·85 ml., which is much too large. The assay is for this reason invalid and the result, however derived, unreliable, though there is some ground for believing the slope-ratio estimate to be nearer the truth.





Occasionally non-linear responses are obtained with both Std. and Test Preps., and the slope-ratio method cannot therefore be applied. Such assays are not necessarily invalid, and an estimate of potency may be made by the direct reading method, provided always that good concordance is obtained at the different dosage levels and there is no evidence of an upward or downward trend in the results.

The choice of procedure in computing the result may thus be summarised as follows. If the points obtained by plotting the Standard mean responses against the corresponding doses lie close to a straight line it is best to plot the Test points also and to follow the slope-ratio method described on pp. 403–404 and illustrated in the examples. The direct-reading method is inferior but may be used if desired. If, on the other hand, the Standard points lie not on a line but on a smooth curve, the slope-ratio method is inapplicable and the direct-reading method must be employed. In this case the reliability of the mean result must be assessed in the light of the concordance between the separate results at the various dose-levels and the absence from them of any regular "drift" of the kind found in Example 2.

Some workers modify the experimental technique and design according to the suggestions of Wood. The essential features are, first, that a trace of the vitamin being assayed is added to the basal medium if necessary to ensure the "blank" falling on the line through the other Standard points; and secondly, that the Test and Standard lines are each defined by only two dose-levels (in addition to the "blank," which is now on both lines), *viz.*, 0·1 and 0·2 μg. of total riboflavine or of nicotinic acid and corresponding "full" and "half" doses of the Test extract. Such a design gives increased accuracy, simplifies the experimental work, and

enables the validity of the assay to be rapidly checked statistically if desired. For further details, Wood's (1946) paper should be consulted.

TABLE VI

COLLABORATIVE RESULTS FOR MICROBIOLOGICAL ASSAY OF RIBOFLAVINE

(All values in μg. per g.)

Laboratory	Basal medium	80% flour	Germ tailings	Malted barley	Malt extract	Malt extract and oil	Meat extract	Dried yeast	Yeast extract
1	X	0·55	3·1	2·7	2·8	2·4	31	44	64
	Y	0·55	3·6	3·0	3·1	2·7	32	43	65
2	X	0·50	2·9	2·1	2·6	2·3	25	34	72
	Y	0·52	3·6	2·8	3·2	2·4	23	36	70
3	X	0·64	2·5	2·7	2·7	1·9	33	40	62
	Y	0·66	3·1	2·9	3·0	2·0	—	42	70
4	X	0·55	4·5	3·1	2·5	—	29	33	—
	Y	—	4·7	3·3	—	—	—	40	66
5	X	0·61	3·6	2·5	2·9	2·4	25	41	64
	Y	—	—	—	—	2·4	—	—	64
6	X	0·55	3·1	2·5	3·2	2·3	28	44	62
	Y	—	3·1	—	—	2·4	—	—	66
7	X	0·56	3·6	2·6	3·0	2·8	33	44	63
	Y	—	3·9	—	—	3·0	—	52	—
8	X	0·51	3·0	2·4	2·9	2·5	31	41	64
	Y	—	—	—	—	—	—	—	—
9	X	0·50	4·0	2·6	2·7	2·4	31	48	61
	Y	—	4·3	—	—	2·7	—	42	66
Mean result	X	0·552	3·37	2·58	2·81	2·38	29·6	41·0	64·0
,, ,,	Y	0·577	3·76	3·00	3·10	2·51	27·5	42·5	66·7

TABLE VII

COLLABORATIVE RESULTS FOR MICROBIOLOGICAL ASSAY OF NICOTINIC ACID

(All values in μg. per g.)

Laboratory	80% flour	Germ tailings	Malted barley	Malt extract	Malt extract and oil	Meat extract	Dried yeast	Yeast extract
1	15·3	136	103	93	84	1040	353	553
2	14·8	126	100	89	76	1100	352	524
3	16·6	162	103	106	92	1100	354	590
4	14·1	129	100	95	—	1080	331	537
5	16·8	155	114	101	90	1190	363	592
6	16·4	132	99	100	83	1180	354	568
7	15·0	135	92	95	74	1030	393	598
8	17·0	149	87	100	89	1080	346	605
9	15·5	158	119	102	90	1120	337	594
Mean result	15·72	142·4	101·9	97·9	84·8	1102	353	573

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The Genetical Requirements of Bio-Assays with Higher Organisms

By KENNETH MATHER

(*Read at the Meeting of the Biological Methods Group in London on May* 27, 1946)

To the analyst using the method of bio-assay, laboratory animals and plants are measuring instruments, and, as of other instruments, he requires that they be both suitable for his purpose and efficient in use. No one would use a barometer for measuring temperature, and similarly an assay organism must be one capable of responding to change in dose of the substance whose concentration is to be measured. Furthermore, since the measurement will involve comparison of the responses given by several, or even many, individuals, efficiency depends on uniformity of response, for in no other way can comparable and repeatable responses be secured.

Every biologist knows that complete uniformity of living organisms is impossible of attainment; there are too many circumstances and chances of development over which no control can be exercised. Equally, however, he knows that many agencies from which variation arises can be controlled, at least in a large measure, and that a nearer approach to uniformity can be gained thereby. The proper care of the organism, its feeding, housing and protection from disease, is obviously necessary in the pursuit of uniformity. We know that individuals raised under varying environmental circumstances and exposed to varying risks will show the effects by varying amongst themselves to a corresponding degree. The organism is, however, not the mere product of its environment; the hereditary materials with which it was endowed by its parents also play their part. Variation of the genotype must therefore also contribute to the causation of variation in the phenotype, and the approach to uniformity in assay organisms will depend on control of the genotype as well as of the environment. Environment and genotype are complementary in the production of the phenotype. Both must be acting throughout the life of any animal or plant, and increase in attention to one will not compensate for neglect of the other. Controls of the two are, however, established by different means, and while the ways of standardising the environment are reasonably familiar, at least in principle, to most of us, those of standardising the genotype demand a knowledge of genetics which is unfortunately imparted only too rarely in the teaching establishments of this country.

Before we proceed to a consideration of the genetical techniques necessary for our purpose, and of the genetical principles upon which they depend, it will be profitable to consider the nature of the task which is before us. What kind of variation arises from differences in the genotype? Indeed, what evidence is there that variation in the responses, upon which biological assays depend, is materially increased by differences in the genotype? For reasons which seem fairly obvious, little attention has been paid by geneticists to the inheritance of these responses. Their determination is much more tedious than is the classification of, say, eye colour or hair type, and the final reaction upon which an assay depends not uncommonly involves death. Nevertheless a little evidence exists and we may also draw on the wide experience of geneticists with other characters in a great variety of organisms.

Even in absence of direct experimental evidence, no geneticist would doubt that heritable variation exists between individuals in their responses to the substances for whose assay the species may be used, or that this variation will, if uncontrolled, lead to a serious loss of efficiency in assays. Such confidence is not unfounded, for in no instance where adequate tests have been made on material exhibiting a particular character has the character failed to show heritable variation. The list of contents of Grüneberg's *"Genetics of the Mouse"*[1] will serve to illustrate the range of characters known to show genetical variation in one animal. It includes colour, pattern and texture of the hair, endocrine organs, brain and nerve organs, blood, skeleton, alimentary tract, urogenital systems, size and fertility, serological properties, development of spontaneous and transplanted tumours and resistance to diseases caused by bacteria, viruses and tapeworm infestation. This list could be matched from any organism to which similar attention has been devoted.

With such a background, little direct evidence is required to convince us that heritable variation exists for the responses used in assays. It was provided as long ago as 1927, when

Winton[2] showed, first, that comparable male and female rats differed in their lethal doses of red squills; and secondly, that inbred rats, which are genetically more uniform than rats of mixed parentage, were equally more uniform in their response to the same poison. It must be stated, however, that a portion of the variation in Winton's mixed rats may well have been environmental in origin, since these animals came from various sources and had doubtless been raised originally under somewhat different conditions. The difference between males and females of the inbred line could not be attributed to such causes and must have been essentially genetical in nature. The preference for inbred material, which is now gaining ground, has presumably been based on experiences of this kind.

Granted that heritable variation occurs, it may take either of two forms. It may be due to a few gene differences each of large effect. Such a difference was that found by Mendel between his tall and dwarf peas, and laboratory genetics has revealed a wealth of major genes, of this kind, in a great variety of organisms. The science of genetics is founded upon the study of these genes, the handling of which is now fully understood. Variation due to their action offers no problem; the true breeding types can easily be recognised and stocks uniform for the desired allelomorph built up from them. Such genes have recently assumed great significance in bio-assay, as the work of Beadle and others has shown that fungi differing from a wild strain in one such gene may be incapable of carrying out a particular chemical synthesis. The possibility is thereby opened up of producing mutant forms incapable of synthesising a particular substance essential to their full development and therefore utilisable for the purposes of assaying that substance in the medium upon which they are grown (see Pontecorvo, this Vol., p. 412). In the same way susceptibility or resistance to *Bacillus piliformus* infection in the mouse, for example, appears to depend on one gene of major effect. If it were desired to test the adequacy of some measure of controlling this infection, the choice of a stock true breeding for the recessive allelomorph of this gene would be essential or the results might be rendered worthless by heritable resistance.

The second type of heritable variation is, however, likely to be of more general concern to those who aim at producing efficient test organisms. It is that due to the simultaneous action of a number of genes having small, similar and supplementary effects—quantitative or polygenic variation, as I have called it.

Any character apparently can show both major genic and polygenic variation, but, whereas the former is generally rare, the latter is ubiquitous. Thus dwarfs, most likely due to the segregation of major genes, are known as rarities in man, but the variation in stature with which we are all familiar by daily observation is polygenic in its hereditary causation. Furthermore, the degree of manifestation of a major difference will itself be under polygenic control.

Thus the problem of handling polygenic variation faces us at all turns. At the same time it is a more troublesome one than that posed by major genes. The individual components of a polygenic system cannot be distinguished consistently by their action on the phenotype—nor can the allelomorphs of one of these genes—since the effect of one gene difference may be masked both by its fellows and by non-heritable agents. The methods of achieving genetic uniformity cannot therefore be based on recognition of the constitution of an individual in respect of each gene it bears, either directly through the phenotype, or by means of a simple breeding test. They must depend on the utilisation of principles known to be of general application in a way independent of direct knowledge of the genotype.

With many plants, including fungi, the production of a number of test subjects known to be genetically alike within narrow limits, may be accomplished by the practice of asexual or clonal propagation. Nuclei related to one another solely by mitosis are identical, apart of course from mutation. Mutation is an inescapable source of heterogeneity and will thus set an untransgressable upper limit to any approach to homogeneity; but it is sufficiently rare in occurrence for this limit to approximate closely to complete uniformity. With this proviso, asexual reproduction, and also parthenogenetic reproduction, which avoid meiosis and fertilisation, will yield genetically uniform material, irrespective of the genetical constitution of the individual so propagated. Heterozygosity is no obstacle, for meiosis, at which segregation and recombination occurs in heterozygotes, is avoided. In higher animals clonal propagation is rare, though it is known. Identical twins in man and cattle are related in this way. Their consequent value to those interested in assessing separately genetical and environmental effects has been widely recognised. In armadilloes the scope is greater, as eight young, all genetically identical by virtue of their clonal relations, are said to follow each conception.

Armadilloes, cattle and men are, however, seldom used as assay organisms and a more general method of achieving genetical uniformity is needed. This is afforded by inbreeding: in hermaphrodite organisms by self-mating, which is the most efficient method, or in dioecious organisms by the next best means, brother-sister or parent-offspring mating. It is easy to see that under a system where close relatives are mated the proportion of heterozygotes in a population rapidly decreases and the proportion of homozygotes correspondingly increases. Like homozygotes when bred together produce nothing but their kind. Heterozygotes on the other hand always throw a proportion of homozygotes. Two points, however, require notice in relation to inbreeding. In the first place, it is a group of homozygotes, not a particular homozygote, which replaces the heterozygotes in an inbred population. The various lines will ultimately be homogeneous within themselves, but they will differ one from another. Genetically homogeneous material can thus be secured only within the line, though any line once it has attained homozygosity may be subdivided for breeding purposes without an early loss of homogeneity. Even so, such sub-lines will eventually drift apart under the action of mutation, so that it is always better to avoid mixing individuals from long separated ancestries, in spite of their ultimate descent from a common homozygous stock.

The second point concerns the rate of attainment of homozygosity. The calculations relating to this question, published by various authors, generally assume that all genotypes are equally viable and fertile and are taken at random for breeding purposes. If breeding is preferentially from the more homozygous individuals, homozygosis will obviously be attained more rapidly than would be expected from these calculations; but if the heterozygotes form a greater proportion of the parents than equal choice would permit, homozygosis will be attained more slowly. In the extreme case, where only heterozygotes are selected for breeding, the state of full homozygosis will never be attained.

The risk of a mating system, designed to produce homozygosis, failing to do so by reason of such selection is not fanciful. Sismandis[3] brother-sister mated *Drosophila* for 14 generations, a period which would give a high degree of homozygosis in the absence of selection, without attaining anything near to homozygosis. He had been practising selection for the number of scutellar bristles, part of the time for increase and part of the time for decrease. This selection must consistently have resulted in heterozygotes being used as parents of the next generation. Any conscious choice of parents, except that for reduced variability of their offspring, might well have similar consequences. And even where no conscious choice is exercised the risk is still present, as Philip *et al.*[4] have found; for the heterozygotes may be more vigorous and survive the competition of their siblings and the general rigours of life more often than do their homozygous fellows.

The danger of such natural selection partially vitiating an inbreeding programme is the greater, since it is commonly observed in normally cross-breeding organisms, both plant and animal, that inbreeding leads to less vigorous and less fertile individuals. Sometimes this inbreeding depression, as it is called, is very great, sometimes it is much less noticeable or may even be absent, as in the rats recorded by King[5]; but, wherever it occurs, the efficiency of the inbreeding programme is likely to be reduced to a corresponding degree.

A great depression of vigour consequent on inbreeding can also have the consequence of rendering the inbred individuals less desirable for use in assay work. The loss of vitality might outweigh the gain in uniformity and lead to a decline in overall value. Fortunately, though inbreeding depression is widespread, it is not always of such a crippling magnitude, and, provided that sufficient inbred lines are initiated, some of them should retain enough vigour to be satisfactory. The difficulty will vary from species to species, and it appears that in small mammals the production of a reasonably vigorous inbred line is not over-difficult.

An alternative is, in any event, always open. Instead of using the inbred individuals themselves as the test subjects, we may use offspring of the first cross between two lines. These, though heterozygous, will be as uniform genetically as their parents were and, provided that appropriate lines are used to make the cross, will show full vigour. The superiority of F_1's between inbreds can be very marked. In maize, for example, the combination of vigour and uniformity has led to synthetic hybrids replacing the older open bred varieties in a large part of the American crop.

A further possible advantage of F_1's over their inbred parents has recently come to light as a result of experiments with the Chinese Primrose. This plant is adapted to cross-breeding by virtue of its heterostyly. Inbred lines have, however, been produced through continued

artificial self-pollination, and these lines now show all evidence of being genetically homogeneous. Nevertheless, at least in some important floral characters, they show a marked variation, though one that is obviously not genetical in its causation since it appears as much between flowers of one plant as between those of different plants. This variation is much reduced both within and between plants in F_1's between the lines (Mather, unpublished). Thus inbreeding can have the further consequence of rendering the individual less able to accommodate itself to chance changes of its circumstances, can lead in fact to a reduction in its developmental stability. Stability is regained in heterozygotes produced by crossing the inbred lines. The extent of this advantage of F_1's over their parental inbreds, for use in work which puts a premium on uniformity, cannot be gauged until the phenomenon has been more widely explored over a range of species, but the possibility of its utilisation is one which should not be ignored.

The genetical principles upon which the provision of adequate assay organisms can be based are thus clear. We know how to secure genetical uniformity by inbreeding, and we can recognise the difficulties and dangers that may arise in inbreeding programmes. We also know how some of these troubles can be overcome by the production of F_1's from inbred lines. The experiments from which these principles are derived are of diverse kinds, carried out on a wide variety of organisms, both animal and plant. The principles are therefore firmly grounded and we may apply them without misgiving. The way of applying them must, however, vary with the particular genetical features of the species in question. This information is still scanty for the characters and organisms of interest to the assayist. Until it becomes more fully available we must be working to some extent in the dark. There is an obvious need for knowledge concerning the inheritance of variation in the responses upon which assays are based, of behaviour under inbreeding and of the comparison between F_1's and their inbred parents. Any body which sets out to supply high quality living material for the assayist should seriously consider undertaking investigations of the kind necessary to provide this knowledge. In doing so it would benefit itself by providing means of improving its own products and it would be performing a more general service in extending genetical knowledge to still wider fields. The reports of the assayist on the performance of the material with which he was supplied, if made available to the breeding establishment, would ensure both that the genetical operations were based on wide testing and that the essentially practical aim of the programme was not overlooked. At the same time the genetical information gained thereby would have its repercussions over wide fields—as wide, in fact, as those that have jointly given us the genetical basis for initiating a programme aimed at providing genetically adequate assay organisms. Genetical organisation is fundamentally the same for all higher organisms, plant or animal, and for many of the lower ones. Experience with one helps us to a better understanding and greater control of them all.

A final example will serve to illustrate this point. It was observed with *Drosophila* that selection for one character, in this particular instance hair number, resulted also in a correlated response in other characters, such as fertility, for which no selection was practised directly (Wigan and Mather).[6] This finding has been extended to fishes by Svärdson,[7] and recent work (Mather and Harrison, unpublished) has shown that such widely diverse characters as fertility, mating behaviour and spermatheca structure may be affected simultaneously in flies. It has also been confirmed that the correlated responses are due to the linkages of the genes affecting the characters which show them to those genes that control variation of the character for which selection is primarily exercised. Selection affecting one set of genes also affects other genes linked to them. Attempts are now being made to apply this principle in plant improvement, though it is as yet too early to say with what success.

It may well be that the principle can also be put to good use in raising homozygous lines by inbreeding. If in small mammals, for example, the inbreeding programme were started with a piebald stock, whose pattern shows considerable heritable variation, rather than with animals whose hair variation is completely marked by a gene such as that for albinism, selection could be practised in favour of those matings giving the progenies least variable in respect of this easily recordable coat character. Such selection would favour homozygosity for the genes controlling pattern. Homozygosity of such genes affecting the assay character as were linked to the pattern genes should also be favoured, though admittedly to an extent reduced by the freedom of recombination between them and the pattern genes. In this way it might well be possible to use an easily recordable character of no direct interest in order to speed up the attainment of homozygosity for the genes controlling the character of primary

concern, through ensuring that the inbreeding was not being vitiated by some unsuspected selective force.

The proof of the pudding lies in the eating, and until it has been tried we cannot be confident that this scheme would be helpful. The necessary test should be well within the power of an establishment breeding animals for assay work, and even a negative result would have its genetical interest, while a positive finding would have a clear practical importance. It is only by undertaking such experiments, suggested by experience with other organisms, that the production of assay material can take full advantage of the tools that genetics offers. The results of such experiments would go far towards repaying any debt that may be contracted to genetics.

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The Genetical Aspects of Bio-Assays with Micro-Organisms

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MOST microbiological assays are based on a quantitative and specific growth response of populations of suitable micro-organisms to particular substances. The response may be stimulation of growth, as in the assays of pantothenic acid with a mutant strain of the mould *Neurospora* or of tryptophan with *Lactobacillus casei*, or it may be inhibition of growth, as in the *Staphylococcus* assay of penicillin. In both instances it is clear that what we want is maximum specificity of response, *i.e.*, ideally, response to one and only one substance, and minimum variability in this response.

There are accordingly two main genetical aspects of microbiological assays. One is the control of specificity in the response, and this links up with the recently discovered possibility of deliberately producing strains in which the response is as specific as biologically possible. The other aspect is the control of variability.

Part of the variability in a microbiological assay is due to inheritable differences between cells of the micro-organism used. The control of this inheritable variability in micro-organisms is as much the job of genetics as is its control in higher organisms. It is often said, however, that to speak of the "genetics" of micro-organisms without sexual stages (like most moulds, let alone micro-organisms without a proper nucleus like bacteria or, to go even further, viruses) is a contradiction in terms. I do not agree with this view. Modern genetics has developed techniques and concepts applicable to all kinds of particulate cell components having the two essential properties of the genes: mutation and reproduction in the mutated form. The properties and transmission of viruses are as much the concern of modern genetics as the properties and transmission of genes.

The control of variability in such micro-organisms as moulds and yeasts presents aspects somewhat different from those in higher organisms. In the latter, every individual starts from the fusion of two cells of which each contributes one set of genes. Even in a self-fertilised species, like the pea, the two germ cells will very seldom be identical in their genes. Each individual will therefore be (*a*) heterozygous for some or many of its genes, and this will result in variability *in later generations*, and (*b*) genetically different from every other individual. In the moulds and yeasts we can easily overcome this original source of variability by starting a strain from a single cell of absolute genetical purity, *i.e.*, from a cell with only one set of genes. This we do by isolating a single ascospore. But the cells of such a "pure" strain are

subject to mutation just as much as those of our inevitably heterozygous higher organisms; it takes place in both at rates as far as we know not substantially different when calculated per cell-generation. If we repeat the process of starting a new culture again from a single spore, this spore may be, and almost always will be, on account of mutation, genetically different from that of the previous generation. This difference will be immediately expressed in the culture we rear from it, even though it may be small enough to pass unnoticed. What we gain in uniformity of a culture, if we start from a single spore, we cannot, therefore, preserve between successive cultures if we go on single-sporing. Thus in higher organisms variability due to heterozygosity in previous generations can only be reduced, but not completely eliminated, by inbreeding, whilst in certain micro-organisms it can be eliminated by single-sporing. On the other hand, in higher organisms variability due to new mutation is to a large extent buffered, and its effects delayed, owing to the fact that the cells carry two sets of genes.

With bacteria genetic investigation has barely begun, but it promises to be extremely fruitful. Because of its implications for the most important microbiological assay of the present time, I must mention the work of Demerec[1] on the mutation of *S. aureus* towards resistance to penicillin. His results show that the resistant *Staphylococci* arise repeatedly, in a sensitive strain, *not* as a response to penicillin; the latter acts only as a selective agent. There is at present no reliable criterion on which to base a method for keeping down the frequency with which resistant organisms arise in a culture. It is likely, on *a priori* grounds, that even the freeze-drying technique of preserving strains of micro-organisms will not appreciably reduce this frequency. But this will have to be settled experimentally. A working hypothesis about the mechanisms of heredity and variation in bacteria is that they are not substantially different from those based on "heterokaryosis" occurring in asexual moulds, *i.e.*, a particulate mechanism with each kind of particle or "gene" present in the cell in many replicas.

I come now to the second, and potentially more important, aspect of the genetics of micro-organisms in relation to assays: namely, the possibility of producing strains best suited for a particular assay. This possibility is only a by-product of advances in our knowledge of the genetical control of biochemical reactions, to which advances the work of Beadle and his collaborators on the mould *Neurospora* has made a fundamental contribution. Briefly, the position is that for most water-soluble diffusible essential metabolites for which an assay is wanted, the geneticist may be able to "create" a micro-organism with the necessary specificity of response.

In organisms as diverse as man, the rabbit, the fly *Drosophila*, the sweet pea, the mould *Neurospora* and yeast, the genes, or at least most genes, act by controlling specific steps in metabolic reactions, presumably *via* specific enzymes. By means of irradiation it is possible to inactivate a gene and therefore block the step controlled by that gene in a chain of metabolic reactions. This block is analogous to, but far more specific and efficient than, one produced with an enzyme poison. In addition, once a gene has been inactivated in a cell, all descendants of that cell will carry the inactivated gene. We can therefore produce a strain in which all cells carry the inactivated gene and which will be unable to perform the corresponding metabolic process. Two examples will illustrate this point. In the biological synthesis of arginine by *Neurospora* there are two genes controlling the step ornithine→citrulline and one controlling the step citrulline→arginine. In the synthesis of tryptophan,[2] also by *Neurospora*, there is one gene controlling the formation of anthranilic acid from an unknown precursor, and another controlling the formation of indole from anthranilic acid. Many other examples of this kind are at present known. If the step blocked is one in the synthesis of an essential metabolite, obviously if we want the strain to grow at all it will be necessary to supply this metabolite in the culture medium, or any one of the intermediates between this and the step in the chain of reactions which has been blocked. In other words, the block will determine a nutritional requirement, and this is what we need for an assay.

Even though we are not entitled to think in terms of genes in bacteria, the results obtained by Tatum and collaborators[3,4] show that these organisms react in the same way as moulds and higher organisms to irradiation; that is, irradiation produces inheritable blocks at specific points in metabolic reactions.

The types of mutant strains with specific nutritional requirements obtained by some such methods by Beadle and co-workers in *Neurospora*, by Tatum and collaborators in bacteria, by myself in *Penicillium notatum* and by others in other fungi cover a large variety of

processes, such as syntheses of amino-acids and water-soluble vitamins. Obviously these mutants represent only a fraction of those which could be obtained with further work.

Any one of these artificially incapacitated organisms is a potential test organism for the substance it requires, in the same way that any organism which in nature requires a specific nutrilite may be used for assaying it.

It is evident, however, that of several mutants all failing to synthesise the same essential metabolite (say, an amino acid or a vitamin) some will be more suitable than others for the assay of that metabolite. For example, there have been obtained several genetically different mutants of *Neurospora*[5] and of *Escherichia coli*[3,4] that cannot synthesise aneurine. Of these, some require intact aneurine and some can grow equally well on aneurine or thiazole. Those requiring aneurine secrete the thiazole and pyrimidine moieties and those requiring the thiazole secrete the pyrimidine only, showing that the block is indeed a failure to proceed with a bio-synthesis. Thus the specificity of the response is, to a large extent, a function of the position in the chain of reactions at which the block occurs. This is, of course, equally true for strains in which a nutritional requirement occurs naturally and for those in which it has been induced artificially. But the artificial production of such blocks presents the following advantages:

(1) We can induce requirements for new substances which no known natural strain requires, and therefore extend the field of biological assays.

(2) We can use the same organism for producing an array of mutants each requiring a different nutrilite, and thus unify the procedure. The only condition is that the organism must have as simple nutritional requirements as possible to begin with. This should also make it possible to use assay media less expensive than those used at present (*cf.* Stokes *et al.*[6]).

(3) We can produce blocks at the most suitable positions in the chain of synthetic reactions leading to the substance to be assayed. For instance, in the previous example of aneurine we shall choose those strains which require intact aneurine, presumably because the step blocked is the final one in the synthesis of aneurine.

This last is the most important of all these advantages. It implies that in many instances we can improve the specificity of our assay by going on producing mutants until we find the one with the block at the right place.

However, non-specificity of response may be of various kinds, not all of which are genetically circumventable. There are instances in which the block is not absolute, as in a riboflavine-requiring mutant of *Neurospora*,[7] in which there is no requirement at certain temperatures but there is at others. And there are instances in which the block is not in the synthesis but in the utilisation of an essential metabolite. However, all these difficulties occur also in organisms as found in nature. Again, the advantage of the genetic technique is that, if mutants with these unsatisfactory properties arise, we can go on trying to produce better ones and often we may be successful.

In conclusion, it is for the assayist to state for which substances better test organisms are wanted and for which additional substances the development of an assay is required. The geneticist can try to supply these tools. Once a "perfect" strain is produced it can be used for an indefinite time all the world over. Its chance of becoming unsuitable through reverse mutation is smaller than for a "natural" strain.

The genetical technique is simple and work of this kind in collaboration between the geneticist and the assayist would certainly be reciprocally fruitful.

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The Determination of Small Amounts of Arsenic

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Milton and Duffield[1] describe an alternative to the Gutzeit method, in which the arsine after absorption in alkaline iodine solution is converted into arsenomolybdate, reduced to form an intense blue colour and measured by the Spekker absorptiometer. Following their procedure we obtained discrepant results and further investigation was considered necessary.

Experimental

Generation of arsine with zinc and acid—Milton and Duffield use "zinc shot" for the generation of hydrogen and arsine but do not specify the size. We were unable to obtain a supply of zinc shot of uniform diameter, and used "zinc pellets, free from arsenic, for forensic purposes," the diameter of which ranged from 4 to 10 mm. Under identical conditions repeat determinations with 5 g. of pellets gave results which differed up to 15 per cent., and only about 60 per cent. of added arsenic was found. Using 5 g. of AnalaR granulated zinc instead of pellets we obtained 80 per cent. recovery of added arsenic.

It appeared, therefore, that if all the arsenic was being evolved some must have been escaping through the absorption solution. It was decided to use a stronger absorption solution and a larger amount of zinc. The subsequent experiments were all done with AnalaR granulated zinc, as this gave better recoveries than the zinc pellets.

In order to obtain at least 90 per cent. recovery of added arsenic it was found necessary to use 10 g. of zinc and absorption solutions containing twice the concentration of iodine and bicarbonate recommended by Milton and Duffield. Increasing the acid concentration by 50 per cent. and 100 per cent. above that recommended by Milton and Duffield and using 5 g. of zinc gave lower results, the hydrogen evolution rate being extremely vigorous.

Alkaline hypobromite as recommended by Jacobs and Nagler[2] was tried for the absorption of the evolved arsine, but the results obtained were erratic owing to formation of a greenish tint in some of the tests.

Generation of arsine by electrolysis—The surface area of the zinc used is thus a very critical factor in the generation of hydrogen from acid. As we were unable to obtain zinc pellets of a uniform size it was decided to use an electrolytic cell in conjunction with the absorption vessel recommended by Milton and Duffield.

The type of cell having a parchment diaphragm was unsatisfactory, as it was impossible to obtain the necessary pressure of hydrogen to force the gas through the absorbing solution. The design of the cell was altered so that the oxygen liberated at the anode could be used to balance the pressure in the cell. A U tube cell, without a diaphragm, was made, the final form of which is shown in Fig. 1. It was found that the gas liberated at the anode attacked the rubber bung holding the delivery tube, so a glass joint was fitted on this limb. The rubber bung was retained on the cathode limb to facilitate adjustment of the delivery tube. A description of the cell and its preparation for use is given below.

Description of cell—The cell (Fig. 1) is of Pyrex glass. Each limb is 7″ long and fitted with a B.14 standard ground glass socket. The cathode limb is of 1¼″ tubing and has a bulb 1¾″ in diameter, within which an annular well is formed by means of an internal seal. A length of 0·5 mm. platinum wire is sealed through the wall of the bulb to make electrical contact with the mercury in the well. The other limb is ¾″ in diameter and contains the anode, which is of platinum sheet (2″ × ⅝″ × 0·005″) welded to a platinum wire lead sealed through the wall of the vessel. A tap is fitted to the bottom of the cell for removal of electrolyte.

The delivery tubes are of 3/16″ glass tubing and their jets are drawn down to 0·5 mm., gauged with a wire of this diameter. The absorption vessels are about 10″ long and the graduation mark for 10 ml. is on the narrow part which is of ⅜″ diameter. Above the graduation mark the vessel widens to form a cup ¾″ in diameter × 2″ long. A small glass cone, through which the delivery tube will just slide, is fitted on the cathode absorption vessel to prevent loss by spraying.

Experiments with known amounts of tervalent arsenic showed that, with a current of 1 to 1·2 amps., 1½ hours were required to evolve all the arsenic when less than 0·05 mg. was added, and 2 hours when the amount was 0·05 to 0·10 mg.

It was also established that when the arsenic was added in the quinquevalent form, as it would be with samples requiring a preliminary treatment with nitric and sulphuric acids,

the whole of the arsenic was recovered without addition of a reducing agent, such as hydroxylamine sulphate as recommended by Lockwood,[3] or potassium iodide and stannous chloride as recommended by Milton and Duffield.

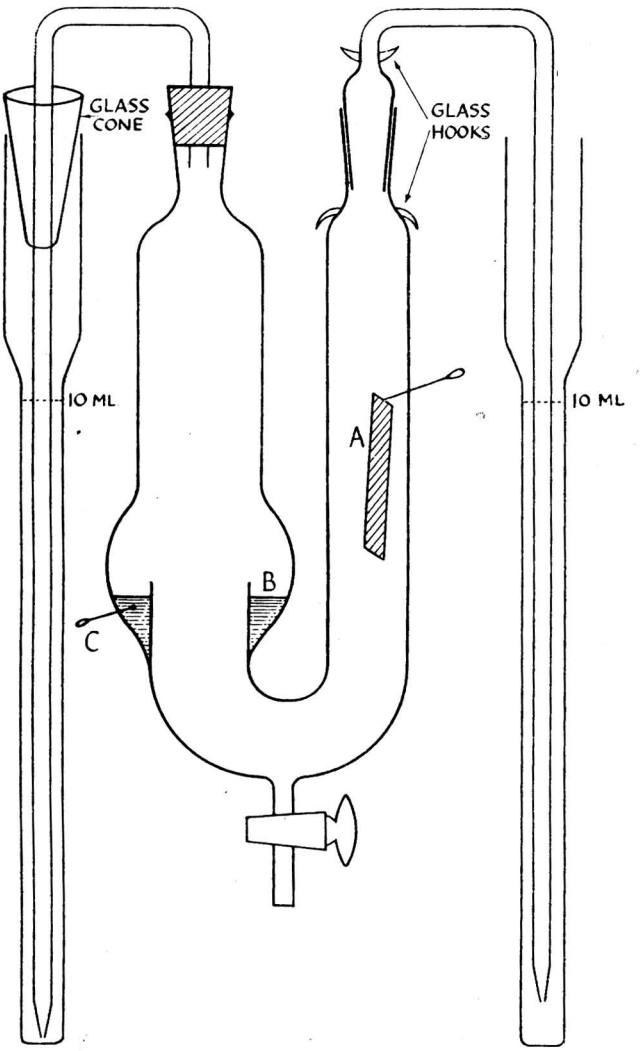

Fig. 1. To scale. (½ size)
A Platinum anode. B Mercury cathode.
C Platinum contact to cathode.

Recoveries of added arsenic (both tervalent and quinquevalent) ranging from 95 to 102 per cent. were obtained with the cell, following the procedure given below:

Method

(*a*) Reagents—

(1) *N sodium bicarbonate.*

(2) *N*/10 *iodine solution.*

(3) *Sulphuric-molybdate solution*—Mix equal volumes of 13 *N* H_2SO_4 (standardised accurately) and 9·5 per cent. sodium molybdate solution ($Na_2MoO_4.2H_2O$).

(4) *Sodium metabisulphite solution*—Five per cent. Prepare freshly as required.

(5) *Stannous chloride solution (strong)*—Dissolve 40 g. in concentrated hydrochloric acid by warming. Cool and dilute to 100 ml. with concentrated hydrochloric acid.

(6) *Stannous chloride solution (dilute)*—Dilute the strong solution 1 to 200 with water as required.

(7) 4 *M Sulphuric acid solution (arsenic free)*—Make up 220 ml. of AnalaR sulphuric acid (sp.gr. 1·84) to 1 litre with distilled water.

(8) *Standard arsenic solution*—Dry in an air oven for 1 hour at 105° C. about 0·5 g. of AnalaR arsenious oxide. Cool in a desiccator and weigh accurately 0·1000 g. Dissolve in a few ml. of 6 *N* sodium hydroxide, dilute somewhat and neutralise with dilute sulphuric acid, using a small piece of litmus paper as indicator. Dilute to 1 litre. One ml ≡ 0·1 mg. of As_2O_3.

(9) *Dilute standard arsenic solution*—Dilute 25 ml. of the above solution to 250 ml. This solution should not be kept more than a few days. One ml. ≡ 0·01 mg. of As_2O_3.

(*b*) PREPARATION OF CELL FOR USE—

Introduce into the cathode well about 5 ml. of clean mercury. Add to the cell 50 ml. of distilled water and 20 ml. of 4 *M* sulphuric acid. Connect the electrodes, in series with a switch and ammeter, to a 12 volt D.C. supply. Switch on the current with the delivery tubes disconnected and allow to run for a few minutes to warm up the electrolyte. If the ammeter now reads less than 1 amp. add a little 4 *M* sulphuric acid to increase the conductivity. If the reading is more than 1·3 amp. add a little water to decrease the current to 1 to 1·2 amp. Switch off the current. Connect the delivery tubes and absorption vessels as shown in Fig. 1, adding to the cathode absorption vessel 2 ml. of *N*/10 iodine and 2 ml. of *N* bicarbonate solution, and to the anode vessel 4 to 5 ml. of distilled water. Secure the anode tube by means of rubber bands over the small glass hooks, as there is a tendency for the glass joint to blow, owing to the pressure developed in the cell. Switch on the current and adjust the cathode vessel so that the delivery tube is touching the bottom, thus causing the bubbles of hydrogen to disperse as a fine stream of gas through the solution. The anode delivery tube should be quite clear of the bottom to ensure the rapid release of oxygen when the pressure builds up in the cell, thus causing only a momentary interruption in the delivery of hydrogen. It may be necessary to adjust the height of water in the anode vessel to obtain the best pressure balance in the cell. Allow the cell to run for an hour and then switch off. Remove the delivery tube and cone, rinsing with the minimum amount of water (not more than 2 ml.).

Treat the iodine solution as given below for the development of colour due to arsenic and if necessary run further tests until a good "blank" is obtained on the cell.

(*c*) PREPARATION OF SAMPLES—

(1) *Organic substances and samples which require digestion with acid*—Treat 2 to 10 g. of sample with concentrated nitric acid (2 to 10 ml.) in a 100 ml. Kjeldahl flask and heat until solution is obtained, avoiding charring. Add 2 to 3 ml. of concentrated sulphuric acid, and more nitric acid if required. Continue the digestion until all the organic matter is destroyed and evaporate until sulphur trioxide fumes appear. Cool, dilute with water and evaporate again to fuming to remove the final traces of nitric acid. Dilute again with water, and if the arsenic content of the sample is expected to be less than 0·10 mg. of As_2O_3 adjust the acidity and bulk as given under (*b*) and transfer the whole of the solution to the cell. If the amount of arsenic is expected to be more than 0·10 mg. of As_2O_3 dilute to a convenient bulk and measure a suitable aliquot into the prepared cell.

(2) *Samples soluble in water or dilute sulphuric acid*—Dissolve 5 to 10 g. in water or dilute sulphuric acid and transfer either the whole or an aliquot part of the solution to the cell.

(*d*) EVOLUTION AND ABSORPTION OF ARSINE—

Having transferred the sample to the cell, connect up the delivery tubes and absorption vessels as described under (*b*), and run the test for 1½ to 2 hours. When the approximate amount of arsenic present is not known, a second test should be allowed to run for a further half hour to verify the complete evolution of arsine.

(*e*) DEVELOPMENT OF COLOUR AND MEASUREMENT WITH THE SPEKKER ABSORPTIOMETER—

To the solution in the absorption vessel add 2 ml. of the sulphuric-molybdate solution slowly to avoid frothing and loss due to liberation of carbon dioxide. Transfer the solution to a 30 ml. beaker. Add metabisulphite solution drop by drop until the iodine colour is *just* discharged. Pour the solution from the beaker into the absorption vessel and back again into

the beaker. Add slowly, with constant swirling, 1 ml. of the dilute stannous chloride solution, transfer again to the vessel and dilute to the 10 ml. mark with water. Mix by pouring into the beaker and back into the vessel. Allow to stand 5 minutes for the development of full colour intensity and measure the absorption in a 1 cm. cell, using a combination of Chance No. 3 (OY2) and No. 1 (OR2) filters in the Spekker absorptiometer, with the mercury lamp.

Carry out a reagent blank at the same time in exactly the same manner.

Establish a factor by developing the colour from a series of standards containing from 0·01 to 0·10 mg. of As_2O_3 and the amounts of reagents stated. Then

(Spekker reading for test — Spekker reading for blank) × factor = mg. of As_2O_3.

The factor obtained with the Spekker used for this work was 0·104.

SUMMARY—The method of Milton and Duffield for the determination of small amounts of arsenic (0·01 to 0·10 mg. As_2O_3) has been examined and an improved procedure devised. An electrolytic cell is used for the evolution of arsine, which is oxidised to arsenate by absorption in alkaline iodine solution. The arsenate is converted to arsenomolybdate, which is then reduced with stannous chloride and the intense blue colour formed is measured by the Spekker photoelectric absorptiometer. The electrolytic cell eliminates errors due to use of zinc and acid for the generation of hydrogen.

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The Determination of Lead in Creta Praeparata

BY E. C. DAWSON AND A. REES

CRETA praeparata is added to bread-making flour at the rate of 7 oz.* per 280 lb. of flour. The British Pharmacopoeia specifies a limit of 20 p.p.m. of lead in Creta praeparata and it prescribes a general method of analysis[1] which, however, has been shown to be unsatisfactory when applied to Creta.

The outline of the B.P. test is as follows:

For the *primary solution* 4 g. of Creta. For the *auxiliary solution* 2 g. of Creta and 4 ml. of dilute solution of lead (0·0016 per cent. of $Pb(NO_3)_2$). Dissolve in acetic acid, boil, make ammoniacal, add cyanide and filter. Make up to 50 ml., equalise colours with a dilute solution of burnt sugar, add sodium sulphide and compare colours.

In practice, four difficulties are encountered in carrying out the test:

(1) Crystallisation of ammonium acetate during filtration, resulting in choking of the filter, and precipitation of calcium carbonate in the filtrate. This may be overcome by diluting the acetic acid solution to 100 ml. and boiling down to 40 ml., thus volatilising some excess of acid and completing the expulsion of dissolved carbon dioxide.
(2) Darkening of the burnt sugar colour itself by sodium sulphide. This difficulty, which applies to the general method as a whole, is overcome by the substitution, for the burnt sugar solution, of very dilute solutions of methyl orange and/or methyl red.
(3) Adsorption of lead by the acid-insoluble residue present in Creta.
(4) If several test solutions are prepared from the same sample of Creta they frequently have different colours and show an irregular response to sodium sulphide.

The B.P. specifies a limit of 2 per cent. for insoluble matter and commercial samples usually contain a residue of this order. It is siliceous in character and as such may be expected to adsorb lead. Thus, when the standard dilute solution of lead is added to the auxiliary solution before filtration as specified, the darkening with sodium sulphide is much less than when the lead is not added until after filtration. When Creta containing added lead is used for the preparation of the primary solution, the darkening produced by the sulphide

* Increased to 14 oz. as from August 11, 1946 (*cf.*, this vol., p. 382).

is less than that in a solution containing the same quantity of added lead but no Creta. When the insoluble matter from a sample of Creta, after being thoroughly extracted with hydrochloric acid, was added to dilute acetic acid containing lead and the solution was boiled, made ammoniacal and filtered, the filtrate was found to contain only 30–40 per cent. of its original lead.

Experimental

The Society's Sub-Committee on the Determination of Poisonous Metals in Food-colouring Materials[2] reported that lead was recovered from certain siliceous materials by extraction with hydrochloric acid. Such an extract from Creta contained so much iron that the colorimetric test could not be directly applied, and separation was necessary. The lead was therefore precipitated by means of sodium sulphide, the slight contamination with sulphur not being objectionable. The *p*H was adjusted to 4·0–5·0[2] and copper[3] and citrate were added, to ensure precipitation of all the lead and the minimum of iron. After filtration the mixed sulphides were dissolved in nitric acid; cyanide and excess of ammonia were added, colours were equalised, sodium sulphide was added and the colours were compared with standards. It was not necessary to remove the nitric acid, as it did not interfere under ammoniacal conditions. The test solutions were slightly yellow owing to the presence of a small quantity of iron, but the colour was too pale to interfere, and very little preliminary colour-equalising was necessary provided that an approximately equal amount of iron was used in the preparation of the standards. (Creta contains approximately 600 p.p.m. of iron.)

Lead recovery from the standard solutions was found to be complete within the limits of accuracy of the colorimetric test. By the following procedure lead was shown to be absent from the siliceous residue. Hydrofluoric acid, with or without sulphuric acid, broke down the silicates into soluble materials, the silicon tetrafluoride being volatilised. A small portion still resisted solution in hydrochloric acid, but it was oxidised with a mixture of nitric and sulphuric acids, and a clear solution could then be prepared. Volatilisation of lead took place if the sulphuric acid was completely driven off, but complete recovery was obtained after evaporating only a small portion of it. Three 20 g. portions of Creta, (*a*), (*b*) and (*c*), were extracted as described in the first paragraph under "Details of Method," using four times the normal quantities of acid, etc. The residues were washed into platinum basins and 1 ml. of 0·016 per cent. lead nitrate solution was added to residue (*a*). One ml. of hydrofluoric acid and 1 ml. of concentrated sulphuric acid were placed in each basin, and also in a fourth basin (*d*) for a reagent blank. The solutions were heated until dense fumes began to be formed. After cooling, 1 ml. of concentrated nitric acid was added to each, and boiled gently, covered with a watch-glass, till no more brown fumes were formed and the solutions became pale yellow. The covers were removed and heating was continued till sulphur trioxide fumes were again evolved. A further three 1 ml. portions of hydrofluoric acid were added to each basin and evaporated off, and fuming was allowed finally to continue for a few minutes. Two ml. of hydrochloric acid were added and the solutions were boiled, diluted with a little hot water and washed with hot water into beakers, the volumes being made up to about 50 ml., and 1 ml. of 0·016 per cent. lead nitrate solution was added to (*b*). The lead was then precipitated and determined as in the third and fourth paragraphs under "Details of Method." For samples of Creta from each of two sources, the colour produced in (*a*) was equal to that in (*b*), showing complete recovery of added lead; while that in (*c*) was equal to that in the reagent blank (*d*), showing the absence of any measurable amount of residual lead in the insoluble matter after extraction with hydrochloric acid.

Details of Method

To 5 g. of the sample of Creta in a beaker add a slight excess (45 ml.) of diluted hydrochloric acid (1 in 5). Boil, and filter. Wash the residue back into the beaker with a fine jet of hot water (about 20 ml.), add 5 ml. of concentrated hydrochloric acid, boil for a few minutes, filter, and wash with about 10 ml. of hot water. Combine filtrates and washings.

Prepare a standard solution by mixing the appropriate quantity of lead nitrate solution (0·016 per cent., containing a little nitric acid; 1 ml. ≡ 20 p.p.m. of Pb on a 5 g. sample), 1 ml. of 2 per cent. ferric chloride ($FeCl_3$) solution (containing a little hydrochloric acid) and 65–70 ml. of diluted hydrochloric acid (1 in 5).

To all solutions (test and standard) add 5 ml. of 20 per cent. ammonium citrate solution and 1 ml. of 0·1 per cent. copper sulphate ($5H_2O$) solution. From a burette add ammonia until the liquid is nearly neutral to Congo red paper. Heat to about 70° C. Add 1 ml. of

approximately 10 per cent. sodium sulphide (Na_2S) solution, then more ammonia until the colour change of the Congo red paper is almost complete. Filter through a retentive paper (Whatman No. 3) and wash with a little hot water.

Tear up the filter paper and place it in the beaker in which the precipitation was carried out, add 20 ml. of diluted nitric acid (1 in 5) and digest, nearly boiling, for 5 mins. Decant through a filter into a Nessler cylinder, draining the digested paper as completely as possible. Digest the paper again in the same manner but using 15 ml. of water, and then again with a further 10 ml. of water. To the combined extracts add 1 ml. of 10 per cent. potassium cyanide solution and excess of ammonia. The solution develops a yellow colour when alkaline. If necessary the colours may be equalised by addition of a little very dilute solution of methyl red and/or methyl orange. Make up to 50 ml., add a few drops of 10 per cent. sodium sulphide solution, mix, allow to stand for five minutes, and compare the colours. A suitable range of standards must be prepared unless the method is being used as a limit test only.

The accuracy of this method, within the range 10–30 p.p.m., of lead, is ±1 p.p.m. but the range may be extended by the use of aliquots. Judged by this method, commercial Creta praeparata has a lead content of 10–15 p.p.m.

SUMMARY

The general limit test for lead given in the B.P. is shown to be unsuitable for Creta praeparata and a more accurate method, brief enough for routine purposes, is described.

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The Determination of the Pyridine Content of Technical Pyridine

BY A. HAMER, R. POMFRET AND W. V. STUBBINGS

(*Read at the Meeting of the North of England Section on April* 27, 1946)

As far as the authors are aware, no reliable direct method for determining the pyridine content of technical pyridine bases such as "115° to 140° C. Pyridine" has hitherto been available. Their quality has been assessed by such characteristics as total alkalinity and distillation range, neither of which is necessarily simply related to the pyridine content. Moreover, the distillation range is appreciably affected by slight variations in water content.

Before the direct method described in the present communication was evolved, various other lines of investigation were followed, but, being unsuccessful, are described in brief outline only.

EXPERIMENTAL

1. *Laboratory precision distillation*—Separation of the constituent bases from technical pyridine by laboratory precision distillation was insufficiently precise for analytical purposes, the intermediate fractions being unexpectedly large. In a distillation of a typical "115° to 140° C. Pyridine" through a 70-plate modified Widmar column at a reflux ratio of upwards of 20 : 1, the fraction intermediate between pyridine (b.pt. 115° C.) and α-picoline (b.pt. 128° C.) amounted to 11·5% v/v of the charge, and the fraction intermediate between α-picoline and the β- and γ-picoline - 2:6-lutidine fraction (b.pt. 144° C.) to approx. 10% v/v of the charge.

No fractionation was effected by distilling "115° to 140° C. Pyridine" with deliberately added water through a 30-plate column. Almost the whole of the charge distilled as an azeotrope of b.pt. 94·5° to 95° C.

The 70-plate column distillation demonstrated that the "115° to 140° C. Pyridine" examined consisted essentially of a pyridine - α-picoline mixture with probably no more than 10 per cent. v/v of higher boiling bases, essentially the β and γ-picoline - 2:6-lutidine mixture,

b.pt. 144° C. This distillation also provided supplies of relatively pure pyridine and α-picoline for preliminary experimental work on alternative methods.

Note on preparation of pure bases—For more precise work pure pyridine, pure α-picoline and pure "β and γ-picoline fraction, b.pt. 144° C." were prepared by separate laboratory precision distillations of three fractions (b.pts. 115° C., 128° to 129° C. and 143° to 145° C. respectively) from an industrial distillation of pyridine bases, supplied by the courtesy of British Tar Products Ltd., Cadishead. The purity of the precision distilled pyridine and α-picoline was established by the only methods available, *viz.*, boiling point, refractive index and alkalinity. For observations on the purity of the precision distilled "β and γ-picoline fraction, b.pt. 144° C." see under 7 later.

2. *Gravimetric precipitation of pyridine as perchlorate*[1] *or oxalate*[2]—Pyridine perchlorate was shown not to be quantitatively precipitated from a solution of a synthetic mixture of pyridine and α-picoline in 6 *N* hydrochloric acid. In oxalate precipitations, α-picoline oxalate remains only temporarily in solution in acetone (presumably owing to supersaturation) and thus tends to co-precipitate with the pyridine oxalate.

3. *Selective oxidation of pyridine homologues*—Conditions under which the pyridine homologues were completely oxidised to carboxylic acids with potassium permanganate,[3] chromic - sulphuric acid, selenium dioxide in xylene,[4] or potassium periodate, invariably led to partial destruction of pyridine.

4. *Vertanen and Pulkki's "rate of distillation" method*[5]—Water could not be used as co-distillation solvent owing to azeotropism, but the rates of distillation of pure pyridine, pure α-picoline and "β and γ-picoline fraction" with carbon tetrachloride were found to differ considerably when tested separately. Tests on synthetic mixtures showed, however, that, contrary to expectation, the rate of distillation of each component was influenced by the presence of the other components. Fictitious results were therefore obtained and the method failed.

5. *Condensation of α- and γ-picolines with aromatic aldehydes*[6]—Of the aromatic aldehydes tried for this condensation, *m*-nitrobenzaldehyde appeared to be the most promising. Tests on purified pyridine showed, however, that, contrary to expectation, some interaction occurred. With α-picoline the reaction was far from complete, even after a reaction period of 10 hours.

6. *Infra-red absorption methods* were unpromising, since no single component showed maximum absorption at a wavelength free from absorption due to one or more of the other components.

7. *Calculation of pyridine content from alkalinity and water content of sample*—The pyridine content of a mixture of pyridine and the three picolines can be calculated from the alkalinity and water content, but this procedure would not be highly accurate, owing to the relatively small difference between the molecular weights of pyridine and picoline. Application of such a procedure was abandoned after it was found that the alkalinity of the precision distilled "β and γ-picoline fraction, b.pt., 144° C." (see 1 above) indicated the presence in this product of a fairly substantial proportion of 2:6-lutidine—a component which is liable to be present, not necessarily in constant proportions, in the technical pyridines under consideration.

8. *Cloud point in aqueous sodium chloride solution*—Pyridine—either purified or technical—may be "salted out" from aqueous solution by addition of sodium chloride. Further tests showed that:

(i) a 20 per cent. w/v solution of "115° to 140° C. Pyridine" in 10 per cent. w/v aqueous sodium chloride solution is clear at room temperature but clouds sharply on warming to about 40° C., replicate tests giving cloud points reproducible within about ±0·2° C. of the mean value;

(ii) a cloud point - composition graph, constructed by the procedure outlined in (i) from synthetic binary mixtures of purified pyridine and α-picoline could be used to determine the pyridine content of such binary mixtures; but

(iii) on applying procedure (ii) to *ternary* synthetic mixtures containing "β and γ-picoline fraction, b.pt. 144° C." as third component the binary graph was not applicable, the observed cloud points being lower than those corresponding to the known pyridine content.

The method therefore fails, clouding being almost certainly due to the separation, not of pyridine, but of the less soluble homologues, *viz.*, α-picoline from the binary mixtures and one or more of the components of the β and γ-picoline fraction from the ternary mixtures.

9. CLEARING TEMPERATURES OF AQUEOUS SOLUTIONS OF THE PERCHLORATES—Of all the above methods, cloud point determination has the simplest technique and was, therefore, pursued further. The solvent properties of pyridine are such, however, that no readily available organic solvent could conveniently be used in determining a true cloud point—in which a liquid phase separates. The sparingly water-soluble pyridine perchlorate, referred to in 2 above, was accordingly studied further from the point of view of the clearing temperature, *i.e.*, the temperature at which the last crystal redissolves.

Preliminary tests showed that (1) under conditions which lead to a heavy precipitation of pyridine perchlorate, α-picoline and the "β and γ-picoline fraction, b.pt. 144° C." gave no precipitate, (2) the water solubility of pyridine perchlorate increases sharply with rising temperature and (3) under suitable conditions the clearing temperatures of perchlorate preparations from pure or from technical pyridines are readily reproducible in replicate tests within the extreme limits ±0·25° C. from the mean value. The most suitable procedure is described later.

The clearing temperature data given in Tables I and II were obtained by applying Method A—see later—to a series of binary synthetic mixtures of purified pyridine and α-picoline and to a series of ternary mixtures of purified pyridine, α-picoline and "β and γ-picoline fraction, b.p. 144° C.," respectively:

TABLE I

CLEARING TEMPERATURES OF BINARY PYRIDINE - α-PICOLINE MIXTURES

Pyridine, % w/w ..	100	82·45	71·05	61·45	51·55	47·65	37·3	29·95	20·4	16·55
α-Picoline, % w/w ..	0	17·55	28·95	38·55	48·45	52·35	62·7	70·05	79·6	83·45
Clearing temp. °C. ..	56·75	49·3	44·1	39·5	34·4	31·9	24·7	18·6	9·4	4·3

TABLE II

CLEARING TEMPERATURES OF TERNARY PYRIDINE - α-PICOLINE - β AND γ-PICOLINE MIXTURES

Pyridine, % w/w	41·25	51·25	24·5	54·0
α-Picoline, % w/w	52·75	42·75	61·3	30·0
β and γ-Picoline, % w/w ..	6·0	6·0	14·2	16·0
Clearing temp. °C.	27·6	33·9	13·8	35·3

If the data in Table I are plotted, a smooth curve relationship is obtained between pyridine content and clearing temperature. Within the limits of experimental error, all points represented by the data in Table II also lie on the same smooth curve, indicating that the clearing temperatures of these ternary mixtures depend only on the pyridine content. Relatively wide variations in the ratio of α-picoline to β and γ-picoline fraction, deliberately arranged considerably to exceed the variations likely to be encountered in products such as "115° to 140° C. Pyridine," were without effect on the clearing temperature. It may therefore be concluded that the clearing temperature Method A, described in detail on p. 422, interpreted by the aid of a pyridine content - clearing temperature graph constructed from the data given in Table I, is applicable equally to binary mixtures of pyridine and α-picoline, to ternary mixtures of pyridine, α-picoline and "β and γ-picoline fraction" and to technical pyridine bases such as "115° to 140° C. Pyridine" containing these components.

9*a*. MODIFIED METHOD FOR HIGH PYRIDINE CONTENT TECHNICAL BASES—Method A, primarily designed for testing "115° to 140° C. Pyridine" containing approx. 35–55 per cent. of pyridine, is not entirely suitable for testing mixtures containing upwards of about 70 per cent. of pyridine. Under the experimental conditions, such products give a very bulky precipitate of pyridine perchlorate and the clearing temperature is relatively high (45° to 55° C. approx.), at which temperature a thin hard film of solid perchlorate, difficult to bring into solution, tends to deposit above the surface of the test solution.

Tests on the purified pyridine used for Table I were accordingly carried out at a greater dilution as described under Method B. Under these conditions the observed clearing temperature was 43·3° C., deposition of solid perchlorate above the liquid surface was negligible and the smaller amount of separated solid rendered the whole determination more convenient than in corresponding tests on 5·000 g. quantities (Method A).

Synthetic binary mixtures of known compositions varying between 65 per cent. and 100 per cent. of pyridine were prepared, using the same samples of purified pyridine and purified α-picoline as before, and clearing temperatures were determined on each by the modified (3 g.) Method B. Results obtained are given in Table III.

A graph of clearing temperature against percentage of pyridine, constructed from experiments 1–11 of Table III, is almost but not quite linear. The points corresponding to experiments 12 and 13, in which β and γ-picoline fraction was used in place, respectively, of the whole and a part of the α-picoline, lie on this graph, within the limits of experimental error. These results show that, no matter whether the 5·00 or the 3·00 g. modification of the

TABLE III

CLEARING TEMPERATURES OF BINARY PYRIDINE - PICOLINE MIXTURES UNDER THE MODIFIED (3·000 g.) CONDITIONS OF METHOD B

Experiment No.	Pyridine, % w/w	α-Picoline, % w/w	β and γ-Picoline % w/w	Clearing temperature, °C.
1	100	nil	nil	43·3
2	95·4	4·6	,,	41·8
3	92·6	7·4	,,	40·95
4	89·9	10·1	,,	39·9
5	86·5	13·5	,,	38·9
6	83·6	16·4	,,	37·8
7	80·1	19·9	,,	36·5
8	76·7	23·3	,,	35·35
9	72·8	27·2	,,	33·7
10	70·1	29·9	,,	32·7
11	65·4	34·6	,,	30·8
12*	86·5	—	13·5	38·6
13*	73·9	15·3	10·8	34·15

* In test 12 purified β and γ-picoline fraction was used in place of purified α-picoline, and in test 13 both α-picoline and β and γ-picoline fraction were present in the test mixture.

method is used, the clearing temperature depends only on the pyridine content and is independent of the identity and relative proportions of the pyridine homologues present, within the limits of variation tested.

Alternative method of graphing results—It is of interest to note that if the data of Tables I and III are plotted in the form $\log_{10}C$ *versus* $1/T$, where C = percentage of pyridine and T = clearing temperature in °Absolute, a wholly linear relationship is obtained from Table III, whereas the graph obtained similarly from Table I consists of two straight lines, intersecting at a very obtuse angle at a point corresponding to a pyridine content of approximately 70 per cent.

It is difficult to indicate whether the form of this second graph is due to (1) the experimental difficulties already indicated in testing samples containing upwards of 70 per cent. of pyridine by the original 5·000 g. Method or (2) departure of the relationship of $\log_{10}C$ to $1/T$ from strict linearity over the relatively wide range 16–100 per cent. of pyridine. Whatever the explanation, this method of plotting demonstrates the need—already established from other considerations—for following the original 5·000 g. Method, A, for products containing up to 70 per cent. of pyridine, and the modified 3·000 g. Method, B, for samples containing 70 per cent. and upwards of pyridine.

METHOD

A. FOR SAMPLES CONTAINING UP TO 70 PER CENT. OF PYRIDINE—Into the inner, 6 in. by 1 in. tube of a crystallising point apparatus[7] weigh 5·000 g. of the test sample, add from a burette 10·0 ml. of water and add 0·2 ml. of mixed indicator (0·01 per cent. of methyl orange and 0·02 per cent. of xylene cyanole FF in water) and just acidify to a magenta end point by addition, from a burette, of perchloric acid (AnalaR 60%) added dropwise towards the end. Add sufficient water from a burette to bring the total volume (water + perchloric acid + indicator) to 20·0 ml. Warm if necessary to dissolve crystals of pyridine perchlorate, cool with vigorous stirring until small crystals separate and immediately assemble the apparatus, with bath temperature previously adjusted to 10° C. above the expected clearing temperature. Stir briskly and continuously and observe the temperature at which the last crystal dissolves, using a thermometer graduated in 0·1° or 0·2° C. intervals. Read off the pyridine content from a clearing temperature - composition graph constructed from the data given in Table I.

B. FOR SAMPLES CONTAINING UPWARDS OF 70 PER CENT. OF PYRIDINE—Weigh out 3·000 g. of test sample and proceed exactly as described in A above.

Read off the pyridine content from a clearing temperature - composition graph constructed from the data given in Table III.

NOTES

(1.) Over-cooling is to be avoided at the crystallisation stage, otherwise the time required for a determination is unduly prolonged.

(2.) If the test mixture is allowed to stand, large crystals tend to form. These dissolve relatively slowly on warming and high results will then be obtained. In such circumstances redissolve by warming and induce crystallisation as described above immediately before the clearing temperature determination.

(3.) The clearing temperature is more easily observed if a suitable lamp (*e.g.*, a microscope lamp) is mounted towards the back and to one side of the apparatus.

10. CONFIRMATION OF THE PURITY OF THE "PURE" COMPONENTS USED IN TABLES I–III—The authenticity of the data given in Tables I–III depends on the assumption that the purified pyridine and picolines used were 100 per cent. pure.

The purity of the pyridine used was established by comparison of its clearing temperature (3 g. method) with that of pyridine specially purified by (1) precipitation as perchlorate from concentrated aqueous solution, (2) crystallisation of the separated perchlorate once, twice and three times respectively from water, (3) regeneration of pyridine from the resulting products by treatment with aqueous sodium hydroxide, saturation with sodium chloride at 45° to 50° C., separation and drying of the oil over solid potash, and (4) distillation of the dry oil through a 6 in. Vigreux column and rejection of all but the constant boiling main fraction.

The data obtained with these purified samples are given in Table IV.

TABLE IV

PYRIDINE CONTENT OF SAMPLES OF PURIFIED PYRIDINE

Source of purified pyridine	Clearing temp., °C. (3 g. method)	% Pyridine from graph ex data in Table III
Via perchlorate, crystallised once from water	43·3	100
,, ,, ,, twice from water	43·25	100
,, ,, ,, three times from water	43·3	100
Precision distillation. As used for Tables I–III	43·3	100
,, ,, Pyridine fraction from a second distillation	43·0	99·1

The data given in Table IV show that (1) pure pyridine may be prepared from technical pyridine bases by precipitation as perchlorate followed by a single crystallisation from water and (2) the precision distilled material used in Tables I–III was, in fact, 100 per cent. pyridine. Hence precision distillation through a highly efficient column, though unsuitable for an analytical separation, may be used to isolate pure pyridine from technical pyridine bases.

The absence of pyridine from the picoline fractions used in the foregoing tests may be inferred from the data given in Tables I–III, since, within the limits of experimental error, the interchange of various proportions of α-picoline and β and γ-picoline fractions in the synthetic mixtures gave clearing temperatures which all lie on the clearing temperature - composition graphs for binary pyridine - α-picoline mixtures.

11. *Influence of departures from specified experimental conditions*—Tests on technical pyridine samples showed that:

(i) a deficiency of 0·5 ml. of water in the test mixture raised the clearing temperature by approx. 0·3° C.;

(ii) an excess of 0·5 ml. of water in the test mixture lowered the clearing temperature by approx. 0·4° C.;

(iii) a 0·5 ml. excess of perchloric acid (60%) in the test mixture, the total volume (water + perchloric acid + indicator) still being maintained at 20·0 ml., raised the clearing temperature by approx. 1° C., whereas a 0·5 ml. deficiency of perchloric acid (60%) under similar conditions lowered the clearing temperature by approx. 5° C.

Strict adherence to the volumes specified in the method is therefore essential, and the neutralisation must be done carefully. The indicator colour change (green or greenish-grey to magenta) is, however, very well defined and readily permits titration to a one-drop end point.

12. *Reproducibility of the method*—Extreme deviations in replicate tests were within the limits: ±0·25° C., equivalent to approx. ±0·5 in the percentage pyridine figure.

13. *Accuracy of the method*—In the absence of an established alternative method for determining pyridine in pyridine bases, comparative tests by different methods could not be made. Since, however, the method described is based on experiments using synthetic mixtures

of highly purified components, the method would be expected to be accurate within the limits of reproducibility given above.

14. *Tests on technical pyridine bases*—The clearing temperature method has been applied to a variety of samples of technical pyridine bases of various grades. A selection of typical results is given in Table V

TABLE V

PYRIDINE CONTENT OF TECHNICAL PYRIDINE BASES

Sample No.	Nature of pyridine	Clearing temp. °C. 5 g. Method A	Clearing temp. °C. 3 g. Method B	% Pyridine (from graphs)
1	115° to 140° C. pyridine ex supplier A	23·35	—	38·3
2	,, ,, ,,	30·1	—	45·0
3	,, ,, ,,	35·5	—	53·5
4	115° to 120° C. pyridine ex supplier A	48·7	37·1	81·0; 81·7
5	,, ,, ,,	49·0	37·3	81·8; 82·2
6	,, ,, ,,	46·4	35·1	75·8; 76·2
7	115° to 122° C. pyridine ex supplier B	—	38·6	86·5
8	,, ,, ,,	—	38·9	87·5
9	,, ,, ,,	—	41·5	94·7

SUMMARY—After attempts to devise a chemical method of analysis had failed, a physico-chemical method, which is specific for the pyridine component, was developed. This depends on the sparing solubility of pyridine perchlorate in cold water, perchlorates of pyridine homologues being readily soluble. The determination is made by exactly neutralising a concentrated aqueous solution of the sample with perchloric acid, and determining the clearing temperature (the temperature at which the last crystal dissolves) at controlled dilution. The pyridine content is read off from a clearing temperature - composition graph constructed from data on synthetic mixtures of the purified components of technical pyridine. Procedure is described suitable for samples containing (A) up to 70 per cent. of pyridine, (B) upwards of 70 per cent. of pyridine.

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The Determination of Nickel by Precipitation with Dimethylglyoxime

BY J. T. MINSTER

THE need for maximum accuracy in the determination of nickel in alloys containing about 99 per cent. or more of nickel led to an investigation of the errors liable to be encountered in the gravimetric dimethylglyoxime method. Experience with variations of this method described by several authors had shown that a reliable operator, carrying out duplicate determinations by the same process, would normally obtain results showing an error of up to ±0·3 per cent. of the nickel content; occasionally errors of as much as ±1 per cent. would occur. Although such discrepancies might not be of importance if the nickel content of an alloy were relatively low, their effect on the high contents here under consideration was appreciable.

The maximum amount of the bulky precipitate that can be handled conveniently is about 0·5 g., which is derived from 0·1 g. of nickel. A weight of alloy containing this amount of nickel may be dissolved in nitric acid and the whole of the solution used for precipitation. Since the alloys in question contain about 99 per cent. of nickel this involves weighing 0·1 g.,

so that an error of 0·0001 g. will give rise to an error of 0·1 per cent. in the result. To achieve this standard of accuracy the weighing must be carefully carried out on a good balance. A possible alternative considered is to take a larger quantity of alloy, dissolve, make up to a definite volume and measure out a fraction for precipitation; suitable quantities would be 2 g. made up to 1 litre and 50 ml. pipetted out. But in such a process the possibility of mistakes arising due to faulty manipulation is greatly increased and, even if the highest grade glassware is employed, errors arising from calibration tolerances are of significance. Furthermore, the space occupied and time taken in this procedure are objectionable features when large numbers of samples are analysed.

The conditions for precipitation are variously described in the published methods, and errors, some compensating, may be introduced at this stage and in the washing of the precipitate. Treadwell[2] specifies addition of five times the theoretical quantity of 1 per cent. solution of dimethylglyoxime in alcohol; he precipitates with ammonia at nearly boiling, allows to stand an hour if convenient, filters hot and washes with hot water. Vogel[3] adds a slight excess of 1 per cent. dimethylglyoxime solution, amounting to 6 to 7 ml. on a theoretical requirement of 21 ml., precipitates with a slight excess of ammonia at 70 to 80° C., allows to stand on a steam bath for 20 to 30 minutes and on a water bath for 1 hour, filters and washes with hot water. Mellor[4] adds 20 ml. of a 1 per cent. solution of the reagent to 300 to 500 ml. of nickel solution at 50° C. and adds sodium acetate, instead of ammonia, to eliminate mineral acid. The precipitate is allowed to stand for 1 hour before filtering and is washed with water at 50° C., although Nuka[5] is quoted as recommending washing with cold water to reduce solubility losses. In the A.S.T.M. method[1] the liquid is first made alkaline with ammonia and the dimethylglyoxime solution added subsequently. Precipitation is carried out, at 60–70° C., by adding 0·4 ml. of a 1 per cent. solution of the reagent in alcohol for every mg. of nickel, and 5–10 ml. in excess. The liquid is allowed to cool to room temperature and cool wash liquor is employed. Barnicoat[6] shows that the *p*H of the solution after precipitation should be not less than 7·5 to ensure minimum solubility of nickel-dimethylglyoxime precipitate and states that when minute amounts of nickel are being determined the solution should stand at least three days to give the precipitate time to crystallise.

Trikov and Lapshina[7] show that nitrates in the solution inhibit precipitation when the nickel content is 0·0005 g., but that when it is above 0·0025 g. nitrates are without effect. They also prefer to use a 3 per cent. solution of dimethylgloxime in 3 per cent. aqueous potassium hydroxide, instead of the more usual 1 per cent. alcoholic solution, and give figures to show that the alcoholic solution fails to precipitate all the nickel when 0·0005 g. is being determined, the amount precipitated falling with increasing excess of alcoholic reagent. When the reagent amounts to about 38 per cent. of the final volume no precipitation occurs with this small amount of nickel. These authors state that the time of standing does not play a great part, and in their complete method the precipitate is allowed to stand two hours in a warm place and then filtered and washed with hot water. It is then redissolved, reprecipitated, filtered after standing at a moderate heat for 30 minutes and again washed with hot water.

In presence of ferric iron, sodium acetate may not be used for neutralisation so, since iron is always present in the alloys under consideration, ammonia was used for this purpose throughout. Investigation was made into the possible effect of the amount of excess ammonia. A number of determinations on the same alloy were carried out similarly as far as the neutralisation stage. Ammonia was run in slowly, from a burette, until a faint permanent precipitate formed and then various measured quantities in excess were added. The precipitates were filtered off, washed, dried and weighed. It was found that varying the excess of ammonia from 5 to 20 ml. had no appreciable effect on the weight of the precipitate.

Temperatures ranging from 50° C.[4] to nearly boiling[2] have been recommended as suitable for precipitation. Addition of dimethylglyoxime to almost boiling acid solution results in decomposition of the reagent. Precipitation tends to be slow if the temperature is too low, and experiments indicated that 80 to 85° C. was a safe temperature at which to add the reagent solution. No obvious decomposition occurs at this temperature which, at the same time, is high enough to ensure a rapid reaction, the nickel compound being almost entirely precipitated in a short time.

In view of the disparity between the amounts of excess reagent specified by Treadwell and the other authors cited, and the differences between the various temperatures for filtering and washing, experiments were carried out to determine the effect of these variations.

Many determinations were carried out, using 100 ml. of 1 per cent. dimethylglyoxime solution to precipitate 0·1 g. of nickel—2·5 times the theoretically necessary quantity of reagent.

Examination of the filtrates from such determinations showed that a white precipitate of the reagent normally appeared and that frequently it was coloured with the characteristic pink tint of the nickel dimethylglyoxime compound. Evidence was obtained that if, in order not to lose the latter, the filtrate were passed through the filter again some of the precipitated dimethylglyoxime would be held on the filter and not removed by subsequent washing, so giving rise to fictitiously high results.

The solubility of dimethylglyoxime under the conditions of the analysis was investigated. A blank was run in which only the nickel was omitted and the liquid was allowed to stand overnight, its temperature falling to 26° C. A few crystals of the reagent were apparent and on cooling to 15° C. in a water bath their number increased appreciably. The precipitate was filtered, washed with water at 15° C., dried and weighed and was found to amount to 27 per cent. of the original dimethylglyoxime. The amount removed from solution during an analysis by combining with the nickel is about 40 per cent. of the amount added, so that at 15° C. the whole of the remaining 60 per cent. should remain in solution. Another blank was carried out in which the volume of alcoholic dimethylglyoxime solution added was reduced by 40 per cent., this volume being replaced by alcohol. On standing overnight and cooling, as before, to 15° C., no deposition occurred, but a few crystals formed at 12° C.

There is thus no danger of excess reagent co-precipitating with the nickel compound, under the conditions of the analysis, if the temperature of the liquid is not allowed to fall below 25° C., this providing an adequate margin of safety. No heed need then be paid to a white precipitate of dimethylglyoxime in the filter flask, as it will be due to the lowering of temperature owing to rapid evaporation and also to the actual removal of alcohol from the solution. It was confirmed that the removal of alcohol from an aqueous-alcoholic solution does cause precipitation of dimethylglyoxime; and in this connection it should be noted that the method ultimately standardised includes no heating after addition of the reagent. Such heating, *e.g.*, standing for a period on a water bath, as is specified in some published methods, would have a tendency to lower the alcohol content of the solution and thus promote precipitation of the reagent.

In Vogel's and the A.S.T.M. recommended procedures the excess of the reagent solution is reduced to 6–7 and 5–10 ml. respectively, so that to precipitate 0·1 g. of nickel 45–50 ml. of 1 per cent. reagent solution would be required instead of the 100 ml. used in experiments already described. This reduction in the amount of reagent will very materially lessen the possibility of excess precipitating, although the smaller concentration of alcohol in the solution might have an opposing effect. A series of experiments were carried out to investigate this point and to ensure that the small excess of reagent is sufficient to bring about the precipitation of all the nickel. Results are shown in Table I.

TABLE I

Volume of dmg* solution added for 0·1 g. of alloy ml.	Apparent nickel content of alloy %	Appearance of filtrate	Reaction on addition of dmg* to filtrate	Remarks
30	68·39	Clear	nickel present	Known deficiency of dmg.
40	83·43	,,	,, ,,	Dmg just sufficient theoretically. Insufficient in practice.
50	99·08	,,	,, absent	Sufficient dmg.
75	99·18	White haze	,, ,,	Sufficient dmg. Slight precipitate of excess in filtrate.
100	99·02	White precipitate	,, ,,	Considerable precipitate of excess dmg in filtrate.

* Dimethylglyoxime.

It thus appears that an excess of 10 ml. of 1 per cent. dimethylglyoxime solution is sufficient to ensure complete precipitation of the nickel and that with this excess there is no precipitation of the reagent in the filtrate. So that if the nickel content of the material being analysed is known approximately it is undesirable to add a larger excess of reagent than 10 ml. of 1 per cent. solution for 300–400 ml. of nickel solution. If the nickel content is not known and a larger excess of reagent is used, the possibility of the excess co-precipitating with the nickel compound is eliminated by keeping the temperature at filtration at 25 to 30° C. This

holds up to an excess of 40 ml. of 1 per cent. dimethylglyoxime solution in 400 ml. of nickel solution.

The after-precipitation of the nickel compound in the filter flask, which was frequently observed in early analyses, was found to be due to filtering and washing at too high temperatures. When filtered hot, an appreciable amount of the nickel compound passes through the filter in solution, and is precipitated in the flask at the lower temperature. If the liquid is allowed to become quite cold before filtration, no after-precipitation of nickel compound occurs; but, as has been shown, there is a risk of precipitating the reagent if there is a large excess of it present, should the temperature fall too low. Experiments showed that the temperature of 25° to 30° C., found to be high enough to prevent precipitation of excess reagent, was also low enough to ensure complete precipitation of all the nickel compound, so that no pink precipitate formed in the filter flask. Subsequent washing with distilled water at 25° C. also failed to carry any of the precipitate through into the filtrate; but if hot distilled water were used nickel dimethylglyoxime compound was dissolved from the filter and would re-precipitate in the flask either during the filtration or on standing for some hours. This, again, is in substantial agreement with the conditions of the A.S.T.M. method.

The complete method finally adopted is as follows.

Method

Weigh between 0·0900 and 0·1100 g. of the alloy sample into a 1 litre beaker. Add 20 ml. of diluted nitric acid (1 + 1) and dissolve, warming as necessary. Add 250 ml. of distilled water and 25 ml. of tartaric acid solution (1 + 9) and boil. Remove from the Bunsen and immediately add 100 ml. of cold distilled water and then 50 ml. of a 1 per cent. solution of dimethylglyoxime in alcohol, stirring with a rod. Without delay cautiously add ammonia (sp.gr. 0·880) from a burette, stirring, until a pink colour persists throughout the liquid. Add a further 5 ml. of ammonia and stir well. Remove the rod, and allow the beaker and contents to stand overnight in a place where the temperature will fall to between 25° and 30° C. by next morning.

Filter through a prepared and weighed sintered glass crucible (Pyrex SF.2C.4). When all the liquid has been filtered, wash the residual precipitate from the beaker into the crucible with distilled water at 25° C., which takes about six rinsings. Wash the precipitate in the crucible with distilled water as follows. Almost fill the crucible and suck dry; rinse the precipitate from the sides to the bottom of the crucible and suck dry. Repeat both operations. Heat in an oven at 105° to 110° C. for one hour, cool in a desiccator and weigh.

Preparation of Crucible—When this technique had been established it became evident that certain discrepancies were due to erratic behaviour of the crucibles. Mechanical removal of the bulk of the precipitate from a previous analysis, dissolving the remainder with hydrochloric acid, and washing with water was insufficient to ensure constancy of weight under the conditions of filtration. Treatment for 1 hour with hot, strong hydrochloric acid, and sucking through the crucible tap and distilled water, then ammonia (sp.gr. 0·880) and finally distilled water, overcame this difficulty. As previously stated, weighings must be carried out with great care; weights used for this work must be calibrated.

Typical results are shown in Table II.

Table II

Sample	Observer	Results Nickel per cent.	Notes
1	A	99·45 (*a*) 99·28 (*b*)	One crucible was used for determinations (*a*) and another for (*b*); neither was pre-treated with hot HCl and ammonia.
1	A	99·42 (*a*) 99·31 (*b*)	
2	A	99·33 (*a*) 99·31 (*b*)	Crucibles (*a*) and (*b*) as above, pre-treated with hot HCl and ammonia.
3	A	99·22 (*a*) 99·24 (*b*)	
4	A	99·51 99·49 99·49 99·45	
5	B	99·00 99·13	B was the least experienced operator.
6	B	98·87 98·80	
7	C	98·83 98·86	
8	D	99·13 99·10	
9	D	99·17 99·19	

Experience with the method described has shown that the gross errors which previously occurred are eliminated and that a careful worker should obtain duplicate results that do not differ by more than 0·05 per cent.

SUMMARY—Published methods for the gravimetric determination of nickel by precipitation with dimethylglyoxime are reviewed. When alloys containing about 99 per cent. of nickel are analysed, dissolving a weighed amount, precipitating from the whole solution with a small excess of dimethylglyoxime solution and adjustment of the temperatures of filtration and of the wash liquid are shown to result in increased precision. The A.S.T.M.[1] method is less liable to error than other published procedures.

I must express my thanks to the management of this factory, in whose laboratory the work was carried out, for permission to publish this paper, and to Mr. R. C. Chirnside for drawing my attention to certain references in the literature cited.

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THE GENERAL ELECTRIC CO., LTD.
CAPE MILL, SHAW, NR. OLDHAM, LANCS.

February, 1946

The Determination of Silicon in Nickel Alloys

BY J. T. MINSTER

REGULAR analyses of samples of nickel alloy containing about 99 per cent. of nickel and 0·01 to 0·10 per cent. of silicon brought into prominence the desirability of employing a method more rapid than the usual gravimetric method for silicon determination. This was emphasised by the introduction of a combustion - volumetric method for the determination of sulphur in these alloys and the abandonment of the gravimetric process. Silicon removal had been a step in the gravimetric sulphur estimation, and the subsequent ignition and weighing of the silica had taken relatively little time. It was regarded as undesirable to retain the lengthy evaporation and baking process solely for silicon determination if a more expeditious method of sufficient accuracy could be employed.

A colorimetric process seemed most suitable and consideration was given to the development of the silicomolybdate yellow colour and its subsequent conversion to molybdenum blue. When this process is applied to the alloys in question the nickel interferes with the evaluation of the blue colour by visual means, although it might be possible to carry out such measurements in presence of nickel by a photoelectric method. In view of the reactions involved, suppression of the nickel colour by means of cyanide is not a practicable procedure.

The investigation thus divided itself into two sections: (1) solution of the alloy and removal of the nickel, leaving the silicon in solution, and (2) selection of the particular colorimetric method best suited to the special requirements of the case.

Nickel can be separated from many other elements, including silicon, by means of electrolysis with a mercury cathode.[1] Methods for the separation of metals by this process have almost always used solution in sulphuric acid, other acids, if present, being removed by heating to fuming with sulphuric acid. Lundell, Hoffman and Bright[2] use this procedure in the determination of vanadium in steel, as also does Etheridge,[3] who filters off any precipitated silica before electrolysing. Etheridge[4] also follows this course when separating aluminium. Brophy[5] states that only sulphate solutions can be used in the separation of aluminium from other metals, it being necessary to fume with sulphuric acid and filter off any precipitated silica before electrolysis. According to Pavlish and Sullivan[6] sulphuric, perchloric, phosphoric and a mixture of sulphuric and phosphoric acids are all suitable solvents for the removal of iron over a mercury cathode; nitric and hydrochloric acids are not satisfactory for use in mercury cathode analysis. Chirnside, Dauncey and Proffitt[7] fume with sulphuric acid before electrolysing to separate nickel from aluminium, magnesium and beryllium.

The general tenor of the literature thus indicated that electrolysis in presence of nitric acid would probably be unsuccessful. But in view of the impossibility of removing nitric acid, which is necessary to dissolve the alloys, by fuming with sulphuric acid and at the same time maintaining the silicon in solution, it was decided to try electrolysis without previous removal of the nitric acid. It was found that nickel can be removed quite satisfactorily under such conditions. The alloy is dissolved in a small excess of nitric acid, a little sulphuric acid is added, and electrolysis is carried out as previously described.[7,8] Typical results of electrolysing a solution containing 1·4 g. of nickel with a current of 4 amps. are: green colour vanished at the end of 1¼ hours; trace of nickel was indicated by dimethylglyoxime after 1¾ hours; nickel was absent after 2¼ hours; mercury was absent from solution after 1¼, 1¾ and 2¼ hours. Apparatus made from materials normally present in the laboratory, as described by Chirnside, Dauncey and Proffitt,[7] may be used, although in this work the apparatus described by the author[8] was employed. It has been found unnecessary to stir the solution, or to heat or cool it, during electrolysis and a rotating anode is not required. As mentioned later, it was found advantageous to add sulphamic acid during the electrolysis to stabilise the molybdenum blue colour subsequently developed, but this was without apparent effect on the separation.

In the estimation of silicon by the silicomolybdate - molybdenum blue method phosphorus is an interfering element. It is not normally a constituent of the alloys being examined but it was regarded as desirable to allow for the possibility of its presence in quantities sufficient to affect the silicon determination. Several of the methods which have been proposed for the suppression of phosphorus were investigated and Vaughan's procedure[9] was finally selected. In this the yellow phospho- and silicomolybdates are formed in dilute acid solution; on acidifying more strongly the phosphomolybdate only is decomposed, leaving the silcomolybdate unaffected. Subsequent addition of stannous chloride reduces the silicomolybdate to molybdenum blue, which is compared with standard colours.

Fading of the blue colour occurs in presence of nitric acid and, although some decomposition occurs during electrolysis, the residual nitric and nitrous acids render prompt matching of the colours essential. If the blue colour is developed in a solution prepared without nickel and without electrolysis, and containing only silicon and the amount of nitric acid that would have been employed to dissolve the usual weight of nickel, fading is very rapid; the blue vanishes in a period of tens of seconds leaving a deep orange solution. Under normal conditions of electrolysis and development the colour is stable for 5 minutes, but may fade appreciably in 10 minutes, after which fading is rapid. Comparison of the test solution with standards had therefore to be made within 5 minutes of adding the stannous chloride. Increasing the amount of this reagent to reduce the oxidising agents results in an olive green tint unsuitable for comparison purposes.

The nitrous acid may be destroyed by treatment with urea, but Silverman[10] has described the advantages of sulphamic acid for this purpose in connection with the electrolytic deposition of copper. Its reaction with nitrous acid is rapid and complete and no organic residues remain to introduce possible complications. Addition of 5 ml. of a 10 per cent. solution of sulphamic acid to the electrolyte when the green colour of the nickel has just vanished, and continuation of electrolysis for a further 15 minutes, resulted in elimination of nitrous acid and a marked increase in the stability of the molybdenum blue when it was developed. No change was apparent 15 minutes after the addition of the stannous chloride, and at the end of 30 minutes fading was very slight. Comparative results are shown in Table I.

TABLE I

FADING OF BLUE COLOUR WITH AND WITHOUT ADDITION OF SULPHAMIC ACID

	Time after addition of stannous chloride solution: 0	5	10	15	30	40	50	60	120 minutes
	Si standards required to match test solutions								
Sulphamic acid added	5	5	5	5	4·5	4	3·5	3·25	2·5 ml. std. Si soln.
	4·5	4·5	4·5	4·5	4	3·5	3·25	3·0	2·25 ,, ,, ,, ,,
	5	5	5	5	4·75	4	—	3·5	2 ,, ,, ,, ,,
Sulphamic acid omitted	5	5	3·25	2·5	1	0*	0	0	0† ,, ,, ,, ,,
	5	5	4	3	1	0*	0	0	0† ,, ,, ,, ,,
	5	5	5	3·25	1	0*	0	0	0† ,, ,, ,, ,,

* Deep orange colour. † Very pale yellow.

The method finally adopted is as follows:

METHOD

REAGENTS—

Sulphamic acid solution—10 per cent. in distilled water.

Ammonium molybdate solution—1·4 per cent. in distilled water; neutralise if necessary.

Stannous chloride solution—Heat 2·5 g. of stannous chloride with 3 ml. of concentrated hydrochloric acid until clear and dilute to 250 ml. with distilled water.

Standard silicon solution—Weigh 0·075 g. of pure silica into a platinum dish. Add 10 ml. of distilled water, 0·5 g. of potassium hydroxide, A.R., and warm gently until a clear solution is obtained. Dilute with distilled water, add a few drops of phenolphthalein indicator and dilute sulphuric acid until just acid and make up to 1 litre. One ml. of this solution contains 0·000035 g. of silicon, which is equivalent to 0·01 per cent. in the alloy when the quantities specified below are used.

PROCEDURE—

Dissolve 1·40 g. of the nickel alloy in 10 ml. of diluted nitric acid (1 + 1) and add 5 ml. of diluted sulphuric acid (1 + 3). Transfer to an electrolytic cell with a mercury cathode and platinum anode and make up the volume to 50 ml. Pass a current of 3·5–4 amps. until the solution is colourless, which takes about 1–1¼ hour. Add 5 ml. of the sulphamic acid solution and electrolyse for a further 15 minutes. Run off the aqueous layer into a 100 ml. graduated flask before switching off the current. Rinse the cell and mercury three times with distilled water into the flask, avoiding transference of any mercury. If a bead should run out with the aqueous layer, return the whole solution to the cell, electrolyse for 5 minutes and separate again. Cool the solution and make up to 100 ml.

Measure 25 ml. of the solution into a 100 ml. Nessler cylinder. Add phenolphthalein and freshly prepared sodium hydroxide solution (1 + 4) to faint permanent pink. Turbidity at this stage indicates the presence of mercury due to the solution of a bead removed from the cell with the aqueous layer. Add 10 ml. of diluted sulphuric acid (1 + 30) and mix; then add 10 ml. of ammonium molybdate solution, mix and allow to stand for 5 minutes. Add 30 ml. of diluted sulphuric acid (1 + 3), mix and allow to stand for 5 minutes. Add 10 ml. of stannous chloride solution, make up to 100 ml. with distilled water and mix. Compare the colour with standards prepared by pipetting into Nessler cylinders quantities of standard silicon solution ranging from 1 to 10 ml., diluting to 50 ml. with distilled water, adding 10 ml. of diluted sulphuric acid (1 + 30) and proceeding as in the determination. Make the comparison within 15 minutes of adding the stannous chloride to the first cylinder. It is preferable to start the preparation of the standards before adding reagents to the test cylinder and then to add each reagent to all the cylinders in succession so that the colours are developed with a minimum time difference between the test and standard cylinders.

Typical results are shown in Table II (p. 431).

To confirm that silica is not precipitated when the alloy is dissolved in nitric acid, experiments were carried out in which various amounts of standard silicon solution were added to the alloy before this was dissolved. The analyses were then carried through normally. The results are shown in Table III (p. 431).

Although, as has been stated, phosphorus is not found in the alloys in question, the investigation was extended to discover the amount of phosphorus that will be suppressed by this method, since it was found that if relatively large amounts are present the development of blue colour due to phosphorus is not entirely prevented. Nickel solutions containing known amounts of silicon and phosphorus were analysed and results showed that concentrations of up to 0·01 per cent. of phosphorus in the metal caused no appreciable error. In presence of larger amounts than this errors gradually begin to appear, although a content of 0·2 per cent. of phosphorus in the alloy gives rise to an apparent increase of only 0·005 per cent. in the silicon content which, in view of the precision of the method, is almost negligible.

The presence of phosphorus in any significant quantity is indicated by a decrease in intensity of the yellow colour which occurs when the 30 ml. of diluted sulphuric acid (1 + 3) are added to the Nessler cylinder in the analysis; 0·01 per cent. of phosphorus, or less, which is without effect on the silicon determination, gives rise to only a faint yellow tint, the destruction of which by the sulphuric acid results in a very slight paling. When the content of phosphorus is sufficient to affect the silicon results the decrease in intensity of the yellow colour is obvious. Snell and Snell[11] mention the use of a solution containing 0·63 g. of potassium

TABLE II

SILICON ADDED AND FOUND IN ABSENCE AND PRESENCE OF 0·01 PER CENT. OF PHOSPHORUS

Form of nickel used	Silicon added, per cent. of nickel	Total silicon found, per cent. of nickel	Added silicon found, per cent. of nickel
	Phosphorus absent		
		0·005 (reagents only)	
Nickel nitrate A.R.	nil	0·01	—
,, ,, ,, ...	0·01	0·02	0·01
,, ,, ,, ...	0·05	0·06	0·05
,, ,, ,, ...	0·10	0·11	0·10
,, ,, ,, ...	0·12	0·13	0·12
Spectroscopically pure nickel . .	nil	0·01	—
,, ,, ,, . .	0·02	0·035	0·025
,, ,, ,, . .	0·04	0·055	0·045
,, ,, ,, . .	0·07	0·09	0·08
,, ,, ,, . .	0·10	0·12	0·11
	0·01 per cent. of phosphorus added		
Nickel nitrate A.R.	nil	0·01	—
,, ,, ,, ...	0·02	0·035	0·025
,, ,, ,, ...	0·04	0·06	0·05
,, ,, ,, ...	0·07	0·08	0·07
,, ,, ,, ...	0·10	0·11	0·10
Spectroscopically pure nickel . .	nil	0·01	—
,, ,, ,, . .	0·02	0·03	0·02
,, ,, ,, . .	0·04	0·05	0·04
,, ,, ,, . .	0·07	0·075	0·065
,, ,, ,, . .	0·10	0·10	0·09

chromate per litre as a colour standard for the determination of silicon as silicomolybdate. This solution may also be employed to indicate the presence of phosphorus in concentration sufficient to interfere with the silicon determination by the present method. Nessler cylinders containing distilled water, a few drops of ammonia and potassium chromate solution are prepared to match the test cylinder of silico- and phosphomolybdate before and after the 30 ml.

TABLE III

RESULTS WITH STANDARD SILICON SOLUTION ADDED TO NICKEL ALLOY BEFORE DISSOLVING IN NITRIC ACID

	Silicon added, per cent. of alloy	Total silicon found, per cent. of alloy	Added silicon found, per cent. of alloy
Series 1	nil	0·07 (blank)	—
	0·015	0·085	0·015
	0·03	0·10	0·03
	0·045	0·115	0·045
Series 2	nil	0·07 (blank)	—
	0·01	0·08	0·01
	0·02	0·09	0·02
	0·03	0·10	0·03
	0·045	0·11	0·04
	0·06	0·12	0·05

of diluted sulphuric acid (1 + 3) are added. The difference is due to phosphorus, some relationship existing between the two volumes of potassium chromate solution and the phosphorus content, although this aspect has not been further investigated. Table IV shows the results of one series of experiments.

SUMMARY—After removal of nickel from solutions of alloys containing about 99 per cent. of nickel and 0·01–0·10 per cent. of silicon by electrolysis, using a mercury cathode and without eliminating nitric acid, silicon is determined colorimetrically by means of the silicomolybdate - molybdenum blue reaction. Sulphamic acid, added during electrolysis, eliminates nitrites and reduces the rate of fading of the blue colour. The presence of phosphorus is indicated by the paling of the yellow molybdate colour that occurs on increasing the acidity, which also prevents interference by this element.

TABLE IV

EFFECT OF PHOSPHORUS ON APPARENT SILICON CONTENT; PALING OF PHOSPHOMOLYBDATE ON STRONG ACIDIFICATION

Phosphorus added, per cent. of alloy	Determined silicon content, per cent. of alloy	Volume of potassium chromate soln. required to match yellow colour: As formed ml.	Volume of potassium chromate soln. required to match yellow colour: After addition of 30 ml. of sulphuric acid (1+3) ml.
0	0·045	4·0	4·0
0·005	0·045	4·0	4·0
0·01	0·045	4·0	4·0
0·05	0·045	5·5	4·0
0·10	0·05	6·0	4·0
0·20	0·05	9·0	4·0

I must express my thanks to the Management of this factory, in whose laboratory the work was carried out, for permission to publish the paper, and to Mr. J. Paterson and Mr. J. Dearden for carrying out much of the practical work.

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THE GENERAL ELECTRIC CO., LTD.
CAPE MILL, SHAW, NR. OLDHAM, LANCS.

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The Direct Photometric Determination of Aluminium with 8-Hydroxyquinoline

BY C. H. R. GENTRY AND L. G. SHERRINGTON

THE methods in general use for the photometric determination of aluminium are based upon the formation of strongly coloured lakes with aurintricarboxylic acid, alizarin S, eriochromcyanin or haematoxylin.[1,2,3,4] None of these methods is markedly superior to the others, all are very sensitive, none is free from interference, and all require very close attention to experimental detail if the results are to be at all precise. Despite the work of numerous investigators, none of these methods is entirely satisfactory, and there appears to be a need for an accurate method of determining traces of aluminium which is capable of a wide range of application.

8-Hydroxyquinoline has been used for the indirect colorimetric determination of aluminium either by coupling the separated hydroxyquinolinate with diazotised sulphanilic acid[5] or by the estimation with Folin's reagent.[6]

The direct colorimetric determination of aluminium with 8-hydroxyquinoline is dependent on the solubility of aluminium hydroxyquinolinate in chloroform to give a yellow solution.[3] Moeller[7] estimated a number of elements by repeated extraction of their aqueous solutions with a solution of 8-hydroxyquinoline in chloroform and observation of the diluted extracts colorimetrically. With aluminium, complete extraction from the aqueous solution occurred only in the very limited *p*H range, 4·3 to 4·6, under the extraction conditions employed. However, Moeller showed that the chloroform solution of the aluminium complex deviated only slightly from Beer's law in a range up to 20 mg. of aluminium per litre of chloroform, and that the accurate determination of aluminium was possible.

Evidently a more extensive investigation, with particular regard to improved methods

for the extraction of the complex and to possible interferences and methods for their elimination, was justified.

Experimental

Spectrophotometric and Absorptiometric Studies—In preliminary experiments, the absorption spectra of chloroform solutions of 8-hydroxyquinoline and its aluminium and ferric salts were recorded; the results were in good accord with those of Moeller.[7]

The transmission curve of aluminium hydroxyquinolinate showed maximum absorption at about 3950A, and suggested that the photometric determination of aluminium was possible in a wavelength range of about 3850A to 4100A. Solutions of 8-hydroxyquinoline in chloroform absorbed strongly below about 3700A and showed slight absorption in the range suggested for the determination of aluminium.

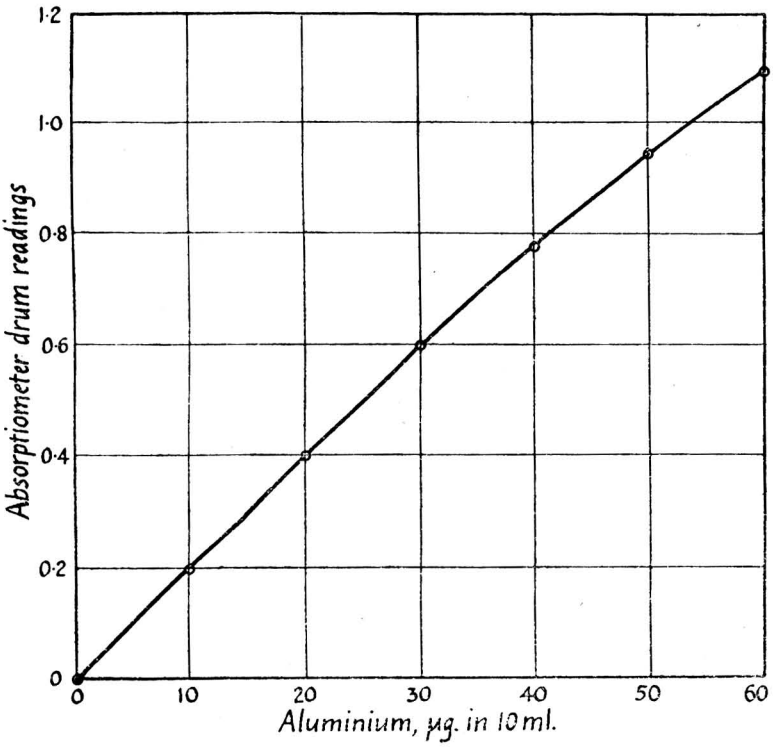

Fig. 1. Photometric determination of aluminium as the hydroxyquinolinate in chloroform solution.

Absorptiometric studies on aluminium hydroxyquinolinate were made with the Spekker Absorptiometer, using the mercury vapour lamp in conjunction with Chance No. 8 (OV1) and Wratten No. 2 filters. With this filter combination it was possible to isolate the 4047A mercury line and thus use the instrument as an "abridged spectrophotometer." The setting of the absorptiometer was made in the usual manner[8] and need not be described further.

The first attempts to obtain a standard calibration curve from solutions of aluminium hydroxyquinolinate in chloroform gave very erratic results. These were due to acid decomposition products of the chloroform, which presumably reacted with the hydroxyquinolinate. Accordingly the solvent was purified by shaking with an aqueous solution approximately 2 *M* in ammonia and ammonium chloride. The acid-free chloroform was separated and used for the preparation of standard solutions.

A solution of 0·1363 g. of aluminium hydroxyquinolinate, previously dried at 140° C., in 100 ml. of purified chloroform was prepared and 10 ml. of it were diluted further to 100 ml. Various quantities of this dilute solution were introduced from a micro-burette into 10-ml. graduated flasks and diluted to the mark with the acid-free chloroform. Absorptiometer readings of these solutions in 1 cm. cells were recorded (see graph in Fig. 1).

It should be recorded here that the absorption of violet light by a solution of aluminium hydroxyquinolinate in chloroform was accompanied by an intense greenish-yellow fluorescence, which might be made the basis of a fluorimetric method for the determination of aluminium.

EFFECT OF REAGENT CONCENTRATION—A standard aluminium solution was prepared by dissolving a known weight, approximately 0·05 g. of spectrographically pure aluminium in a minimum of *aqua regia*, evaporating to moist dryness and diluting to exactly 250 ml. The strength of this solution was confirmed by a gravimetric determination of aluminium on a suitable aliquot.

A "dilute standard" aluminium solution was prepared daily, as required, by diluting 5 ml. of the standard solution to 100 ml. Five ml. of the dilute standard solution contained 48·6 μg. of aluminium.

A series of reagent solutions was prepared by dissolving AnalaR 8-hydroxyquinoline in AnalaR chloroform to give concentrations ranging from 0·01 to 2 per cent. w/v. Extractions were carried out on 50 ml. of aqueous solution at *p*H 9 containing 48·6 μg. of aluminium, using 10 ml. portions of the various reagent solutions. Forty-five ml. of an ammonia - ammonium chloride buffer solution were put into a clean dry 250 ml. separating funnel, and 5 ml. of the dilute standard aluminium solution were added from a pipette. Exactly 10 ml. of the chloroform reagent solution were added, and the funnel was shaken steadily by hand for the specified time of six minutes. The excess pressure was released occasionally by carefully withdrawing the stopper. After the layers had been allowed to settle, the chloroform layer was run off into a clean dry 20 ml. stoppered flask containing 1 g. of anhydrous sodium sulphate. This removed traces of water whose presence might have caused slight turbidities.[9]

A blank was run under the same conditions, with water in place of aluminium solution.

Table I shows the results with reagent strengths from 0·01 to 2 per cent.

TABLE I

EFFECT OF REAGENT STRENGTH ON THE EXTRACTION OF ALUMINIUM

*p*H of aqueous solution 8·90 at 20° C.

Reagent strength, %	Spekker readings		Total aluminium found, μg.		Aluminium found, μg. Corrected for blank
	Solution	Blank	Solution	Blank	
0·01	0·069	0·038	3·4	1·8	1·6
0·05	0·678	0·054	34·0	2·6	31·4
0·1	0·798	0·078	40·4	3·9	36·5
0·5	0·991	0·110	52·2	5·4	46·8
1·0	1·020	0·111	54·2	5·4	48·8
2·0	1·010	0·116	53·5	5·7	47·8

The Spekker readings in this Table were translated into μg. of aluminium by reference to Fig. 1. Such a procedure did not take into account the loss of chloroform by solution in the aqueous layer and by evaporation to saturate the air in the separating funnel. However, the error involved was small, so Fig. 1 was generally used as the standard of reference in the experiments to establish the best conditions for determining aluminium.

It was concluded that under the conditions used a 1 per cent. solution of 8-hydroxyquinoline in chloroform gave effectively complete extraction of aluminium from the aqueous layer. The use of more concentrated reagent solutions offered no advantage, whilst weaker solutions than about 0·5 per cent. gave incomplete extraction. Proof that the extraction with the 1 per cent. reagent was virtually complete was provided by carrying out a second extraction on an aluminium solution from which the first chloroform extract had been separated; no detectable amount of aluminium was found.

EFFECT OF *p*H ON THE EXTRACTION OF ALUMINIUM—A series of buffer solutions was prepared covering a *p*H range of 3 to 11, using mixtures of *M* acetic acid and *M* sodium acetate for the acid range and mixtures of *M* ammonia and *M* ammonium chloride for the alkaline range. To 45 ml. of each of these buffers in 250 ml. separating funnels, 5 ml. of the dilute standard aluminium solution were added. An extraction was then made with 10 ml. of the 1 per cent. 8-hydroxyquinoline reagent, using a shaking time of three minutes.

The chloroform layer was separated and its absorption value measured by the method previously described. The *p*H of the aqueous layer was measured by means of a glass electrode assembly and the Cambridge portable *p*H meter. Blanks were carried out by the same procedure.

The Spekker reading was converted into weight of aluminium by reference to Fig. 1 and, after subtraction of the blank for the impurities in the buffer solution, the weight of aluminium found was calculated.

Further experiments were made in the same way, using dilute hydrochloric acid or sodium hydroxide solutions to extend the *p*H range.

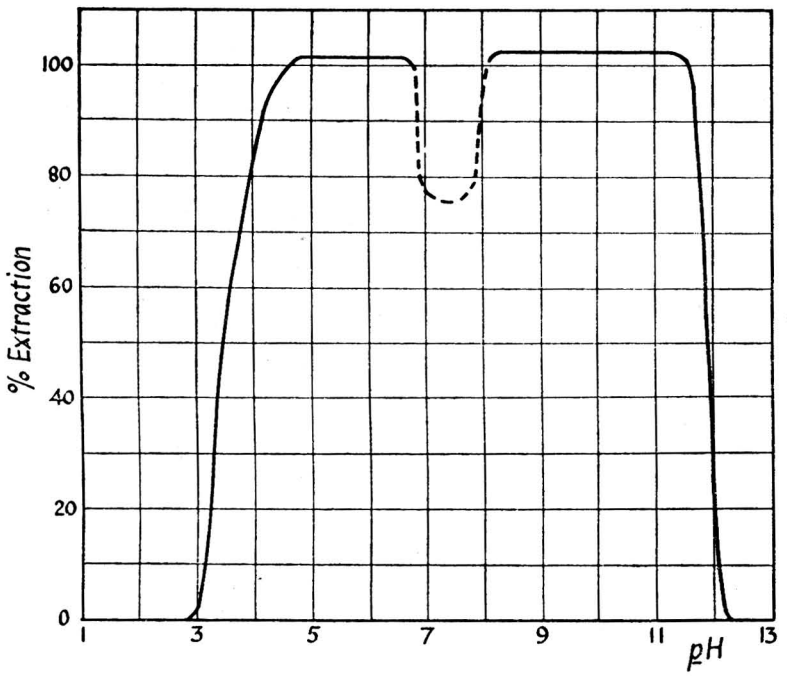

Fig. 2. The effect of *p*H on the extraction of aluminium hydroxyquinolinate with chloroform.

The complete results obtained are plotted in Fig. 2, while Table II presents typical results.

TABLE II

INFLUENCE OF *p*H ON THE EXTRACTION OF ALUMINIUM

Final *p*H of aqueous layer	Aluminium taken, μg.	Aluminium found, μg.	Apparent extraction, %
2·60	50·6	0·0	0
3·64	50·6	16·1	31·6
4·00	50·6	43·4	85·6
4·10	48·6	44·2	90·8
4·74	48·6	49·3	101·4
6·00	48·6	48·7	100·2
6·70	50·6	38·6	79·5
7·11	50·6	37·8	77·8
7·42	48·6	36·7	75·4
8·37	50·6	52·5	103·5
9·02	48·6	49·3	101·5
10·1	50·6	51·9	102·3
11·0	50·6	52·0	102·6
11·8	50·6	46·0	91·0
12·3	50·6	0·0	0·0

Evidently the extraction of aluminium was effectively complete in a *p*H range of 4·5 to 11·5 except between approximately 6·5 and 8. No attempt was made to define the limits of these *p*H ranges with any exactitude as the results would only have been of significance under the precise experimental conditions used. Double extractions were made on some of the solutions, but the extraction of aluminium was still incomplete from solutions with *p*H

approximately 7. Extraction from a solution of *p*H 7·0 was almost complete after very prolonged shaking.

It should be recorded that the *p*H had a very marked effect on the colour of the aqueous layer. At approximately *p*H 7 the aqueous layer was colourless, but as the acidity or alkalinity increased, so the solution became progressively more yellow as more 8-hydroxyquinoline passed into the aqueous layer.

Consideration of these results showed that solutions of *p*H approximately 5 or 9 were of most significance for the photometric determination of aluminium.

THE PRECISION OF THE METHOD—The reproducibility of the method was investigated by carrying out a series of determinations in triplicate with various quantities of the dilute standard aluminium solutions in 50 ml. of a solution buffered at *p*H 9·0. The results, corrected for the blank, are given in Table III.

TABLE III

THE PHOTOMETRIC DETERMINATION OF VARIOUS AMOUNTS OF ALUMINIUM

Aluminium taken, μg.	Aluminium found, μg. 1	2	3	Mean, μg.	Apparent error, μg.
9·7	9·0	9·4	9·0	9·1	−0·6
19·4	20·6	19·8	19·4	19·9	+0·5
29·2	28·9	29·7	29·6	29·4	+0·2
38·9	40·2	39·5	39·4	39·7	+0·8
48·6	48·8	49·3	48·6	48·9	+0·3

As a further test of the precision of the method, fourteen identical determinations were carried out on known solutions containing approximately 50 μg. of aluminium. The final *p*H of the aqueous solution was 9·02 and three minutes' shaking was used. The standard deviation for the group was found to be ±0·47 μg.

INTERFERENCES—A qualitative investigation was made of the reactions of other metals in a sodium acetate - acetic acid buffer of *p*H 5·0 and in an ammonia - ammonium chloride buffer of *p*H 9·0. The general procedure was to place 25 ml. of the buffer solution in a stoppered cylinder and add a solution of about 20 mg. of a salt of the metal. After mixing, 5 ml. of 1 per cent. reagent were added, and the cylinder was shaken for a few seconds; any coloration in the chloroform layer was noted. The cylinder was then shaken vigorously for about a minute and any further changes were noted. When slight colorations were found, comparison was made with a blank.

It was found that tungsten, calcium and tervalent chromium showed no reaction at either *p*H. Zirconium, molybdenum added as molybdate, and vanadium added as vanadate reacted in only the acid buffer and gave respectively yellow, yellow and bluish-grey chloroform layers. Beryllium, magnesium, manganese, tervalent cerium, neodymium and praseodymium gave yellowish chloroform layers at *p*H 9, but showed no reaction at *p*H 5. With magnesium the colour of the extract faded and a precipitate formed on continued shaking.

Antimony, bismuth, cadmium, quadrivalent cerium, cobalt, copper, ferrous and ferric iron, lead, mercurous and mercuric mercury, nickel, stannous tin, titanium, uranium and zinc reacted in both acid and alkaline media. Most of them gave yellow or orange extracts but cerium gave a reddish chloroform layer and iron a black extract.

Cadmium gave only slight extraction at *p*H 5·0, while the extract obtained at *p*H 9·0 faded on shaking and a precipitate formed. The chloroform solutions of zinc hydroxyquinolinate also faded, with simultaneous formation of a precipitate, on continued shaking.

An ammonia - ammonium nitrate buffer was used when investigating the reactions of mercury. The presence of chloride prevented extraction of both mercuric and mercurous hydroxyquinolinates.

A quantitative determination of aluminium in presence of a number of different cations was next undertaken. It was possible to determine 50 μg. of aluminium in presence of 0·1 g. of chromium, magnesium, beryllium or manganese as their chlorides or sulphates in buffered solution at *p*H 5·0. Similarly, aluminium was successfully determined in presence of a large excess of both molybdenum and tungsten at *p*H 9·0.

In investigating cationic interferences large amounts of acetate, chloride, nitrate and sulphate had been shown to be without sensible effect on the determination of aluminium. The effects of fluoride, phosphate and tartrate were demonstrated in a series of quantitative experiments, summarised in Table IV.

TABLE IV

ANIONIC INTERFERENCES

Anion	Salt added	Final *p*H	Aluminium added, μg.	Aluminium found, μg.
Fluoride	0·17 g. Ammonium fluoride	5·00	48·6	0·0
,,	0·17 g. ,, ,, ...	9·00	48·6	1·3
Phosphate	0·15 g. Disodium hydrogen phosphate	5·02	50·6	46·5
,,	0·15 g. ,, ,, ,, ...	8·98	50·6	53·5
Tartrate	0·2 g. Rochelle salt	5·06	50·6	43·0
,,	1·0 g. ,, ,, ...	5·06	50·6	23·5
,,	0·2 g. ,, ,, ...	9·02	50·6	42·4

ELIMINATION OF INTERFERENCES—The value of this method for determining aluminium depends on successful elimination of the large number of cationic interferences. While some of these could be overcome by adjusting the *p*H of the solution, many others would require either preliminary separations, for which methods are well known, or suitable "masking" of the interferences. The latter alternative was investigated.

A valuable method that suggested itself was the use of potassium cyanide in alkaline solution. A solution was prepared containing 2 g. of ammonium nitrate, 1 g. of potassium cyanide and 1 ml. of diluted ammonium hydroxide (1 + 1) in 45 ml. of water. The required quantity of the salt under investigation was added to this solution in a separating funnel; then 5 ml. of dilute standard aluminium solution were added and an extraction was made in the usual manner. A blank was carried out on the reagents. In this manner it was possible to determine 50 μg. of aluminium in presence of 0·1 g. of copper, nickel, cobalt, zinc or cadmium as their nitrates or sulphates, with no loss of precision.

Interference by iron could not be masked by addition of thioglycollic acid, but 50 μg. of aluminium was successfully determined in presence of 0·25 g. of ferric alum after conversion of the iron into ferrocyanide. This was effected by addition of 2 g. of potassium cyanide to the mixture of ferric alum and aluminium solutions in a small beaker, warming at 50° C. for 3 minutes, and adding 10 ml. of 10 per cent. sodium sulphide solution. The cooled solution was transferred to the separating funnel and diluted to 50 ml., 2 g. of ammonium nitrate were added and an extraction was carried out in the usual manner.

Preliminary experiments showed that aluminium could also be determined in presence of a moderate amount of iron, by first extracting the iron with a chloroform solution of 8-hydroxyquinoline from an aqueous solution at *p*H 2. At this *p*H no aluminium is extracted, and complete extraction of iron is possible.

THE DETERMINATION OF ALUMINIUM IN TUNGSTIC OXIDE—As an example of the application of the proposed method a procedure will be described for the determination of traces of aluminium in tungstic oxide.

If this determination is attempted with reagents such as aluminon, the aluminium must first be separated from the traces of iron which are also present. The usual sodium hydroxide or sodium carbonate separation is not complete, some iron passing into the filtrate with the aluminium, possibly by formation of an iron - tungstate complex.[10] This complication makes the determination of aluminium in tungstic oxide with the usual reagents a tedious procedure.

The improved procedure developed for this analysis as a result of the present investigation may be briefly described as follows. Fuse 6·3 g. of tungstic oxide (equivalent to 5 g. of tungsten metal) with 10 g. of sodium carbonate. Leach with water and dilute to 500 ml. in a graduated flask. Pipette a 50 ml. aliquot into a 100 ml. beaker. Add 1 g. of potassium cyanide and 0·5 g. of sodium sulphide. Warm to 70° C. for 3 minutes. Cool and transfer to the separating funnel with a minimum of water. Add 3 g. of ammonium nitrate and shake until it is dissolved. Add exactly 10 ml. of a 1 per cent. solution of 8-hydroxyquinoline in chloroform. Shake steadily for 3 minutes. Allow layers to separate and run off most of the bottom layer into a 20 ml. flask containing 1 g. of anhydrous sodium sulphate. Stopper and shake the flask. Determine the aluminium on the Spekker Absorptiometer. Carry out a blank, omitting the sample, and make appropriate correction.

This method was tested on tungstic oxide samples and it was found possible to determine aluminium down to 0·0002 per cent. in presence of all the impurities usually found in tungstic oxide samples.

DISCUSSION

The use of the standard graph obtained by dissolving aluminium hydroxyquinolinate in chloroform, for the conversion of Spekker readings to μg. of aluminium, is not strictly correct

owing to changes in volume of the chloroform reagent on shaking with the aqueous solution. This fact explains the apparently high aluminium results shown in Fig. 2. However, this error disappears if the standard graph is constructed by carrying out the complete procedure on known quantities of aluminium under conditions identical with those to be used for the subsequent estimation of the unknown. Using this method of preparing the standard graph, the accuracy of the aluminium determination should approximate to the precision which has been found, and the error on 50 μg. of aluminium should be less than 1 μg.

An unexpected feature of the effect of *p*H is the existence of a range around *p*H 7 in which extraction of aluminium is incomplete. This may be due to the non-reactivity of aluminium hydroxide near its isoelectric point or to the minimum aqueous solubility of 8-hydroxyquinoline in this *p*H range, or to a combination of both these factors. It would appear that more complete extraction could be obtained by more prolonged shaking, or possibly by the use of a stronger reagent.

The qualitative experiments showed the lack of selectivity of the method and emphasised the importance of either developing adequate methods of separating aluminium from interfering elements or preventing interferences by "masking". Mercury cathode electrolysis could be used to separate many of the heavy metals before determination of aluminium.

In alkaline cyanide solution the method becomes reasonably selective and is widely applicable, particularly if the interference of iron is overcome by the method which has been described (p. 437).

The method now proposed can be used in presence of many anions, but fluoride and organic hydroxy-acids cause serious interference and must be removed.

A hitherto unreported fact is the anomalous behaviour of the hydroxyquinolinates of cadmium, magnesium and zinc, the chloroform extracts of which fairly rapidly give precipitation on continued shaking with water. It would seem that the anhydrous hydroxyquinolinates are first extracted into the chloroform layer and there react with water, on shaking, to give the insoluble dihydrates. Some evidence for this hypothesis is the fact that zinc hydroxyquinolinate dried at 160° C. can be dissolved in chloroform to give a stable yellow solution which on shaking with water rapidly gives a precipitate.

The present studies have been concerned solely with the determination of aluminium. However, the extraction and the subsequent photometric determination of many metals with chloroform solutions of 8-hydroxyquinoline should be possible. The general application of such methods is chiefly dependent on the establishment of satisfactory methods of eliminating interferences.

SUMMARY—A method for the determination of traces of aluminium is described based on photometric measurement, on the Spekker Absorptiometer, of the hydroxyquinolinate after extraction with chloroform. The effect of the strength of the reagent and the *p*H of the aqueous solution on the completeness of extraction is quantitatively demonstrated. The precision of the method under certain conditions has been found. Interferences have been investigated in detail, and methods for masking certain cationic interferences are described. Attention is drawn to the anomalous behaviour of the hydroxyquinolinates of zinc, cadmium and magnesium. Finally the determination of aluminium in tungstic oxide is described as an example of the application of the method.

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MATERIAL RESEARCH LABORATORY
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MITCHAM JUNCTION, SURREY

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The British Pharmacopoeia

NOTICE has been received of the following alterations and amendments, here abbreviated.

SYRUPUS FERRI PHOSPHATIS CUM QUININA ET STRYCHNINA—The Amendment to the B.P., 1932, authorised in the Sixth Addendum, pp. 26–28 (ANALYST, 1943, **68**, 280) is annulled. Easton's Syrup is again required to contain quinine.

IPECACUANHA RADIX—The dried root or rhizome of *Cephaëlis acuminata* Karsten, known in commerce as Cartagena, Nicaragua or Panama Ipecacuanha, may be used as an alternative to the dried root of *C. Ipecacuanha*. Ipecacuanha contains not less than 2% of the total alkaloids of Ipecacuanha, calculated as emetine, but the requirement of a definite proportion of non-phenolic alkaloids is deleted.

IPECACUANHA PULVERATA—Contains 2% of the total alkaloids of Ipecacuanha, calculated as emetine (limits 1·9 to 2·1), but the requirement of a definite proportion of non-phenolic alkaloids is deleted.

PENICILLINUM—The anti-infective acid produced when *Penicillium notatum* or related organisms are grown under appropriate conditions on or in a suitable culture medium; converted into the sodium or calcium salt. Non-specific impurities are removed as completely as possible, and the purified penicillin salt is dried under conditions designed to ensure the sterility of the final product. When pure, Penicillin (sodium salt) is a white powder which may occur as granules or scales; it contains 1666 units in 1 mg. The sodium and calcium salts that have not been completely purified are pale yellow to light brown amorphous hygroscopic powders containing not less than 300 units per mg.

Penicillin is very soluble in water and insoluble in fixed oils and in liquid paraffin. A solution in sterilised water, after addition of a quantity of sterile penicillinase or other suitable inactivating agent adequate to ensure complete inactivation of the penicillin present, complies with the tests for sterility.

Tests are specified for undue toxicity and pyrogens. Two methods, the "Cylinder-Plate" and "Broth-Dilution" methods, are described for the biological assay. Formulae are specified for the preparation of Cremor Penicillini, Cremor P. Sterilisatus, Injectio P. (since amended), Injectio P. Oleosa, Oculentum P., Trochiscus P., and Unguentum P.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Use of an Ion-exchange Resin in Determination of Traces of Copper with Special Reference to Powdered and Fluid Milk. H. A. Cranston and J. S. Thompson (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 323–326)—Raw milk from several sources has been reported to contain from 0·1 to 0·5 p.p.m. of copper. The usual copper content of premium grade powdered whole milk is now found to be less than 1 p.p.m. The ideal method for determination of copper in foods would be one in which preliminary ashing was avoided and a sufficiently large sample could be taken to ensure a measurable concn. of copper. Copper proteins lose both cuprous and cupric ions in acid soln. below *p*H 3 (Ames and Dawson, *Id.*, 1945, **17**, 249), the cuprous ions being first oxidised to cupric ions. It has also been shown that copper in powdered whole milk is combined with the milk protein, and, presumably, this is true also of raw milk. It is therefore sufficient to convert the copper in milk to the electrovalent form of cupric ions by reducing the *p*H below 3, and thus, under optimum conditions, the protein and fat can be removed and the copper rendered available for determination by simple filtration. The filtrate so obtained, when corrected for added acid and loss of protein and fat, contains copper in the same concn. as the original milk. Synthetic ion-exchange resins afford a satisfactory means of removing copper from the filtrate, but apparently there have been no reports of the use of such resins for concentrating metals in amounts of the order of μg. The general equation applicable to the separation of cations from anions by any ion-exchanger in the hydrogen cycle may be represented as

$$2H^{\cdot}A' + Cu^{\cdot\cdot} + SO_4'' \rightleftharpoons Cu^{\cdot\cdot}A_2' + 2H^{\cdot} + SO_4''.$$

The usual protein precipitants such as metaphosphoric acid, trichloroacetic acid and tungstic acid proved unsatisfactory owing to the difficulty of removing copper from them. Glacial acetic acid had the disadvantage of its buffering capacity, an unreasonably large amount being required to reduce the *p*H sufficiently. Perchloric acid proved an ideal precipitant, only a small amount being required to reduce the *p*H to 1 and provide an easily filtered ppt. Amberlite IR-100 (Resinous Products and Chemical Co., Philadelphia, Pa.) was found entirely satisfactory as an ion-exchanger. It was supplied as the hydrogen derivative and was used in the hydrogen cycle. It was easily purified from its trace of copper by washing with 10% hydrochloric acid and rinsing with water, and back washing was not necessary. A column 150 mm. long and of 12 mm. diam. absorbed up to 1 mg. of copper independently of the amount of filtrate and was more than adequate for 100 ml. of milk filtrate. Passage of the filtrate through the column was rapid and one rinsing with 100 ml. of water was sufficient. The absorbed copper was readily dissolved by hydrochloric acid. Polarographic analysis of the eluate proved the most rapid and simple method, but spectrophotometric methods may be used.

Whole milk requires no preparation beyond thorough mixing. For powdered milk and ice cream reconstitute 25 g. in 250 ml. of water. To the prepared sample in a 400-ml. beaker add 4 ml. of double vacuum distilled 72% perchloric acid, stir well, allow to stand for 30 min., stir gently and filter. Allow the pptd. curd to drain; it should not retain more than 25% of the filtrate. If the paper has been washed free from copper before use, discard the first few ml. of filtrate. To 100 ml. of filtrate add 3 drops of cresol purple indicator and diluted ammonia (10 ml. of conc. ammonia + 100 ml. of redistilled water) slowly with stirring until the colour changes to grey or grey-purple (*p*H 5), the slight ppt. being another indication of the end point. Pass the neutralised filtrate through the column, adjusting the flow so that the dropping rate is 2 or 3 drops per sec. Always stop the flow so that a small amount of fluid remains above the surface of the column. Rinse residual filtrate into the

column with water and stop the final washing so that about 5 ml. of liquid remains above the resin. Wash the adsorbed copper from the column with 40 ml. of 6% hydrochloric acid (1+5) in 4 portions, maintaining the fluid level above the resin. In the final rinsing allow the resin to drain thoroughly. Evaporate the combined filtrate and washings to dryness on a water bath, cool, add 5 ml. of electrolyte (28% ammonia, 200 ml.; ammonium chloride, 40 g.; gelatin, 10 ml. of a 1% soln.), stir and allow to stand for 10 min. Prepare two polarographic cells. To cell A add 1 ml. of redistilled water and 2 ml. of the prepared sample; to cell B add 1 ml. of a copper standard (1 ml. ≡ 20 μg. of copper) and 2 ml. of the prepared sample. Remove oxygen by bubbling hydrogen or nitrogen through the cells for 5 min. and record the polarograms. Run a reagent blank daily on redistilled water and perchloric acid and correct for any copper found. If a is the step height due to the concn. of copper in cell A and b the step height due to that in cell B, $(b - a)$ is due to the added standard, which represents a concn. of 6·67 μg. of copper per ml. Then $6{\cdot}67a/(b - a)$ is the concn. of copper per ml. in the unknown soln. and $50a/(b - a)$ represents the total copper (μg.) in the aliquot of milk filtrate taken for analysis.

For a spectrophotometric procedure collect the eluate and washings from the resin column in a 300-ml. Kjeldahl flask, concentrate by boiling, cool, add 4 ml. of conc. sulphuric acid and a few ml. of redistilled nitric acid. Complete the destruction of organic matter in the usual way, make the soln. up to 50 ml. and determine the copper by the method of Bendix and Grabenstetter (*Id.*, **1943, 15**, 649; ANALYST, 1944, **69**, 63). In this manner a concn. four times that possible by direct digestion of the sample is effected. Phosphates will have been eliminated as anions in passing through the column the first time.

To prepare the resin for use, remove fine material by means of a stream of water, place the resin in a large column tube previously filled with water and slowly pass hot 10% hydrochloric acid through the column until a 75 ml. portion of eluate gives no reaction for copper with a specific reagent (*e.g.*, dithizone). Wash the column free from hydrochloric acid with water and store the resin under water.

The method should have wide application in food analysis, and its possible adaptation to the determination of metals other than copper is being investigated. A. O. J.

A Colorimetric Determination of β-Erythroidine. E. M. Dietz and K. Folkers (*J. Amer. Pharm. Assoc.*, 1946, **35**, 48–49)—β-Erythroidine, isolated from species of *Erythrina* (Folkers and Major, *J. Amer. Chem. Soc.*, 1937, **59**, 1580), has a curare-like physiological activity and its clinical application has been studied. It has been found that a solution of the alkaloid in concentrated sulphuric acid gives an intense purple colour with ferric chloride and this reaction has been utilised for its determination.

Method—Prepare a standard solution by dissolving 12 mg. of β-erythroidine in 100 ml. of water. To 5 ml. of this solution (≡ 0·6 mg. of alkaloid), contained in a 50-ml. volumetric flask, add 15 ml. of a mixture of 2 volumes of sulphuric acid and 1 volume of water very slowly from a pipette. Suspend the stoppered flask in a large oil bath maintained at 115° C. for 1 hour and then add 0·3 ml. of a 10% aqueous soln. of ferric chloride, $FeCl_3.6H_2O$, shake, and continue the heating for a further period of 8 min. Cool the clear purple solution and dilute to the mark with 40% sulphuric acid. Treat the unknown solution in the same manner and evaluate the colour produced with a Klett-Summerson photoelectric colorimeter, using tubes of 15 mm. internal diameter and a No. 54 green filter (absorption maximum 539 mμ). The instrument should be set to zero, using a blank on the reagents alone. The standard and test solutions should be submitted to the test simultaneously, as slight variations in the temperature during the heating may cause the intensity of the colour to vary by ±5%. It is stated that the scale readings follow the Beer-Lambert Law over a wide range and that the colour produced is stable for at least 24 hr. The test is practically specific for β-erythroidine; other *Erythrina* alkaloids give a slight positive reaction, but it is stated that a 10% contamination of β-erythroidine with any of these causes a positive error of only 3%. J. A.

Chemical Investigation of *Spilanthes acmella*. V. G. Gokhale and B. V. Bhide (*J. Indian Chem. Soc.*, 1945, **22**, 250–252)—The flower head of *S. acmella*, which is used as a popular remedy for child stammering, is a powerful mosquito larvicide. Its pungent principle has been extracted by cold percolation with ether. After removal of the ether and treatment of the residue with 60% alcohol, an orange-coloured gum separated and a dark green oil was obtained after removal of alcohol from the extract. The gum had no pungent taste; from its unsaponifiable portion a sterol of m.pt. 184°–185° C. was isolated. An ethereal extract of the dark green oil yielded as main product a substance identified with the spilanthol isolated from *Spilanthol Olerecea* by Asano and Kanematsu (*Ber.*, 1932, **65B**, 1602). The flowers give no appreciable quantity of any essential oil on distillation. Alkaloids were absent from extracts of the flower head with Prolius fluid. E. B. D.

Biochemical

Improved Medium for Microbiological Assays with *Lactobacillus helveticus*. E. C. Roberts and E. E. Snell (*J. Biol. Chem.*, 1946, **163**, 499–509)—The medium devised by Landy and Dicken (*J. Lab. Clin. Med.*, 1942, **27**, 1086) for the estimation of six vitamins by means of *L. helveticus* has not been widely used, largely because it is lacking in substances which, though not essential, greatly stimulate the growth of the organism. An improved medium has now been devised, in which an enzymic digest of casein is used to produce optimal growth. Maximum growth is obtained in 16 hours and maximum acid production in 72 hours.

To prepare the enzymic digest, dissolve 120 g. of "vitamin-free" casein in 2 litres of 0·8% sodium bicarbonate solution. Add 600 mg. of "pancreatin" suspended in 15 to 20 ml. of water, layer with toluene and incubate for 48 hr. at 37° C. Heat in a steamer for 20–30 mins. to remove the toluene, cool and adjust to *p*H 6·0 by addition of glacial acetic acid (about 7 ml.). Filter and add 60 g. of activated charcoal (Darco G-60 was used). After 30 mins., filter, adjust the *p*H of the filtrate to 3·8 with glacial acetic acid (about 75 ml.), stir for 30 mins. with 24 g. of activated charcoal and again filter. Wash the residue from each filtrate with 50–75 ml. of water, and add the washings to the filtrate. The charcoal treatment removes the vitamins present in the initial digest. Finally dilute the filtrate to

2·4 litres, so that 1ml. ≡ 50 mg. of casein. Actually the solution should contain 40 to 45 mg. of solids per ml. and, if markedly lower quantities are present, the preparation should be repeated. The basal medium has the following composition: casein digest 4 ml. (≡ 200 mg. of casein); sodium acetate 200, KH_2PO_4 25, K_2HPO_4 25, glucose 200, cystine 1 mg.; uracil 100, adenine sulphate 100, guanine hydrochloride 100, *p*-aminobenzoic acid 1, biotin 0·04, calcium pantothenate 5, folic acid 0·02, nicotinic acid 5, pyridoxine hydrochloride 10, riboflavine 5, aneurine hydrochloride 5 μg.; Salts C ($MgSO_4.7H_2O$ 10, NaCl 0·5, $FeSO_4.7H_2O$ 0·5; $MnSO_4.4H_2O$ 2·0 g. in 250 ml. of water) 0·2 ml.; all per 10 ml. of final medium. The vitamin to be estimated is, of course, omitted from the medium.

To prepare the inoculum transfer 10-ml. quantities of the complete medium (containing all the vitamins) to test tubes, plug the tubes, sterilise and inoculate from a stab culture grown on a medium containing 1% of Difco yeast extract, 1% of glucose and 2% of agar for 24–48 hr. at 37° C.; store in the refrigerator until required. Incubate the inoculum at 37° C. for 16–24 hr., centrifuge, remove the supernatant liquid aseptically and replace by 10 ml. of sterile saline. Add a sufficient amount of the resulting suspension to a second 10 ml. portion of saline to give a suspension which is just visibly turbid, and use 1 drop for inoculating the assay tubes.

Prepare the assay tubes in the usual way by putting suitable aliquots of the solution to be tested and of standard solutions into test tubes, diluting to 5 ml. with water and then adding 5 ml. of the double-strength basal medium (minus the vitamin being assayed). Sterilise at 15 lb. for 10 min., inoculate and incubate at 37° C. for 16 hr. if the turbidimetric method is to be used, or for 72 hr. if the acidimetric method is to be used. Both methods gave similar results when riboflavine and folic acid were assayed in various foodstuffs and biological materials. Fatty substances affected the response of the organism in the same way as with other media, but measures such as solvent extraction and filtration at *p*H 4·5 appear to eliminate the interference. The advantages of the new method are: (i) the results can be obtained within 24 hr. by means of the turbidimetric procedure; (ii) a single basal medium can be used for the assay of several vitamins; and (iii) enzymic digests of casein are easier to prepare than acid hydrolysates. F. A. R.

Preparation of Samples for the Microbiological Assay of Pantothenic Acid. M. Ives and F. M. Strong (*Arch. Biochem.*, 1946, **9**, 251–258)—Since acid and alkaline hydrolysis destroy pantothenic acid, neither of these methods can be used to liberate combined pantothenic acid preparatory to microbiological assay. Enzymatic digestion suffers from the disadvantage that most enzyme preparations contain appreciable amounts of pantothenic acid, leading to high results. Following an investigation into the amounts of pantothenic acid present in various enzyme preparations and the recovery of calcium pantothenate after incubation with these enzymes, it was found that satisfactory results could be obtained by incubating with "mylase P" (Wallerstein Labs., New York), which acted more quickly and in smaller amounts than the other enzymes tested; its pantothenic acid content was also relatively low and the blank correction negligible. The recommended procedure is as follows. Suspend a quantity of the finely-divided material, containing 3–10 μg. of pantothenic acid, in 50 ml. of water, adjust to *p*H 6·8–7·0 and autoclave for 15 mins., at 121° C. Allow to cool to room temperature and add a quantity of "mylase P" equivalent to one-tenth the total solids content of the sample, 2 ml. of 2·5 *M* sodium acetate and then sufficient hydrochloric acid to reduce the *p*H to 4·8. Layer the mixture with toluene, cork the tube and incubate for 12–24 hrs. at 50° C. Dilute to 100 ml. with water, filter through a fluted Whatman No. 40 paper, re-adjust to *p*H 4·8 if necessary and dilute to a convenient concentration for assay. F. A. R.

Determination of Vitamin A in Liver. W. D. Gallup and J. A. Hoefer (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 288–290)—Procedures for the extraction of vitamin A and removal of interfering substances before colour development with antimony trichloride vary with different materials and for the most part require considerable time and careful manipulation. Simplification of these procedures without sacrifice of accuracy is desirable for routine work and may be accomplished in the analysis of some materials relatively high in vitamin A content and low in fat content. A simplified procedure similar to that developed for blood (Kimble, *J. Lab. Clin. Med.*, 1939, **24**, 1055), but with preliminary saponification of the fat (Kaser and Stekol, *Id.*, 1943, **28**, 904), has been applied to liver after a study of the sources of error involved.

Aldehyde-free alcohol, required for the preparation of alcoholic 5% potassium hydroxide soln., may be prepared as described by Woodman ("*Food Analysis*," 3rd Ed., p. 523, McGraw-Hill Book Co., New York, 1931). To prepare the antimony trichloride reagent dissolve 100 g. of the fresh C.P. salt in enough chloroform of reagent grade and satisfying the Amer. Chem. Soc. specification to make a 25% solution.

Method—Digest 1 g. of liver and 5 ml. of 5% aldehyde-free alcoholic potassium hydroxide soln., in a 50-ml. heavy-walled centrifuge tube having a constricted neck, in a water bath at 75° C. until all the tissue has disintegrated. Adjust the cooled liquid to its original volume with alcohol and add an equal volume of water. This concn. of alcohol and potassium hydroxide in presence of not more than 50 mg. of original fat allows practically complete extraction of vitamin A (and carotene) by light petroleum (b.pt. 40° to 60° C., redistilled Skellysolve F) without appreciable change of volume. If large amounts of vitamin A are present, dilute an aliquot of the digest to 10 ml. with 50% alcohol. Shake the mixture vigorously for 2 to 3 min. with 10 ml. of light petroleum and centrifuge. Transfer 5 ml. (or less if the liver has a high vitamin A content) of the upper layer to an Evelyn colorimeter tube containing a glass bead and evaporate to dryness at 45° C. under reduced pressure. Determine carotenoids, when present, before evaporation of this aliquot by an accepted procedure (Boyer, *et al., Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 101; Oser, *et al., Id.*, 1943, **15**, 724; Kimble, *loc. cit.*; Koehn and Sherman, *J. Biol. Chem.*, 1940, **132**, 527; ANALYST, 1940, **65**, 303). Evaporation should be complete in 5 to 10 min. and a stream of nitrogen may be used as an added precaution against oxidation of the vitamin. Dissolve the residue in the tube in 1 ml. of chloroform and, after adding 1 ml. of C.P. acetic anhydride develop the colour with 9 ml. of antimony trichloride reagent in the usual way. Take galvanometer readings at the point of temporary stability within 5 sec. after adding the reagent.

Consistent results were obtained from day to day when the method was applied to replicate samples of liver of unknown vitamin A content. Likewise, when vitamin A was added to liver in the form of distilled esters recovery values were within 5% of the calculated amount present. Moderate amounts of fat in the original material interfered with the quantitative extraction of the vitamin with light petroleum, but in presence of not more soap than is equivalent to 0·05 g. of fat 95% of the vitamin is removed from solution in 50% alcohol by a single extraction with light petroleum. Although the vitamin could be extracted with ethyl ether in presence of saponified fat, several extractions followed by thorough washing and drying are necessary and it seemed desirable to avoid these steps. Although triple extraction with ethyl ether apparently effected good extraction independently of the amount of saponified fat present, the recovery of known amounts of vitamin A added to vegetable oil was low (91·1 to 94%), probably owing to loss of the vitamin during the washing and drying of the extract. Expts. showed that the recovery of vitamin A from oils containing known amounts was not improved by alterations in the concentrations of alcohol and potassium hydroxide or by variations of the details of the method described.

The use of alcohol containing aldehydes introduces errors of from 10 to 50% in some determinations. Light petroleum extracts from new rubber stoppers (and from some old ones) material that gives a blue-green colour with the antimony trichloride reagent. The intensity of this colour, unlike that of the vitamin A colour, increases during the first few min. If the chloroform used is of doubtful quality it should be suitably treated (Koehn and Sherman, *loc. cit.*, Oser *et al.*, *loc. cit.*). Other sources of error are presence of peroxides in ethyl ether (when this solvent is used) and exposure of solns. of vitamin A to bright indoor light.

A. O. J.

Spectrophotometric Determination of Vitamin A and the Conversion Factor. P. Müller and M. Reinert (*Nature*, 1946, **157**, 876)—A new determination of the conversion factor relating the extinction factor to the biological activity of vitamin A has been undertaken. Vitamin A concentrates and crude liver oils were subjected to the chromatographic purification procedure of Müller (*Helv. Chim. Acta*, 1944, **27**, 443) before being used for the spectrophotometric and biological investigations. It has been found that the growth produced by feeding β-carotene or equivalent quantities of vitamin A concentrates to rats is subject to great variations depending on the diet used, the amount of the dose, the test animals and the season of the year. The growth is not proportional to the amount of vitamin fed, but is considerably greater per unit of vitamin fed, for small doses than for large ones. Under standardised conditions, the best dosage to give reproducible results was found to be 4 to 5·6 I.U., whereas at the Laboratory for Vitamin Control of the Institute for Physiological Chemistry in the University of Basle, the most suitable daily dose was 2 to 3 I.U. On suitably modifying the conditions, similar results were obtained at both places. The average conversion factor obtained was 1700, but in tests carried out without observing the precautions described values were obtained ranging from 1000, for high daily doses, to 3600, for low daily doses of vitamin A. If the chromatographic purification procedure was omitted, low factors (1000 or less) were obtained, owing to impurities which interfered with the spectrophotometric assay and were quantitatively removed by the chromatographic method. The biological equivalence of vitamin A ester and vitamin A alcohol, provided the necessary precautions concerning the dosage are observed, has been demonstrated. It is suggested that since vitamin A is now available in a crystallised and fully characterised form the old international standard of crystallised β-carotene will be replaced by an equally well-defined vitamin A preparation. Such a preparation would be absorbed and utilised better by the test animals and would therefore be preferable to β-carotene for the comparative assay of vitamin A by biological tests. J. A.

Micro-method for the Estimation of Vitamin A in Liver Biopsies in Man and Larger Animals. T. K. With (*Biochem. J.*, 1946, **40**, 249–252)—By carrying out the Carr - Price reaction in special small cuvettes, a layer can be obtained sufficiently thick to give measurable extinctions with the minute amounts of liver tissue obtainable by microbiopsies.

Saponify the weighed tissue by heating carefully with 3 ml. of 5% ethanolic potassium hydroxide solution in a small flask, transfer the solution to a 50-ml. separating funnel and rinse out the flask with water. Extract the solution with three 10-ml. portions of peroxide-free ether, wash the extract and filter through a 1 cm. layer of anhydrous sodium sulphate. Evaporate the solution to dryness in an inert atmosphere, avoiding excessive heating, and dissolve the residue in 1 ml. of freshly distilled chloroform. Transfer the solution to a 10-ml. graduated cylinder, rinse out the flask with three 0·5-ml. portions of chloroform and adjust the volume of the combined solution and washings to 2·5 ml. Transfer 0·1 ml. of the solution to a test-tube and add 0·1 ml. of antimony trichloride reagent. If a pale blue solution results, transfer 1·25 ml. of the vitamin A solution to a 5 cm. micro-cuvette and add one or two drops of acetic anhydride and then 1·25 ml. of the reagent; evaluate the colour in a Pulfrich photometer, using filter S61. If the colour is dark blue, use 0·1 ml. or less of the vitamin A solution with 1 ml. of the reagent and evaluate the colour in a 2, 1 or 0·5 cm. micro-cuvette, so that the extinction lies between 0·3 and 0·8. Whenever possible, the average of four readings made with separate portions of the solution is taken. Calculate the results in the usual way. The differences between the results of micro- and macro-analyses on the same samples of liver never exceeded ±11% and the average difference was 4·65%. F. A. R.

Direct Determination of Diacetyl in Tissue and Bacterial Filtrates. A. G. C. White, L. O. Krampitz and C. H. Werkman (*Arch. Biochem.*, 1946, **9**, 229–234)—The method is based on the condensation of diacetyloxime with urea in strong acid to form a coloured compound. Dilute a trichloroacetic or metaphosphoric acid filtrate of the tissue or bacterial juice so that each ml. contains not more than 100 μg. of diacetyl. To 1 ml. of the diluted extract add 1 ml. of 1% hydroxylamine solution, 1 ml. of 3% urea solution and 2 ml. of a mixture (1 : 3 by vol.) of conc. sulphuric acid and syrupy phosphoric acid. Dilute the mixture to 6 ml. with water. Treat in a similar manner tubes containing 100, 80, 60, 40 and 20 μg. of diacetyl. Immerse the tubes in a boiling water-bath for 35-40 mins., cool and evaluate the colour in a photoelectric colorimeter using a 470 mμ filter. Calculate the

diacetyl content of the extract from the standard curve prepared by plotting the results obtained with the standard solutions. When diacetyl was added to minced liver in amounts up to 100 μg., recoveries were 92–96% of the theoretical. The method can be used for the estimation of acetoin by oxidation to diacetyl by means of ferric chloride solution, followed by distillation as described by Stotz (*J. Biol. Chem.*, 1943, **148**, 585; ANALYST, 1943, **68**, 337) and Stotz and Raborg (*J. Biol. Chem.*, 1943, **150**, 25; ANALYST, 1944, **69**, 23). Recoveries were 95–106% of the theoretical. F. A. R.

Methionine Determination in Proteins and Foods. F. A. Csonka and C. A. Denton (*J. Biol. Chem.*, 1946, **163**, 329–338)—The method suggested is a modification of that of McCarthy and Sullivan (*J. Biol. Chem.*, 1941, **141**, 871). Hydrolyse the protein material by boiling with 20% hydrochloric acid for 24 hours, cool, and dilute 4- to 5-fold. Add a solution of phosphotungstic acid (1 g. per ml.) drop by drop until precipitation is complete and then add a few drops in excess. Make up to a suitable volume and leave in an ice-water bath for 2 hours, centrifuge again, immerse in ice-water for ½-hour and filter. Allow the filtrate to warm up to room temperature and transfer an aliquot, containing not more than 300 mg. of the original protein material, to a 25 ml. volumetric flask. Adjust the *p*H to 4–5 by adding 5 *N* sodium hydroxide. Dilute to a suitable volume, decolorise with 50 mg. of charcoal and filter. Transfer a 4 ml. portion or less of the filtrate to a large test tube, add 2 ml. of 5 *N* sodium hydroxide and sufficient water to bring the volume to 6 ml. Add 1 ml. of a freshly prepared 1% sodium nitroprusside solution (it is essential that exactly 10 mg. be present in the reaction mixture, because this is the quantity used in making the calibration curve), immerse the tube in a water-bath at 40° C. for 8 minutes, cool in ice-water for 5 minutes and then slowly add 2·5 ml. of 20% hydrochloric acid with vigorous shaking. Shake for about 3 minutes and then 1 or 2 minutes later evaluate the red colour in a Coleman spectrophotometer at 510 mμ. Calculate the methionine content from a calibration curve obtained by the same procedure, using pure methionine. The results obtained were lower than those obtained by the original McCarthy - Sullivan method, but in reasonable agreement with those reported by other workers. F. A. R.

A Primary Standard for the Colorimetric Determination of Haemoglobin. B. L. Horecker (*J. Lab. Clin. Med.*, 1946, **31**, 589–594)—The use of crystalline haemin as the primary standard for the determination of haemoglobin is the basis of several methods recently suggested. Clegg and King (*Brit. Med. J.*, 1942, ii, 329) and King, Gilchrist and Delory (*Lancet*, 1944, i, 239) have used solutions of crystalline haemin in 0·1 *N* sodium hydroxide in the alkaline haematin procedure, while Drabkin (*Amer. J. Med. Sc.*, 1945, **209**, 268) recommends haemin dicyanide prepared from haemin as the primary standard for the determination of haemoglobin and cyanmethaemoglobin. Although the latter is probably the most precise method, the routine use of cyanide is an objection. It has been found that a more satisfactory standard for use in the alkaline haematin method is a solution of haemin in 0·1 *N* borate buffer of *p*H 9·4. This is prepared by dissolving crystalline haemin in the buffer to give a stock solution containing about 1 mg. per ml., ageing overnight in the refrigerator and diluting to a concentration of between 10 and 50 mg. per litre just before use. The stock solution is stable for about a month and its absorption spectrum, determined with a Beckman spectrophotometer using 10 mm. Corex cells and an effective slit width of 40A, much more closely resembles that of alkaline haematin prepared from human blood by the method of Clegg and King (*loc. cit.*) than does the spectrum of a solution of haemin in 0·1 *N* sodium hydroxide. For the colorimetric determination of haemoglobin as alkaline haematin, a photoelectric colorimeter with a filter with maximum transmission at 5700A was used; this is considered to be superior to the Chance green filter used by Clegg and King (*loc. cit.*) and with it, 29·6 mg. of crystalline haemin per litre of 0·1 *N* borate buffer has an absorption equal to that of a blood sample, containing 16·7 g. of haemoglobin per 100 ml., diluted in the ratio of 1 to 251 with 0·1 *N* sodium hydroxide. It is stated that the alkaline haematin method is accurate to within 0·2 g. per 100 ml. J. A.

Organic

Determination of Small Amounts of Aromatic Hydrocarbons in Aqueous Solutions. H. E. Morris, R. B. Stiles and W. H. Lane (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 294–295)—The determination of aromatic hydrocarbons in plant effluents and other aqueous solns. is frequently necessary. A moderately accurate method depends upon the development of a brown colour when conc. sulphuric acid containing a small amount of formaldehyde is brought into contact with an aromatic hydrocarbon under specified conditions.

To prepare the formaldehyde - sulphuric acid reagent mix 1 ml. of 37% U.S.P. formaldehyde with 100 ml. of conc. sulphuric acid.

Shake 100 ml. of the sample in a separating funnel with 25 ml. of carbon tetrachloride (previously treated if necessary with sulphuric acid until it remains colourless in contact with the reagent), withdraw the carbon tetrachloride layer and add it to 5 ml. of the reagent in a 200-ml. flask. After vigorous shaking for 1 min. allow the reactants to stand for 5 min. whereupon a brown colour develops in the acid layer. As the depth of colour of this layer changes on standing, observe it at some standard interval of time after shaking. Estimate the amount of aromatic hydrocarbon present by comparison with a sample prepared at the same time and under exactly similar conditions from an appropriate quantity of an aqueous soln. of known concn. of the aromatic hydrocarbon in question. Determine by trial and error the exact amount of the known hydrocarbon soln. required to give an exact colour match with the unknown sample and compute the concn. in the sample.

This procedure was applied to the determination of styrene in an aqueous liquid in the following manner. The standard aq. soln. of styrene used contained 15 p.p.m. Three trials were made in which (*a*) 5 ml. of the unknown were compared with 25 ml. of the standard, (*b*) 3 ml. of the unknown were compared with 50 ml. of the standard and finally (*c*) 3 ml. of the unknown were compared with 60 ml. of the standard. In the last trial an exact colour match was secured and the concn. of styrene in the unknown soln. was thus 300 p.p.m.

Concns. of aromatic hydrocarbons in water, ranging from 1 to 10 p.p.m., may be determined with an accuracy of ±1 p.p.m. With higher concns. it is necessary to dilute the sample with distilled water until it falls within this range and

then to multiply by the dilution factor. Since the test will detect 1 p.p.m. of hydrocarbon in 100 ml. the sensitivity is actually 0·0001 g. of aromatic hydrocarbon. The formaldehyde and sulphuric acid reagent has been applied to aqueous samples containing benzene, toluene, ethylbenzene, and styrene alone or in combination and in concns. ranging from 1 to 500 p.p.m. These hydrocarbons give different degrees of colour with the reagent, and for the most accurate work it is necessary to know the identity of the hydrocarbon. This is not necessary, however, if only a rough estimate of the concn. is required. For routine control work it was found advantageous to use a set of permanent colour standards prepared by mixing appropriate amounts of inorganic salt solns. to obtain colour matches with known samples containing 1, 2, 5 and 10 p.p.m. of aromatic hydrocarbon. The partition coefficient of aromatic hydrocarbons between water and carbon tetrachloride is such that one extraction with the solvent effects complete removal of the hydrocarbon from the aqueous layer. Other extraction agents may be used to remove the hydrocarbon from the aqueous layer. The use of diethyl ether lowers the sensitivity of the test, but may be useful when high concns. of the hydrocarbon are frequently encountered.

Expts. showed that when the concn. of formaldehyde in the reagent was varied from 0·5 to 5·0 ml. per 100 ml. and the reagent was applied to solns. containing 2 to 6 p.p.m. of ethylbenzene the lower concns. of formaldehyde rendered the test more sensitive than the higher. Obviously one concn. must be accepted and maintained as standard to prevent any possible variation from this source.

A. O. J.

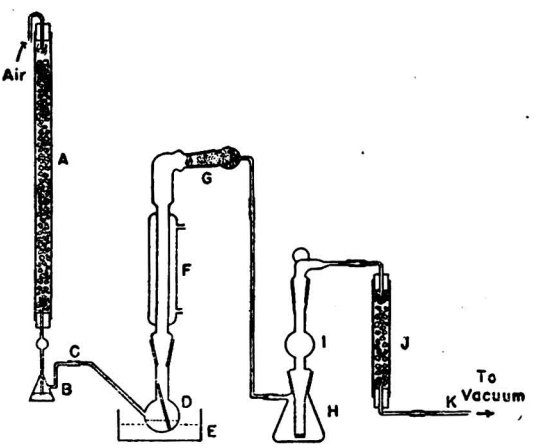
The Determination of Organic Peroxides in Hydrocarbons. E. M. Tanner and T. F. Brown (*J. Inst. Pet.*, 1946, **32**, 341–350)—Three methods for the determination of organic peroxides, those of Lea (*J. Soc. Chem. Ind.*, 1945, **64**, 106), Yule and Wilson (*Ind. Eng. Chem.*, 1931, **23**, 1254) and I.P. 88/44 (Tentative), were found to give low results when used for testing blends containing known amounts (up to 3%) of various peroxides in straight-run gas oils. A method has been developed, accurate to ±4%, which is based on the oxidation of ferrous salts, avoids the tedium of titanous chloride titrations and needs no special colorimetric apparatus.

Procedure—0·5 ml. of the gas oil is weighed into a 100 ml. conical flask, the air of which has previously been replaced by nitrogen, and 65 ml. of glacial acetic acid, de-aerated by passage of a stream of nitrogen for 5 to 10 min., are added and mixed with the oil to form a homogeneous solution. Five ml. of 0·1 *N* ferrous ammonium sulphate solution (de-aerated with nitrogen) are run in from a burette. A brisk stream of nitrogen is passed through the head-space of the flask for 1 to 2 min., the gas supply is considerably cut down, and the flask is heated in a water-bath at 60° to 70° C. for 15 min. Flask and contents are then cooled and the contents are transferred to a 250 ml. flask and diluted with 60 ml. of distilled water. Ten ml. of a solution containing 30 ml. of phosphoric acid (sp.gr. 1·7), 30 ml. of sulphuric acid (98%), and water to make up 200 ml. are added, together with 4 or 5 drops of indicator (0·5 g. of diphenylamine in 50 ml. of 98% sulphuric acid. The mixture is titrated with 0·02 *N* potassium dichromate solution to an intense violet-blue. A blank test is made. The percentage of peroxide is $0{\cdot}05(a-b)fN\mathrm{M}w/$ and the peroxide number is $1000(a-b)fN/w$, where f is the normality factor of the dichromate, *N* its normality, M the molecular weight of the peroxide present, w in grams is the weight of sample used, and *a* and *b* are the blank and test titrations in ml.

The method is applicable to the determination of peroxides formed by autoxidation in cracked gasolines and of non-hydrocarbon peroxides of the type R–O–O–R′ in synthetic mixtures in hydrocarbons boiling in the gasoline range. For these the vol. of acetic acid is reduced to 40 ml., and the reagents are not de-aerated if the iron solution is freshly prepared. With "aged" gasolines the reaction mixture may stand at room temperature for 15 min. instead of being heated to 60° to 70° C.

The effects of oxygen, acetic acid concentration, reaction time and temperature are described.

Determination of Uronic Acids. R. M. McCready, H. A. Swenson and W. D. Maclay (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 290–291)—Theoretical amounts of carbon dioxide were obtained from all uronic acids and their derivatives by heating the sample with 19% hydrochloric acid in an oil bath at 145° C. for 1½ to 2 hr. The yield of carbon dioxide is determined by the following method.



Apparatus (see Fig.)—Air, the carrier gas, is passed through a column (A) of Ascarite or soda-lime and through a mercury trap (B) into the reaction flask (D) by means of a tube (C) fused into the side of the flask. The reaction flask is a 100-ml. long-necked boiling flask with a 24/40 ground joint attached. From the flask the carrier gas passes upwards through a 20 cm. reflux condenser, (F), then through a trap (G) containing 25 g. of 20-mesh granulated zinc or tin and finally into the absorption flask (H), which is a 250-ml. Erlenmeyer flask equipped with a 24/40 ground joint and a side tube attached a little below the joint. Above the joint is an absorption tower (I), the lower part of which is an 18-mm. tube fitted with a medium sintered Pyrex disc and sealed to the lower end of the ground joint. The bubbling disc should be 1 or 2 mm. above the bottom of the absorption flask when the joint is in place. The upper part of the joint leads to a 100-ml. bulb which serves as a trap to prevent loss of alkali by frothing, and the outer portion of a 24/40 ground joint is sealed on above the bulb. The absorption tower measures 30 cm. from the disc to the top of the upper joint. The

upper joint carries a hollow ground stopper with a side-tube leading into a soda-lime tower (J). A water pump attached to the tower through a capillary regulator (K) sweeps 1700 to 2000 ml. of carbon-dioxide-free air through the apparatus during the period of heating.

Method—Place the sample to be analysed in the dry reaction flask; the optimum amount of sample used depends upon its uronic acid content, *e.g.*, about 250 mg. are sufficient for analysing pectin. Add 30 ml. of 19% hydrochloric acid and a small boiling tube. Lubricate the ground joint with syrupy phosphoric acid and attach the flask to the reflux condenser and mercury valve. Draw a stream of carbon-dioxide-free air through the reaction flask and reflux condenser to remove traces of carbon dioxide before the absorption tower is attached. Lubricate the ground joints of the absorption tower with stopcock grease and insert into the absorption flask (H). Sweep the flask and tower free from carbon dioxide and introduce 25 ml. of 0·25 *N* sodium hydroxide and 5 drops of butanol-1 into the absorption tower. Attach the side-tube of the absorption flask to the zinc trap (G), and the top of the absorption tower to the soda-lime tower (J), by means of rubber connections. Place the oil bath (E), previously brought to 145° C., in position with the level of the oil 1 or 2 mm. below that of the liquid in the reaction flask. After the initial rapid evolution of gas through the absorption tower has ceased, attach the capillary tube (K) to the soda-lime tower (J) and to the vacuum line. Keep the oil bath at 145° C. while the apparatus is swept out for 1½ hr. Then remove the bath, disconnect the absorption flask and tower from the apparatus and rinse down the alkali from the absorption tower into the absorption flask. Three or four rinsings are needed to remove the last traces of alkali from the absorption tower. Gentle pressure with carbon-dioxide-free air applied to the top of the tower can be used to hasten the rinsing. Add 10 ml. of 10% barium chloride soln. and two drops of phenolphthalein indicator to the contents of the flask and titrate the excess of alkali present with 0·1 *N* hydrochloric acid. Take normal precautions to exclude carbon dioxide during the storage, addition and subsequent titration of the absorbent alkali.

A control standardisation should be made without the sample and used in calculations of the carbon dioxide evolved from the uronic acids. Results are expressed on the moisture- and ash-free sample, moisture being determined by drying *in vacuo* for 24 hr. at 70° C. and ash by heating the dry samples for 3 hr. at 600° C. A sample of galacturonic acid monohydrate was dried over phosphoric anhydride at 25° C. and analysed without further treatment. Pectin and galacturonic acid yielded the theoretical amount of carbon dioxide in 1 hr. Pectic acids required heating for 1½ hr. and alginic acids for 2 hr. Expts. showed that 19% hydrochloric acid is more effective than acid of lower concn. and requires a shorter heating period for complete decomposition of the uronic acids. The amounts of carbon dioxide evolved from various non-uronide substances such as inulin, gelatin, glucono-δ-lactone, starch, sucrose and oxalic and mucic acids were of the same order as that from glucose (0·3%), so none of these substances, when present in moderate amounts as impurity, would vitiate determinations of pectic substances. A. O. J.

A Reaction of Lead Soap with Sodium Iodide. R. S. Barnett (*Ind. Eng. Chem., Anal. Ed.*, **1946, 18, 333**)—It has been shown that reactions of the double decomposition type can occur in insulating solvents, such as dry benzene, between heavy metal soaps and dry hydrogen chloride or metallic chlorides, such as stannic chloride, which are also soluble in benzene. In developing a method for the determination of free organic acids in lead soap, it was found that addition of a soln. of sodium iodide in acetone to an acetone - benzene soln. of lead soap causes double decomposition with formation of a lead iodide and sodium soap. The mixture becomes bright greenish yellow upon addition of the sodium iodide soln. and flocks of soap-like material separate when the solvents are removed by evaporation. The residue is then bright yellow and gives with water an opalescent, soapy extract alkaline to phenolphthalein and forming a white curdy ppt. with barium chloride soln. Absence of lead from benzene extracts of the dried residue shows that all the benzene-soluble lead soap has been converted into a benzene-insoluble lead iodide, possibly a basic iodide. Both commercial lead oleate and lead naphthenates have been treated in soln. with sodium iodide with the same general result, the sodium oleate formed separating more rapidly upon solvent removal than the sodium naphthenate, *i.e.*, appearing less soluble in these solvents.

Warm 95% acetone (5% water) is an excellent solvent for extracting free organic acids, including oxidised fatty acids, from the lead soap of lead soap lubricants. A small amount of lead soap is dissolved at the same time, and this invalidates the usual titration for free organic acids in the combined extracts because the heavy metal soap is titrated as free acid. The foregoing lead soap and sodium iodide reaction was used to transform such dissolved lead soap in combined acetone extracts into sodium soap and a lead iodide. By extracting the dried residue left after removal of the acetone with hot benzene it is possible to isolate the free organic acids (together with a small amount of sodium soap) leaving behind the lead iodide, excess of sodium iodide and most of the sodium soap. The extracted material, dissolved in alcohol, can then be titrated for free organic acids in the usual manner, as the sodium soap present does not interfere. Semi-quantitative results for free acids in lead naphthenates made from naphthenic acids of different mol. wts., obtained by the procedure described, indicate a relatively high free acid content for the soap made from acids of high mol. wt. For example, with soaps made from acids of saponification value 176 (calculated mol.wt. 318) the amount of organically combined lead was 18·5% and the free fatty acids (as oleic acid) 10%, whereas with soaps made from acids of saponification value 282 (mol. wt. 199) the organically combined lead was 41·3% and the free fatty acids 3%. The greater reactivity of the acids of lower mol. wt. towards lead may account for the lower free acidity of this soap.

There are indications that sodium iodide in acetone soln. does not react with lead oxide, which may be present in some lead soaps, to give free caustic alkali and so invalidate the determination of free acidity, although this reaction does occur when lead oxide is heated with aqueous sodium iodide soln. Work is being continued to ascertain whether the lead iodide compound is a basic salt or the normal di-iodide and thus to gain some idea of the structure of the dissolved lead soap. It is also proposed to complete work on the analysis of lead soaps containing known additions of free organic acids, to define the accuracy and reproducibility of the method. A. O. J.

Detection and Determination of Rosin in Paper and Paperboard. Anon. (*Paper Trade J.*, 1946, **122**, April 25, *T.A.P.P.I. Sect.*, 187–188)—Revised *T.A.P.P.I.* Standard T408 m-44. *Qualitative*—(1) Boil 1 g. of paper, cut into small pieces, with 5 ml. of acetic anhydride in a dry test-tube. When approx. 1 ml. remains, pour it into a dry crucible, cool it to room temp., and filter off any solid matter; add to the solution, down the side of the crucible, 1 drop of conc. sulphuric acid. A fugitive rose-violet colour at the liquid junction is a positive reaction. (2) Apply 1 drop of an almost sat. sugar soln. to the paper on a glass plate. After 1 min., blot off the excess, and add 1 drop of conc. sulphuric acid to the same spot. A raspberry-red colour is a positive reaction. *Quantitative*—(For rosin and/or rosin soap added as sizing plus vegetable resins natural to the paper.) If mineral matter which dissolves in or reacts with hydrochloric acid is present, immerse the sample (in strips 60 × 40 mm.; weight 5–7 g. ± 10 mg.) in approx. *N* hydrochloric acid for 5 min., drain, wash with water and dry at room temp. Extract the strips in a Soxhlet apparatus (15 siphonings per hr.) with a mixture of 1 litre of 95% ethyl alcohol and 4 ml. of conc. hydrochloric acid for 2 hr. (2·5 hr. for coated or surface-sized papers). Evaporate off the solvent on a water-bath, and heat the residue at 100–105° C. for 15 min. These conditions must be observed strictly in order to avoid the formation of non-resinous substances, *e.g.*, by hydrolysis of the cellulose. Cool, add 20 ml. of anhyd. ether, and stir to dissolve the rosin. Allow the mixture to stand for 20 min., and then filter it through a fine paper on a Gooch crucible into a tared beaker; if necessary, filter twice through the same paper. Rinse and wash with 20 ml. of ether, evaporate off the ether, dry the residue at 100–105° C. for 15 min., and weigh. Dry until the wt. is const. to ± 1 mg. (*a* g.). If paraffin or a similar wax is present, add 25 ml. of approx. 0·5 *N* alcoholic potassium hydroxide soln. to the residue, heat to 55–60° C. for 15 mins., cool, wash the liquid into a separating funnel, using 25 ml. of ether and 50 ml. of water in succession, and add ether and water so that 25 and 150 ml. are present, respectively. Add 2 g. of sodium chloride, extract, remove the water layer, wash it with 25 ml. of ether, and bulk the ether layers and ether washings (20 ml.). Evaporate off the ether, and weigh the residue as described above (*b* g.). This gives the wax plus the unsaponifiable matter from the rosin, which normally may be assumed to be 5% of the rosin content of the paper. Thus, wt. of rosin = $(a—b)/0{\cdot}95$ g. If waxes or oils volatile at 100–105° C. are present, and drying at a lower temp. is impracticable, boil the rosin - wax residue with 25 ml. each of ether and approx. 0·5 *N* alcoholic potassium hydroxide soln. and 100 ml. of water, under reflux, for 30 min., cool, add 25 ml. of ether, and extract in presence of 2 g. of sodium chloride. Remove the water layer, and re-extract the ether with 50 ml. of water containing 2 g. of sodium chloride. Add 2 drops of methyl orange indicator solution to the combined water extracts, acidify carefully with 5 *N* sulphuric acid, and add 1 ml. of this acid in excess. Cool, extract with 25 ml. of ether (plus 10 ml. of ether wash liquor) in presence of 5 g. of sodium chloride. Separate and wash the water layer twice, using 20 ml. of ether each time. Evaporate off the ether, and weigh the residue as described above. The wt. of rosin so obtained can be assumed to be 95% of that in the paper. High results are obtained in presence of saponifiable fats, and the method is limited to pine rosin and, therefore, cannot be used in presence of synthetic resins. Report the results to the nearest 0·1% by wt. of the paper dried at 100–105° C. Duplicate determinations should agree to within 0·2%. J. G.

Peroxide Bleaching of Mechanical Pulps. Influence of Bacteria and Enzymes. II. [Determination of Catalase Activity.] R. T. Mills, W. S. Hinegardner and W. O. Stauffer (*Paper Trade J.*, 1946, **122**, May 16, *T.A.P.P.I. Sect.*, 216–220)—Place the sample (in the present instance 225 g. of a 5% pulp slurry) in a 500-ml. bottle, in the wide neck of which is fitted a rubber stopper carrying a thermometer and a capillary connecting tube leading to a calibrated 25-ml. gas receiver containing water and inverted over the end of the tube in a reservoir of water. Cool the system to the temp. desired for the test, ± 0·4° F., (usually 70–75° F.) by means of water-baths. Then add a mixture of 215 ml. of distilled water and 25 ml. of 1% hydrogen peroxide at the same temp., mix, and measure the vol. of gas evolved at intervals during the period of the test. A correlation exists between the catalase activity of the mechanical wood pulps and their bleachability by sodium peroxide. J. G.

Measurement of the Degree of Polymerisation for Works Control in the Bleaching House, and Sampling for Ascertaining Damage due to Bleaching. R. Kling (*Melliand Textilber.*, 1946, **23**, Aug., 393–394)—The increase in the cuprammonium viscosity over that of the pure solvent is approx. proportional to the degree of polymerisation of a cellulosic fabric dissolved in the cuprammonium (Staudinger). Cuprammonium is, however, sensitive to acids, and a 10% sodium hydroxide soln. is preferable for testing regenerated celluloses, including "*Zellwolle.*" Cotton does not dissolve in this solvent, and therefore, by leaching the sample with it and testing the residue by the cuprammonium method, mixtures may be evaluated. The method is suitable for routine control of bleaching operations. The sampling error inherent in such determinations is emphasised, and a statistical method suitable for a large rectangular sample and a modification suitable for smaller or valuable samples, are outlined. The fall in the degree of polymerisation on bleaching is approx. proportional to the bleaching time; it is greater for a high than for a lower original degree of polymerisation, *e.g.*, a val. of 600 fell to 390, whilst 250 fell to 205, under the same bleaching conditions. If a fall from 2000 to 1000 is taken as unity, then a fall (calc. on this basis) of more than 0·7 is regarded as unsatisfactory. J. G.

Testing Analytical Filter Papers. T.A.P.P.I. Suggested Method T 471 sm-46. Anon. (*Paper Trade J.*, 1946, **122**, Mar. 21, *T.A.P.P.I. Sect.*, 131–132)—Existing T.A.P.P.I. Standard Methods are used for the determination of thickness and substance (from which the density is calculated), α-cellulose, copper number and *p*H. The last three indicate the degree of degradation (resulting in brittleness and water solubility) of the cellulose. *E.g.*, a doubly acid-washed paper that had become brittle on storage gave vals. of α-cellulose 72%, copper number 3·3 and *p*H 4·5, and a good paper of the same type and grade, 96%, 0·4 and 6·0 respectively. New additional tests are as follows.

Retention of Fine Precipitates—To a soln. of 0·55 g. of potassium sulphate in 275 ml. of water

add 1·0 ml. of conc. hydrochloric acid, heat to boiling, and add slowly, with stirring, 25 ml. of a 5% soln. of barium chloride ($2H_2O$). After 2 hr. ± 15 min. on the water bath (without agitation), stir well, and filter about 50 ml. through each of 4 normally folded circles of the sample paper in 60°-funnels (stem length, 6 in.). Collect the filtrates in 250-ml. conical flasks, swirl to bring any ppt. to the centre, and view from above against a black ground; 0·3 mg. is detectable, and if it is present the paper is regarded as unsatisfactory. *Filtration Time*—Filter some water through at least 2 paper filters in series, the filtration rate of which is at least as slow as that of the sample. Prepare a 60°-filter cone in the usual way, fill it with the water, press down the folds and expel air pockets. Reject the water after 75% has passed through. Approx. 2/3 fill the same cone with the water at 23 ± 2° C., and when 20% of this has passed through, time the filtration rate of 50% of the remainder. Make the test on at least 10 circles, re-using the water so far as possible. *Ash*—A large sample (*e.g.*, 6 g. pre-dried to const. wt. at 100–105° C., and weighed to 0·01 g.) and as small a platinum dish (with cover) as possible (*e.g.*, 20 ml. capacity) are required, in order to obtain the necessary degree of accuracy. Pre-heat the crucible and a similar tare crucible at about 925°C., cool and weigh it. Wet the paper with water containing not more than 10 p.p.m. of solids after ignition, and roll it into a compact mass. Start the ignition in a cold muffle and finish it with the cover removed, for 2 hr. at 925° C.; weigh from a desiccator. Ignition is complete when the wt. remains const. after re-ignition for 30 min. Agreement between duplicates should be within 0·003, 0·01 and 0·02% for ash contents of less than 0·025, 0·025–0·1, and over 0·1%, respectively. *Wet Bursting Strength*—Clip a pack of 5 circles of samples together, immerse it in distilled water at 23 ± 2° C. for 10 sec., hang it up to drain for 10 sec. and immediately determine the bursting strength by the standard method. J. G.

Inorganic

Semimicro Scheme of Qualitative Analysis for the Cations without the Use of Hydrogen Sulphide. J. T. Dobbins and E. S. Gilreath (*J. Chem. Education*, 1945, **22**, 119–122)—Precipitating anions other than the sulphide ion do not give clear-cut separations of cations, since the solubility products do not differ sufficiently. The scheme described combines separation by difference in solubility products with control of the cation concn. by complex-forming agents. After separating the Group I cations (those with chlorides insol. in dil. acids) in the usual way (see Dobbins, "*Semi-Micro Qualitative Analysis*," p. 272, New York, 1943), two groups are separated by the phosphate ion, using lactic acid as a complexing agent, while a third is pptd. as insol. pyridine complex. The original material is tested for the ammonium ion by heating with potassium hydroxide soln.*

TABLE I

SEPARATION AND ANALYSIS OF GROUP II CATIONS

Centrifugate from Group I—Add 1 drop of conc. HNO_3. Heat in water bath for 3 min. Add NH_4OH until slightly alkaline, then add 3 *N* HCl dropwise until slightly acid, when the total vol. should be approximately 1 ml. Add 3 drops of 25% lactic acid and 3 drops of 1 *M* $NH_4H_2PO_4$. Heat in water bath for at least 5 min., centrifuge and wash with 5 drops of water, adding washings to centrifugate, which is examined as Table II.

Residue—Dissolve in not more than 4 drops of conc. HCl, warming if necessary. Add 5 drops of water.

(*a*) To 2 drops, add 5 drops of 3 *N* HCl and 1 cm of Mg ribbon. Allow to dissolve completely, then transfer 2 drops to filter paper moistened with 3 drops of phosphomolybdic acid. A deep blue colour shows **Sn**. (Fe^{II} gives a very faint bluish-green.)

(*b*) To the remainder add 6 *N* KOH until alkaline, then 4 drops in excess. Heat in water bath for 3 min., add 1 drop of satd. NH_4Cl and heat for 1 min. (Longer if Cr occurs alone.) Centrifuge.

Residue—Dissolve in 3 *N* HCl and dilute to 1 ml. Divide into 2 portions.

(*a*) Add 5–10 drops of cinchonine–KI reagent. An orange ppt. shows **Bi**.

(*b*) Make ammoniacal with conc. NH_4OH and add 6 drops of H_2O_2. Warm gently, then heat in water bath for 5 min. Centrifuge.

Residue—Dissolve in 3 *N* HCl and add 1 drop of NH_4CNS. A wine-red colour shows **Fe**.

Centrifugate—If yellow, contains **Cr**. To confirm, place 3 drops on filter-paper and add in immediate succession 2 drops of H_2O_2 and 2 drops of benzidine; a blue colour results.

Centrifugate—Add 6 *N* CCl_3COOH until barely acid and 2 drops in excess. Add 3 drops of 1 *N* K_2CrO_4. A yellow ppt. shows **Pb**.

TABLE II

SEPARATION AND ANALYSIS OF GROUP III CATIONS

Centrifugate from Group II—Make strongly ammoniacal. Heat in water-bath for 3 min., adding drops of conc. NH_4OH at intervals. Centrifuge while hot. Wash with 3 drops of water and 3 drops of 3 *N* NH_4OH. Add the washings to the centrifugate, which is examined as Table IV

* Particulars of the preparation of the reagents used are not given—ABSTRACTOR.

TABLE II—*continued*

Residue—Add 4 drops of 6 *N* KOH and 10 drops of H_2O_2. Stir thoroughly and warm gently for 1 min., then heat in water-bath for at least 5 min. Cool and centrifuge.

Residue—Add 5 drops of water and 5 drops of 3 *N* HNO_3. Heat for 1 min. and centrifuge.

Residue—Add 1 drop of 6 *N* KOH and 5 drops of water. Shake and add 3 drops of benzidine. A blue colour shows **Mn**.

Centrifugate—Add NH_4OH until alkaline, then conc. CH_3COOH until strongly acid. Add 3 drops of 1 *N* K_2CrO_4. Leave for 1 min. and centrifuge.

Residue—May be $BaCrO_4$. Confirm **Ba** by flame test.

Centrifugate—Examine as in Table III.

Centrifugate—Make slightly acid with 3 *N* HCl and add 2 drops of Alizarin Red S. Add 3 *N* NH_4OH until colour is permanent wine red. Heat in water bath for 1 min., cool, acidify with 3 *N* CH_3COOH. Centrifuge. A pronounced red gelatinous ppt. shows **Al**.

TABLE III

Identification of Strontium, Calcium and Magnesium

Centrifugate from Table II—Make alkaline with 3 *N* NH_4OH, centrifuge, wash with 5 drops of water plus 1 drop of NH_4OH. Discard centrifugate and washings. Dissolve the ppt. in the least amount of 3 *N* CH_3COOH, add an equal vol. of water and 4 drops of 2 *N* $(NH_4)_2SO_4$. Leave 3 min., test for complete pptn. and centrifuge.

Residue—May be $SrSO_4$. Confirm **Sr** by flame test.

Centrifugate—Add 2 drops of 5 *M* citric acid and 2 drops of 0·5 *N* ammonium oxalate and centrifuge.

Residue—May be CaC_2O_4. Confirm **Ca** by flame test.

Centrifugate—Use one half. Add an equal vol. of 6 *N* KOH and 3 drops of *p*-nitrobenzeneazo-α-naphthol. Heat in water-bath for 3 min. then centrifuge. A bluish-green ppt. shows **Mg**.

TABLE IV

Separation and Analysis of Group IV Cations

Centrifugate from Group III—Make barely acid with 3 *N* CH_3COOH. If warm, cool, then add 5 drops of pyridine and NH_4CNS reagent. Shake, leave for 2 min. and centrifuge. Examine the centrifugate as Table V.

Residue—Add 5 drops of water and 5 drops of conc. CH_3COOH. Shake and centrifuge.

Residue—If green, is **Cu**. Confirm by adding 1 drop of conc. HCl; residue should turn black.

Centrifugate—Add 6 *N* KOH until alkaline, then 4 drops in excess. Heat in water-bath for 3 min. and centrifuge.

Residue—Dissolve in 3 *N* HCl and dilute to 1 ml. Divide into 3 portions:—

(*a*) Make ammoniacal and add 2 drops of dimethyglyoxime. A red ppt. shows **Ni**.

(*b*) Add 5 drops of satd. NH_4CNS soln. A blue colour shows **Co**.

(*c*) Add 1 *N* KCN until soln. is decolorised and any ppt. has redissolved. Add 5 drops of 6 *N* KOH, 2 drops of "cadion" reagent and 6 to 10 drops of HCHO. A red ppt. shows **Cd**.

Centrifugate—Make slightly acid with CH_3COOH. Add 2 drops of pyridine and NH_4CNS reagent and 1 drop of Rhodamine B. Shake well. A pink ppt. shows **Zn**.

TABLE V

Analysis of Group V Cations

Centrifugate from Group IV—Divide into 2 portions:—

(*a*) Add 4 drops of 3 *N* H_2SO_4 and evaporate to dryness, heating just below redness until fuming ceases. Cool, add 1 drop of water and make flame tests for **Na** and **K**.

(*b*) Heat over boiling water and add conc. HNO_3 dropwise until brown fumes cease. Evaporate almost to dryness, add 5 drops of 3 *N* HNO_3, stir thoroughly and centrifuge.

Residue—Dissolve in 6 drops of conc. HCl.

(*a*) Place 1 drop on a spot plate. Add 0·05 g of $NaNO_2$. After the latter has completely decomposed, add 2 drops of Rhodamine B. A purple colour shows **Sb**.

(*b*) Place 2 drops in a centrifuge tube, add 1 drop of satd. KI soln. and 0·5–0·75 ml of conc. HCl. An orange ppt. shows **As**.

Centrifugate—Add 3 drops of diphenylcarbazide and add dropwise satd. K_2CO_3 soln. until almost neutral. Foam tinged a deep blue colour and a bluish violet ppt. shows **Hg**.

J. T. S.

Analysis of Cable Sheathing Alloys. G. M. Hamilton (*Nature*, 1946, **157**, 875)—The existing methods for the analysis of lead - antimony - tin alloys are mostly subject to drawbacks by reason of the methods used for the solution of the metal. A satisfactory procedure has been evolved for the analysis of a lead alloy containing up to 2% of tin, 0·8% of antimony and 0·25% of cadmium. The reagent used for the dissolution of the metals consists of a mixture of 20 ml. of 30% hydrogen peroxide and 5 ml. of glacial acetic acid and is capable of disintegrating 2·5 g. of lead alloy, which may be in lump form, in a few minutes. Antimony is not attacked and, if present, the finely divided metal gives the suspension a grey colour. Addition of hydrochloric acid dissolves the antimony, converts the other metals to chlorides and decomposes the greater part of the hydrogen peroxide. From this point, the analysis of the solution follows conventional methods except that it is necessary, when reducing the antimony with sulphur dioxide, to digest the mixture on the boiling water-bath for 10 min. before boiling off the excess sulphur dioxide. This treatment removes the last traces of peroxides, which would otherwise adversely affect the antimony determination. Tin may be determined by the method of Evans (ANALYST, 1931, **56**, 171; also 1944, **69**, 201). The procedure fails with alloys rich in tin, such as solders, which are only slowly attacked by the reagent. J. A.

Agricultural

Determination of Nitrogen, Phosphorus, Potassium, Calcium and Magnesium in Plant Tissue. Semi-micro Wet Digestion Method for Large Numbers of Samples. O. J. Kelley, A. S. Hunter and A. J. Sterges (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 319–322)—Several of the numerous methods for individual determination of nitrogen, phosphorus, potassium, calcium and magnesium have been adapted and combined so that these elements can be determined rapidly in one digest of the plant material, the digestion being effected by an adaptation of the rapid wet-digestion, micro-Kjeldahl method for nitrogen determination of Pepkowitz and Shive (*Id.*, 1942, **14**, 914).

Solution A: To a suitable wt. of the material in a 100-ml. Pyrex flask add 3 ml. of a soln. of 32 g. of salicylic acid in a litre of conc. nitrogen-free sulphuric acid and allow the mixture to stand for 30 min. Add 4 drops of 50% sodium thiosulphate monohydrate soln. and 5 ml. of selenium oxychloride soln. (2·4 g. per litre of conc. sulphuric acid) and, after mixing, heat gradually to boiling and digest until clear. If the cooled soln. is not colourless after rinsing down the sides of the flask with water, add 2 drops of 10% perchloric acid, shake immediately, heat first at low temp. and then digest without boiling until colourless. Cool and dilute to 100 ml.

Solution B: Shake soln. A thoroughly to resuspend all pptd. material, immediately withdraw a 25-ml. aliquot, evaporate it to dryness in a 50-ml. beaker and heat at low red heat to remove excess of sulphuric acid and ammonium salts. Cool and dissolve in 2 drops of conc. hydrochloric acid and 10 ml. of water and dilute to volume in a 25-ml. flask.

Determine *nitrogen* by the method of Fraps and Sterges (*Texas Agr. Expt. Sta. Bull.*, 1931, 439). After the aliquot for soln. B has been removed allow the suspended matter in soln. A to settle completely. To 2 ml. of the supernatant liquid of soln. A in a 100-ml. flask add 50 to 60 ml. of water and 2 ml. of *N* sodium hydroxide and shake thoroughly. Add 2 ml. of 1% gum Ghatti soln., shake, then add 2 ml. of Nessler's reagent, mix thoroughly and dilute to 100 ml. Using a 420-mμ filter compare the colour in a colorimeter with that of a series of ammonium hydroxide solns. treated similarly.

Determine *phosphorus* by a modification of the method of Parks *et al.* (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 527) and Fiske and Subbarrow (*J. Biol. Chem.*, 1925, **66**, 375; ANALYST, 1926, **51**, 205). To 5 ml. of the supernatant liquid of soln. A in a 50-ml. flask add 5 ml. of ammonium molybdate soln. (2·5% in 5 *N* sulphuric acid) and shake. Add 2 ml. of 1 : 2 : 4-aminonaphtholsulphonic acid soln. (prepared by adding 0·125 g. to 49 ml. of filtered 15% sodium bisulphite soln. and then adding 1·25 ml. of 20% sodium sulphite soln.). Prepare a set of standards simultaneously since the slope of the standard curve is not constant, allow the colour to develop for 30 min. and read in a photoelectric colorimeter, using a 660-mμ filter for colour comparison.

Determine *potassium* by a modification of the method of Brown *et al.* (*Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 652). To 1·5 ml. of 95% alcohol in a 15-ml. centrifuge tube add a 5-ml. aliquot of soln. B and mix thoroughly. Place the tube in a water-bath at 20° C. When it has attained this temp. add slowly with constant shaking 2 ml. of sodium cobaltinitrite soln. (46·2 g. of sodium cobaltinitrite, 18·9 g. of sodium acetate, 120 ml. of water and 18 ml. of glacial acetic acid, the soln. being prepared 48 hr. beforehand, stored tightly stoppered in the dark and centrifuged before use) and allow to stand for exactly 1 hr. at 20 ± 1° C. Centrifuge for 10 min. at 2000 r.p.m., decant the supernatant liquid from the tightly compacted ppt., invert the tube at an angle of 45° and allow it to drain for several min. Wash the ppt. with 5 ml. of 70% alcohol, centrifuge for 5 min. and drain as before. Dry the ppt. at 80° C. until free from alcohol, add 5 ml. of 0·02 *N* ceric sulphate (more, if more than 0·5 mg. of potassium is present) and 1 ml. of diluted sulphuric acid (1 + 1). Heat in a water-bath at 90° to 95° C. until all the ppt. is oxidised, cool and titrate the excess of ceric sulphate with 0·02 *N* ferrous ammonium sulphate, using a drop of 0·025 *M* *o*-phenanthroline ferrous complex soln. as indicator. To prepare the indicator dissolve 0·173 g. of ferrous sulphate and 0·125 g. of *o*-phenanthroline in 25 ml. of water. The change from pale blue to red is very sharp. Apply the procedure simultaneously to a set of standard potassium solns. containing amounts of potassium of the order present in the aliquots of soln. B. Assuming complete recovery of the potassium in the standard solns., determine a factor for converting ml. of 0·02 *N* ceric sulphate into mg. of potassium.

Remove manganese, iron, aluminium and phosphorus by the method of Peech (*Id.*, 1941, **13**, 436). To a 5-ml. aliquot of soln. B in a 15 ml. centrifuge tube add 0·2 ml. of ferric chloride soln. (1·22 g. of the hexahydrate in 250 ml. of water containing 1 ml. of conc. hydrochloric acid), 3 ml. of water and 2 ml. of 10% sodium acetate soln., mix, add 1 ml. of 0·01 *N* sodium hydroxide and mix again. Add 1 ml. of sat. bromine water and maintain the temp. at 95° C. in a water-bath for at least 1 hr. Add 2 ml. of 25% ammonium chloride soln. and digest for 15 min. Add 1 drop of methyl red soln. (0·02%) and, if the persisting colour of the indicator indicates complete expulsion of bromine, remove the tube from the bath, cool, add 0·6 *N* ammonia until

the colour changes to deep yellow and 2 drops in excess. Make to a vol. of 13 ml., add 5 drops of water in excess to allow for evaporation, mix well and digest in the water-bath at 80° C. for 5 min. to flocculate the ppt. Centrifuge while hot for 10 min. (Solution C).

Determine *calcium* by the method of Peech (*loc. cit.*). To 10 ml. of the supernatant liquid of soln. C in a 15-ml. centrifuge tube add 1 ml. of 60% ammonium acetate soln., heat the tube in a water-bath at 70° C., add 2 ml. of sat. ammonium oxalate soln. mix and digest for 30 min. Cool, adjust to 13 ml. and centrifuge for 10 min. at 2000 r.p.m. Decant the supernatant liquid into a dry test tube (Solution D). Allow the tube to drain at an angle of 45° for several min., wipe the lip of the tube with filter paper, wash the ppt. with 5 ml. of 1% ammonia saturated with calcium oxalate and centrifuge again. Decant and discard the supernatant liquid, and allow the tube to drain again for several min. Add 3 to 5 ml. of 0·02 *N* ceric sulphate and 1 ml. of dil. sulphuric acid (1 + 1), heat to 70° C. in a water-bath and titrate with 0·02 *N* ferrous ammonium sulphate using the *o*-phenanthroline indicator as previously described.

Determine *magnesium* by an adaptation of the method of Reitemeier (*Id.*, 1943, **15**, 393). To 5 ml. of soln. D in a 15-ml. centrifuge tube add 1 ml. of 25% ammonium chloride, 1 ml. of ammonium dihydrogen phosphate and 1 drop of phenolphthalein indicator. Heat to 90° C. in a water-bath and add conc. ammonia until the liquid is pink, cool, add 2 ml. of conc. ammonia, stir with a glass rod, rinse the rod with a small stream of water, stopper the tube and set it aside in a cool place overnight. Centrifuge, decant the supernatant liquid, drain the tube at an angle of 45° for several min., and wash the ppt. and sides of the tube with 5 ml. of a mixture of 20 ml. of ammonia, 80 ml. of water, 100 ml. of alcohol and 100 ml. of ether. Decant and drain and repeat the washing and draining once. Dissolve the ppt. in 5 ml. of 2 N sulphuric acid, transfer into a 25-ml. flask and develop the phosphate colour as previously described. Prepare a photometric calibration curve by simultaneously subjecting a series of magnesium sulphate solns. to the same operations each time a group of samples is analysed. From this curve calculate the amount of magnesium in the unknown sample.

The accuracy of the method is satisfactory; the greatest variations in the determination of known amounts of the elements occurred in the results for magnesium. Most of the error involved is considered to occur in the colorimetric determination of magnesium and not in the digestion procedure.

A. O. J.

Field Surface Test for DDT. H. A. Stiff and J. C. Castillo (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 316–317)—The DDT is removed from a surface by scrubbing with a cottonwool swab impregnated with mineral oil and the swab is then subjected to a modified xanthydrol - potassium hydroxide - pyridine reaction. In presence of DDT a red colour forms. Only two reagents are required, *viz.*, a 0·4% soln. of xanthydrol in pure anhydrous pyridine and potassium hydroxide pellets U.S.P. (Merck or Baker). Some samples of C.P. pyridine may be used without purification but most samples must be rendered anhydrous by treatment with stick sodium hydroxide for several days and subsequent distillation. The xanthydrol reagent is stable for several days when stored in a glass-stoppered bottle.

The apparatus consists of a supply of clean dry 17 × 150 mm. test tubes, a small spirit lamp, a test tube holder, a 2-ml. pipette and a number of oil-impregnated swabs. To prepare the swabs wrap pieces of cottonwool tightly round the end of an applicator stick, dip it in clear liquid paraffin and squeeze out the excess of liquid against the side of the container.

Method—Thoroughly scrub an area of about 12 sq.in. of the surface to be tested with the oil-impregnated swab. Pick up two pellets of potassium hydroxide by inserting the test tube into the container and allowing two pellets to slide to the bottom of the tube. Pipette 2 ml. of 0·4% xanthydrol soln. in pyridine into the tube and boil the liquid gently over the spirit lamp. When the contents of the tube become green insert the swab and boil for a few seconds more. If DDT is present the colour will change to red. If DDT is absent the green will fade quickly to yellow.

A large number of different surfaces known not to contain DDT (*e.g.*, brick, cement, stone, tile, glass, paper, plaster, wood, paint, metal, plastic, canvas, fruit, foliage, grass) were swabbed and tested. No false positive reaction resulted. DDT in ethereal soln., in concns. varying by increments of 5 μg., were applied directly to a series of swabs, the ether was allowed to evaporate at room temp. and the swabs were tested. Twenty-five μg. were detectable in comparison with a blank, and 75 μg. gave a very distinct pink colour. DDT in 5% kerosene soln. and 5% aqueous triton-xylene emulsion was applied quantitatively to 3 × 4 in. surfaces of commonly encountered surface materials to simulate the results of different intensities of spraying. After exposure for 48 hr. at room temp. each entire surface was swabbed and tested for DDT. A positive reaction was considered to be one that matched the colour given by a swab containing 75 μg. of DDT. The following were the minimum amounts of DDT in mg. per sq. ft. that had to be applied to the surfaces to yield a positive reaction, the first figure being for the kerosene soln. and the second for the triton - xylene - water emulsion. Tent canvas (untreated), 1500, 1500; Celotex building board (unpainted), 1000, 1000; rubber matting (ribbed), 750, 50; soft pine board (unpainted), 100, 75; Celotex building board (oil painted), 250, 25; plaster (oil paint surface), 200, 25; soft pine board (oil paint surface), 200, 15; plaster (fresh, smooth, unpainted) 15, 25; sheet metal (galvanised), 5, 5; plate glass, 3, 3.

The reaction is not specific for 2,2-bis-(*p*-chlorophenyl)-1,1,1-trichloroethane but is given by a number of related compounds containing halogen attached to aliphatic groups. With due caution this should not seriously affect the value of the test since the presence of such compounds would usually be known. A statistical evaluation of the application of the test is not yet possible and the data presented indicate orders of magnitude rather than exact data. It is established however that if conditions are such that more than 75 μg. of DDT are picked up by the swab a positive reaction will result. Although the quantitative expts. were made only with building materials, it is believed that the conditions encountered apply to the surfaces of fruit, foliage, grass and vegetation. In general, fruit and green foliage, being relatively non-porous, will retain a higher concn. of DDT, whereas dry vegetation absorbing more of the soln. or emulsion will have a lower surface concn. A. O. J.

Reviews

PHYSICAL METHODS OF ORGANIC CHEMISTRY. Edited by A. Weissberger. Vol. I, 1945, pp. vi + 1–736; Vol. II, 1946, pp. vii + 737–1367. New York: Interscience Publishers, Inc. Price, each volume, $8·50.

The first volume of this publication contains chapters on the determination of melting and boiling temperatures, density, solubility, viscosity, surface and interfacial tension, osmotic pressure, diffusivity, crystal form and the properties of monolayers and duplex films. Calorimetry, microscopy, crystallochemical analysis, X-ray diffraction, electron diffraction and refractometry all have long chapters devoted to them. The various authors are distinguished workers in their chosen fields.

The second volume contains chapters on spectroscopy and spectrophotometry, colorimetry and fluorimetry, polarimetry, dipole moments, conductometry, potentiometry, polarography, magnetic susceptibility, radioactivity and mass spectrometry. Each chapter is written by an authority on the subject.

The preface stresses the increasingly numerous and complex physical methods available for the treatment of organic chemical problems and the danger of using them without adequate preparation. "The object of the authors has been to provide a description of tested methods, the theoretical background for understanding and handling them and the information necessary for a critical evaluation of the experimental results." There is little uniformity of presentation, but apparently the space devoted to theoretical treatment depends upon the availability of "comprehensive modern presentation" in monographs. This is a disarming way to account for C. P. Smyth's 20 pages (about 8,000 words) on dipole moments as against about five or six times as much space for each of the following: calorimetry, microscopy, polarimetry and polarography.

It is claimed that "the book is calculated to appeal to the student who seeks to increase his understanding of the methods described, although he may not practice them himself." For such a purpose half a million words of reading (much of it quite stiff) is excessive. As a work of reference, on the other hand, the book contains many admirable chapters, clear, up-to-date and authoritative. Although 17 dollars is reasonable for a work of this size it is high for private purchasers in this country. Research institutes and university libraries will need to have copies, but it seems very probable that the volumes will disappear for long periods while specialised workers take their time to digest a single chapter. From that point of view it seems a pity that the whole work was not divided into four volumes instead of two.

Perusal of this book may raise important issues in the mind of the reader. First, there is the question of the optimum size of research units. The small unit faces the choice between being under-equipped and carrying rising obsolescence charges on scientific instruments. The large unit runs other risks, such as divorcing direction from experimentation. Secondly, it is clear that efficient control, testing and research require a greater expenditure on new equipment than is even yet at all widely realised in this country. Finally, a re-grouping of the sub-divisions of chemistry is taking place with inevitable repercussions on the teaching of the subject. Those who lecture on Organic, Physical or Biochemistry will find much in these volumes which they would like to use, but they will have to frame selection principles of their own.

In the short time the book has been in the reviewer's hands he has used it in the course of ordinary work on three problems. Each time the necessary information was found, with supporting references to the literature.

R. A. MORTON

SCIENTIFIC PROGRESS IN THE FIELD OF RUBBER AND SYNTHETIC ELASTOMERS. Edited by H. Mark and G. S. Whitby. Vol. II of *Advances in Colloid Science*. Pp. xl + 453. New York: Interscience Publishers, Inc. 1946. Price $7·00.

During recent years many books have been published which describe the technology of plastics, while a few have discussed these substances theoretically. An extensive and detailed account of fundamental investigations on those materials commonly termed "rubbers" is, however, welcome. The present work is therefore an apt place in which to find a twelve-page biography of the late Elmer O. Kraemer, its initiator. After a useful *Introduction* by G. S. Whitby, which includes a brief review of the structures of various elastic polymers, the book is divided into monographs written by specialists in the different fields.

It is pleasing to note that, in an American book, the authors of five of the nine sections are workers in this country.

The subjects treated are as follows: R. F. Boyer and R. S. Spencer, *Second-order transition effects in rubber and other high polymers;* 55 pages. L. A. Wood, *Crystallisation phenomena in natural and synthetic rubbers;* 37 pages. C. W. Bunn, *The study of rubber-like substances by X-ray diffraction methods;* 49 pages. G. Gee, *The thermodynamic study of rubber solutions and gels;* 51 pages. R. H. Ewart, *Significance of viscosity measurements on dilute solutions of high polymers;* 55 pages. E. Guth, H. M. James and H. Mark, *The kinetic theory of rubber elasticity;* 46 pages. E. H. Farmer, *Vulcanisation;* 63 pages. H. P. Stevens, *Rubber photogels and photovulcanisates;* 26 pages. D. Parkinson, *Reinforcing and other properties of compounding ingredients;* 41 pages. Each section concludes with a bibliography (the book contains nearly 800 references) and the complete work has adequate author and subject indexes; a short appendix mentions review articles published since the manuscripts were written, so that the reader is informed of up-to-date opinions.

Most of the book is concerned with physical data and their mathematical treatment and the reader is impressed not only by the number of distinct types of movement ascribed to the rubber "molecule" or to segments of it, but also by the quantitative deductions possible from such apparently simple assumptions. Some of the material now presented has hitherto been unpublished and, in the present state of knowedge, much of the contents of these monographs must be regarded as either not established beyond doubt or not yet quantitatively satisfactory. This is freely admitted by the authors; for example, it is pointed out that after more than a century of technological experience of vulcanisation opinion is still divided over its true nature, while the mechanism of the behaviour of vulcanisation accelerators and zinc oxide, and the precise causes of the reinforcing action of carbon black are very important features of rubber science which remain imperfectly understood. It is tolerably certain, however, that any future account of the rubber-like state will be founded upon hypotheses now discussed. This book can be confidently recommended as providing highly interesting and stimulating answers to such questions as "What is rubber?" "What happens when it is vulcanised?" and "How do you explain its phenomenal elasticity?" All concerned are to be congratulated on a well-produced, readable volume which is remarkably free from misprints.

G. H. WYATT

QUALITATIVE INORGANIC MICROANALYSIS. R. Belcher, F.R.I.C., and Cecil L. Wilson, M.Sc., Ph.D., F.R.I.C. Pp. viii + 68. London: Longmans, Green & Co. 1946. Price 2s. 6d.

Members of the Microchemistry Group of the Society will remember a paper read at one of their meetings by Dr. C. C. Miller (*Chemistry and Industry*, 1946, 26) in which she outlined the course of instruction given at Edinburgh University and showed most encouraging quantitative results obtained by undergraduates learning inorganic microanalysis during their normal studies. We are now presented with the qualitative counterpart of that paper by two more exponents of the theory that microchemistry is more suitably taught before rather than after graduation. Opinion in general seems to be changing towards this point of view and it is obvious that the students concerned require appropriate textbooks; the present "short elementary course" will adequately supply the material indicated by its title.

The authors contend, rightly, that junior students should be given a book within their financial reach; both authors and publishers are to be congratulated in achieving this object. The theory, which "appears in every theoretical textbook," has been omitted, not only to limit the size and cost of the book, but also for the praiseworthy reason that the methods are to be learned as analytical methods and not "merely as pegs to carry the theory of physical chemistry." The student is told first of the various pieces of apparatus needed and how these are used. A set of exercises is then presented to familarise him with the techniques of manipulating them, the examples being selected qualitative analytical reactions. After this he is considered to be capable of learning how to examine unknown substances. Tables are provided showing the usual preliminary tests and the customary group separations, indications being given how these may be best applied on the micro scale. For the confirmatory reactions full use is made of organic reagents and spot tests. There next follows an alternative set of group separations designed to avoid the use of hydrogen sulphide. These are based upon a scheme due to A. J. Mee (*A New Scheme of Elementary Qualitative Analysis;* Dent, 1942). After the usual precipitation of Ag, Hg and Pb as chlorides, Ca, Sr, Ba and residual Pb

are separated as sulphates. Sodium thiosulphate is then used to precipitate the sulphides of As, Hg, Sn, Sb, Cu and Bi. The remaining solution may contain Sn, Al, Pb, Ca, Fe, Mn and Cr, which are precipitated by ammonium carbonate, also Zn, Ni, Co, Cd and Mg, which are then tested for individually. A final chapter describes the examination for acid radicles. Two appendixes give details of the reagents used in the above schemes and a list of textbooks and journals indispensable for those who wish to study the subject more fully.

The authors are wise to emphasise how thoroughly the customary scheme of group separations has now been tested and to give it pride of place over the newer methods. Moreover, one is inclined to ask whether on the micro scale it is so important to avoid the use of hydrogen sulphide, which may be generated in appropriately small volumes without polluting the atmosphere of the laboratory. G. H. WYATT

PHYSICAL METHODS GROUP

A JOINT Meeting of the Physical Methods Group with the Cardiff and District Section of the Royal Institute of Chemistry and the South Wales Section of the Society of Chemical Industry will be held at University College, Cathays Park, Cardiff, on Friday, October 11th, 1946, at 6.30 p.m. The subject will be Electrometric Analysis and the following short papers will be read:

"Recent Developments in Apparatus for *p*H Measurement and Electro-titrations," by A. D. E. Lauchlan, M.A.

"Some Applications of Electrometric Methods to Analysis," by R. J. Carter, B.Sc., A.R.I.C.

"Polarisation End-points," by D. P. Evans, M.Sc., Ph.D., F.R.I.C.

It is hoped that ample time will be available for discussion of these papers.

Facilities will be available for members to take tea together at the Park Hotel, Park Place (about ten minutes' walk from University College) at 5.0 p.m., at a charge of 2/- each. Members wishing to do this should advise Mr. H. F. Adams, Coal Survey Laboratory, 128, Newport Road, Cardiff, before October 4th.

MICROCHEMISTRY GROUP

OWING to unforeseen circumstances the meeting of the Microchemistry Group which was to have been held in Birmingham in September has had to be cancelled.

The next meeting will be held in London in January, 1947.

ADVICE TO AUTHORS

THE Council has approved the following notice by the Publication Committee, which is here given in condensed form.

The Society publishes papers concerned with all aspects of analytical chemistry, inorganic and organic, including chemical, physical, biological, bacteriological and micro methods. Papers on these and allied subjects, by members of the Society or non-members, may be submitted for presentation and publication; they may:

(1) record proposals for new methods and the investigations on which the proposals are based;
(2) record the results of original investigations into known methods or improvements therein;
(3) record analytical results obtained by known methods where these results, by virtue of special circumstances, such as their range or the novelty of the materials examined, make a useful addition to analytical information;
(4) record the application of new apparatus and new devices in analytical technique and the interpretation of results.

Communications.—Papers (which should be sent to the Editor) will normally be submitted to at least one referee, on whose advice the Publication Committee will be guided as to the acceptance or rejection of any communication. Papers or Notes accepted by the Publication Committee may not be published elsewhere except by permission of the Committee.

Abstracts.—The MS. should be accompanied by a brief abstract of about 100 to 150 words indicating the scope and results of the investigation.

Proofs.—Proofs should be carefully checked and returned within 48 hours. Two galley proofs* will normally be sent out, one of which should be retained by the Author.

Reprints.—Ten Reprints are supplied gratis to the Author. Additional reprints may be obtained at cost if the Author orders them directly from the printers, W. Heffer & Sons Ltd., 104, Hills Road, Cambridge, at the time of publication. Details are sent to Authors with the proofs.

Notes on the writing of papers for THE ANALYST

Manuscript.—Papers and Notes should preferably be typewritten.

The title should be descriptive and should set out clearly the scope of the paper. Degrees are now omitted after the names of Authors in the headings of papers.

Conciseness of expression should be aimed at; clarity is facilitated by the adoption of a logical order of presentation, with the insertion of suitable paragraph or sectional headings.

Generally, the best order of presentation is as indicated below:

(*a*) General, including historical, introduction.
(*b*) Statement of object of investigation.
(*c*) Description of methods used. Working details of methods are usually most concisely and clearly given in the imperative mood, and should be given in this form, at least while economy of paper is pressing, *e.g.*, "Dissolve 1 g. in 10 ml. of water and add . . .". Well-known procedures must not be described in detail.
(*d*) Presentation of results.
(*e*) Discussion of results.
(*f*) Conclusions.

followed by a short summary (100 to 250 words) of the whole paper: items (*e*) and (*f*) can often be combined.

Illustrations, diagrams, etc.—The cost of setting up tabular matter is high and columns should therefore be as few as possible. Column headings should be brief or replaced by a number or letter to be used in combination with an explanatory footnote to the table.

Sketches or diagrams should be on white Bristol board, not larger than foolscap size, in Indian ink. Lettering should be in light pencil.

Tables or graphs may be used, but normally not both for the same set of results. Graphs should have the co-ordinate lines clearly drawn in black ink.

References.—References should be numbered serially in the text and collected in that order under "REFERENCES" at the end of the paper. They should be given in the following form:

1. Dunn, J. T., and Bloxam, H. C. L., *J. Soc. Chem. Ind.*, 1933, **52**, 189T.
2. Allen, A. H., "*Commercial Organic Analysis*," Churchill, London, 1882.

Notes on the Presentation of Papers before Meetings of the Society are appended to the "ADVICE," copies of which may be obtained on application to the Secretary, 7/8, Idol Lane, London, E.C.3.

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* During the paper shortage two copies of the MS. will not be insisted on, nor will two galley proofs be sent.

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