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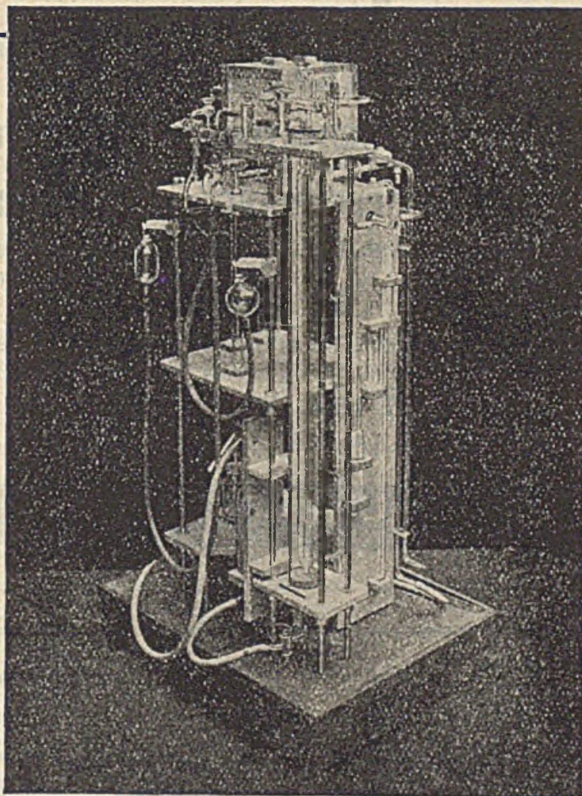

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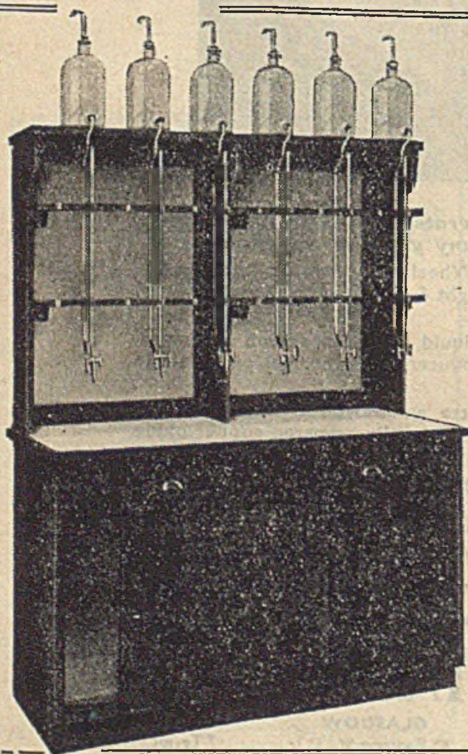

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS
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The Detection and Determination of Auxins in Organic Manures

Part II.—Extraction of Auxins from Manures, and Applications of the Perchloric Acid Test for β-Indolyl Acetic Acid and of the Went Pea Test

By J. HUBERT HAMENCE, Ph.D., M.Sc., F.R.I.C.

(*Read at the Meeting, May 3, 1944*)

BIBLIOTEKA POLITECHNIKI ŚLĄSKIEJ

In Part I of this series[1] a new test based on the use of a perchloric acid reagent was described for the determination of β-indolyl acetic acid and its homologues, and also a new technique employing this reagent in conjunction with chloroform for the determination of β-indolyl acetic acid in presence of the propionic and butyric homologues. This communication is devoted to a description of methods devised in our laboratory for the extraction of auxins from organic manures and the methods of examining the extracts thus obtained.

Although very little work on this subject appears to have been carried out, sufficient knowledge concerning auxins in general was available to predict that the total amount of auxins in fertilisers was not likely to exceed a few mg per 100 g of organic manure. It followed from this and from the general nature of organic manures, farmyard manure, dried blood, etc., that tests could not be applied directly to the manures and that the auxins must be extracted and concentrated before they could be detected and estimated. Moreover, it also became necessary to employ somewhat larger quantities of material than are usual in analytical processes. Amounts ranging from 50 to 500 g have been used in our expts.

At this stage it is appropriate to discuss certain small modifications that it was found necessary to introduce into the original perchloric acid technique in order to make it applicable to all types of organic manures. In some of the early expts. the failure to obtain complete recovery of added β-indolyl acetic acid with some fertilisers was found to be due to an inhibiting effect brought about by impurities extracted together with the auxins.

This fact was established as follows. The final extract from a specimen of hop manure to which a known amount of indolyl acetic acid had been added was divided into halves. In one half the β-indolyl acetic acid was determined by the perchloric acid test; to the second half an additional known quantity of β-indolyl acetic acid was added, and the total β-indolyl acetic was then similarly determined in this portion. The following amounts of β-indolyl acetic acid were found: first half, 0·010 mg; second half plus 0·05 mg of added β-indolyl acetic acid, 0·026 mg.

This proves that some very strong inhibiting action takes place, since in this expt. the auxin added to the final extract could not have been lost in the extraction process.

A very large number of expts. were made to find a method of separating the inhibiting impurities from the auxins. In view of the method of extraction employed it was rightly assumed that the impurities were acidic substances, and accordingly methods of separation as lead and calcium salts were investigated. The separations were carried out in neutral and acid solutions, in water and in alcohol, but without achieving the desired result. It is worth noting at this stage that the lead separation in dil. acetic soln. was very effective in removing colouring matters; this point will be referred to later.

Chromatographic resolution was then tried, employing columns of alumina and magnesium carbonate as the adsorbants; various different solvents were used such as ether, acetone, etc., but still without success. Separation by distribution between immiscible solvents, such as water and petrol, and the use of adsorbent clays and charcoal were also

investigated. It soon became apparent that the interfering impurities had properties very similar to those of β-indolyl acetic acid and that they could not be readily separated.

The difficulty was finally overcome in a comparatively simple manner by modifying the perchloric acid test. It was observed that the interfering bodies tended to form a ppt. when the hot perchloric acid was cooled and that the ppt. adsorbed part of the red colour produced by the action of the perchloric acid on β-indolyl acetic acid. It was found that this pptn. could be prevented if the test was carried out in a solution containing 20% of alcohol.

The use of alcohol gave very much improved recoveries, but the real solution of the problem was found to lie in addition of a trace of ferric chloride to the alcohol soln. The function of the trace of ferric chloride in the perchloric acid reaction is by no means clear; possibly it may act as a catalyst in the perchloric oxidation just as the impurities act as inhibitors.

MODIFIED PERCHLORIC ACID TEST—Place 1·5 ml of the soln. to be tested in a test-tube, add 1·0 ml of alcohol, 1 drop of 0·2% ferric chloride soln. and 2·5 ml of 20% perchloric acid and boil gently for 15 sec. Cool immediately and extract with 5 ml of chloroform. Then match the red colour of the chloroform extract in a 1-cm cell in a Lovibond Tintometer.

Apart from ameliorating the inhibiting action of the impurities the use of ferric chloride together with alcohol has the effect of increasing the sensitivity of the reaction.

The propionic and butyric compounds yield yellow colours in the modified test and, as with the acetic compound, the sensitivity is increased. When the yellow solutions are extracted with chloroform only a very slight pink colour is imparted to the chloroform by the propionic compound, whilst the butyric compound yields a slightly green chloroform extract. The production of this green colour provides a method of detecting the butyric compound in presence of the propionic body, which was not possible by the original process.

Unfortunately the modified procedure suffers from the disadvantage that indole, if present in sufficient quantity, may lead to erroneous results, since it will impart a distinct pink colour to the chloroform extract. Indole may be separated from the indolyl acids by extracting an alkaline soln. of the acids with ether. This stage has been included in the final purification process of the auxin extracts to which reference will be made later. Indole may also be separated by the distillation process of Holt and Callow.[2]

METHODS OF EXTRACTION—As was pointed out in Part I (*loc. cit.*) apparently no attempts have hitherto been made to extract auxins from solid manures. The classical methods of Kogl, Haagen-Smit and Erxleben[3] for the isolation of auxins from urine are too long to be employed as routine analytical processes, but nevertheless give most valuable indications of the type of extraction process most likely to be successful. Holt and Callow[2] described a method of separating indolyl acetic acid from urine by direct continuous extraction with ether. Whilst this process is eminently suitable for the extraction of auxins from urine, preliminary expts. indicated that it would not be suitable for some types of organic fertilisers.

The first process that we tried was alcoholic extraction on the lines of the classical Stas-Otto toxicological technique. Farmyard manure and hop manure were used for the tests. Portions (100 g) were acidified with citric acid and extracted with alcohol, and the process was continued as in the usual determination of aspirin or veronal in viscera. This process was abandoned when it was found that the two manures employed for testing gave unworkable emulsions on treating the residue from the alcoholic extraction with water. Apart from the obstinate emulsions it was considered that, in view of the unstable nature of auxins, a process not involving the evaporation of large volumes of alcohol, even under reduced pressure, would be preferable.

Cold Lime Extraction—As auxins are acidic bodies which form water-soluble salts, it was considered that extraction with dilute alkali should remove the auxins from the bulk of the fertiliser. Lime water removed less fatty material and less pigments from the manure than dil. caustic soda soln. and was accordingly adopted as the solvent for the preliminary extraction of the auxins from the fertiliser. Exhaustive extraction was found to be unnecessary. By adding a known volume of water to a mixture of the fertiliser and lime, filtering, and taking an aliquot portion of the soln., added auxin could be satisfactorily recovered. After a solution of the auxins in lime water had been obtained the auxins could be concentrated satisfactorily by acidifying the soln., extracting with ether and evaporating the ethereal extracts. After the acidification it was found desirable to employ a clearing agent to remove traces of fatty material, etc., prior to extraction with ether. The choice proved very difficult, since most of the usual clearing agents carried down with them part

of the auxin, presumably by adsorption. This difficulty was finally overcome by the use of previously pptd. zinc ferrocyanide. The zinc acetate and potassium ferrocyanide reagents were prepared by the method of Moir and Hinks[4] and were mixed together in a small volume of water before being added to the auxin solution. There was no adsorption of auxin with this reagent, when used in this manner. After acidification of a portion of the filtrate from the lime water extraction with sulphuric acid a suitable quantity of the clearing agent was added, and the solution was made up to known vol., well shaken and filtered. A definite volume of the filtrate was then extracted with peroxide-free ether, and the ethereal extract was washed with water and finally evaporated.

Modified Alcohol Process—When at an early stage in this investigation unsatisfactory recoveries of added β-indolyl acetic acid were obtained with fresh farmyard manure it seemed possible that micro-organisms might be responsible for the small losses that occurred and that therefore an additional process, in which the activity of micro-organisms was suppressed, would be useful. Accordingly the Stas-Otto process was modified in the following manner. The manure was acidified with saturated citric acid soln. and then extracted with alcohol. The alcoholic extracts were united and made slightly alkaline with strong caustic soda soln., and the alcohol was distilled off under reduced pressure. The residue was dissolved in water, the soln. was acidified with sulphuric acid, and zinc ferrocyanide clearing agent was added. Reference was made above to obstinate emulsions frequently obtained at this stage; it was found that these could be avoided by adding sodium chloride to the soln. before making it up to a known volume; clear bright filtrates were thus obtained. The filtrate was then extracted with ether as described in the cold alkali process.

EXAMINATION OF ETHEREAL EXTRACTS—After the auxins have been obtained from a large bulk of organic manure in the form of an extract weighing between 1 and 50 mg, the final stage is to determine their proportion. The β-indolyl acetic acid is determined by the perchloric acid method as follows. The ethereal extract is dissolved in a small known vol. of dil. caustic soda soln. and the modified perchloric acid test is applied direotly to an aliquot portion, say, 1 ml. The remainder of the extract is retained for the determination of total auxins and for other tests.

The problem of the detection of the homologues of β-indolyl acetic acid can now be considered. In Part I of this series[1] the proportion of indolyl propionic and butyric acids was calculated from the number of yellow units required to match the colour in the perchloric acid test before extraction with chloroform. The same technique may be applied to the extracts obtained from organic fertilisers, but extreme care must be exercised in the interpretation of the results. The reason for this is that almost all the extracts obtained by either the lime or the alcohol method from organic manures are slightly yellow, some more so than others, and allowance must be made for this initial colour by matching the colour of the soln. obtained on mixing the reagents for the perchloric acid test prior to boiling.

Experimental work is now in hand to produce a satisfactory method of determining phenyl acetic acid. The odours of warm extracts from a number of organic manures indicate that they contain phenyl acetic acid in small proportions.

Total Auxins—The usual method for the determination of auxins in plant physiology is by the *Avena* or oat test, and the method is ideal for the determination and detection of the comparatively small quantities of auxins involved in that work. When this investigation was undertaken it was decided that the *Avena* test was too sensitive for the problem in hand and that the Went pea test, which is capable of dealing with comparatively gross quantities of auxin, would be more suitable.

The Went Pea Test—This test, described by Went[5] in 1934, is based upon the fact that when elongating organs, particularly stems, are split longitudinally in the growing zone, the two halves curve outwards in water and inwards in auxin solution (see Fig. 1), the split stems being completely immersed in the solutions. The outward curvature is due to tissue tension, the epidermal cells being normally under tension and the pith cells under pressure. The inward curvature is a differential growth phenomenon of a complex nature. It is not proposed to describe here the method of growing the peas and the preparation of the stems in detail, but only to give our method of applying the test. The peas are grown on moist sand in the dark in an incubator at 23° C. They are ready for the test when the plants have developed two nodes each bearing a scale, and one at the top bearing a leaf. In the first place it must be borne in mind that many substances interfere with the

pea test and that therefore only the final ethereal extract from the lime or the alcoholic process must be used for the test. To give a few examples of interfering bodies, Went and Thimann[6] find that traces of copper, nickel, manganese and zinc salts practically inhibit the curvature. Alcohol also interferes.

Little attention appears to have been paid to the reaction of the solution in which the test is carried out. Our expts., however, have shown that the test is most sensitive in slightly alkaline soln. The extract from the organic manure is accordingly treated with water and neutralised with 0·1 *N* sodium hydroxide until the solution is just alkaline to litmus.

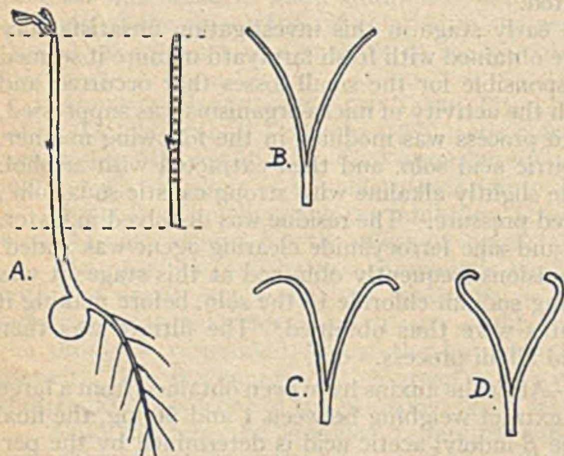

Fig. 1. Went Pea Test
A—Pea seedling B—Split stem ready for test
C—No auxin D—Auxin present

In our experience it is very difficult to assess the quantity of auxin in a solution by comparing the curvature given by a pea stem with standard curvatures given by stems in solns. of known auxin content. We have found that the most reliable method is to prepare a number of dilutions, similar to those used in the McConkey test for *B. coli* in waters, and then determine the dilution which just shows a slight positive inward curvature. The auxin content of this limiting dilution is determined by a control expt. with different dilutions containing known quantities of pure β-indolyl acetic acid.

In our laboratory the dilutions are all made up to a final volume of 10 ml and should be alkaline to the extent of 0·05 ml of 0·1 *N* sodium hydroxide per 10 ml; this volume is sufficient for the complete immersion of the stems. Three split stems are employed in each test dilution, and are observed after standing immersed for 12–15 hr. in the incubator at 23° C. If a total of 15 ml of neutralised extract are available for the pea test, we generally use exploratory dilutions containing 5·0, 1·0, 0·2, 0·04 ml of extract and then make further intermediate dilutions when the limiting range has been found. It is not our practice to standardise each batch of peas grown, but each new batch of seed is checked and also re-checked from time to time. We find that the amount of β-indolyl acetic acid in 10 ml necessary to give a positive curvature varies between 0·001 and 0·002 mg. The distilled water used in the test is twice distilled from glass, a crystal of thiosulphate ("hypo") being added in the first distillation if the tap water used contains much free chlorine.

Details of Procedure

Cold Lime Process—Place 100–300 g according to the nature of the fertiliser in a 1-litre Wagner shaking flask, add 5 g of calcium hydroxide and 500 ml of water. Shake at intervals for 2 hr. and leave overnight. Then shake well and filter through a Whatman No. 12 fluted paper. Place 200 ml of the clear filtrate in a 250-ml flask, acidify with dil. sulphuric acid (a piece of litmus paper may be used as indicator), add 20 ml of pptd. zinc ferrocyanide mixture, prepared by adding 0·5 ml of zinc acetate soln. and 0·5 ml of potassium ferrocyanide soln.[4] to 19 ml of water, and make up to 250 ml. Shake well, leave for a few min. and filter through a Whatman No. 12 fluted filter. Extract 100 ml of the filtrate with 120 ml of ether in a separator; draw off the bottom aqueous layer into a second separator, shake it with a second 120 ml of ether and reject the bottom layer. Place a second 100 ml of the clear filtrate in the first separator and shake well; run off the aqueous bottom layer into the second separator, shake well and reject the bottom aqueous layer. Wash the ether in the first separator with 20 ml of water, draw off the washing liquor into the second separator and shake again. Wash the ethereal layers with a second wash of 20 ml of water. Allow the ethereal layers to drain, evaporate the ether off in a 100-ml flask and add 0·2 *N* sodium hydroxide until the residue has completely dissolved and the resulting soln. is slightly alkaline to litmus. Make the soln. up to definite vol., say, 4 ml., *e.g.*, by adding water until the soln. in the flask weighs approx. 4 g. Use 1·0 ml for the modified perchloric acid test and, if

necessary, repeat the test on 0·5 ml. Dilute 2·5 ml of the soln. to 15 ml with water and use this diluted soln. for the pea test in the dilutions described above under *The Went Pea Test*, adding 0·05 ml of 0·1 N sodium hydroxide to all the dilutions.

If the solution of the ethereal extract in 0·2 N sodium hydroxide is highly coloured, it must be purified as described below under "Highly Coloured Extracts" before the perchloric acid test is applied. When calculating the auxin content of a manure, allowance must be made for its moisture content.

ALCOHOL PROCESS—Place 50–100 g of the organic manure in a 400-ml beaker and stir in 10 ml of saturated citric acid soln. Add 200–300 ml of 90% alcohol and leave in an incubator at 37° C. for several hours. Filter off on a Buchner funnel and suck dry with the pump. Return the residue to the beaker, re-digest with 200 ml of 90% alcohol and filter. Unite the alcoholic filtrates and add 30% sodium hydroxide soln. until the mixture is just alkaline to litmus. Evaporate the alcohol, preferably under reduced pressure. Transfer the aqueous residue in the flask to a 200-ml flask and rinse in with water. Acidify with dilute sulphuric acid, add 20 g of sodium chloride and 20 ml of clearing agent prepared as described under "Cold Lime Process" and make up to the mark. Shake well and filter through a Whatman No. 12 fluted filter. Extract 180 ml of the filtrate in two portions of 90 ml with ether as described for the cold lime process. Then examine the extract as described under the cold lime process.

HIGHLY COLOURED EXTRACTS—One of the advantages of the cold lime process over the alcohol process is that the final extract (containing the auxins) from the former usually contains considerably less pigments than the extracts from the latter. Highly coloured extracts cause trouble in the perchloric acid test; not only is the colour of the test soln. yellow, but occasionally the colour goes into the final chloroform layer. If the final ethereal extract contains appreciable quantities of these pigments it must be purified before the perchloric acid test can be applied.

One method of purification is as follows. Dissolve the ethereal extract in 0·2 N sodium hydroxide, make up to 10 ml, and acidify with dil. sulphuric acid. Stir until the ppt. has coagulated (a little sodium chloride assists coagulation), filter and wash the filter with 5 ml of water. Make the filtrate distinctly alkaline with 10% sodium hydroxide soln. and extract twice with 20 ml of ether to remove any indole. Wash the ethereal extracts separately with 3 ml of 0·2 N sodium hydroxide. Add the small amount of alkaline washings to the bulk of the soln., acidify with dil. sulphuric acid, extract twice with ether, wash the ethereal extracts with water and evaporate.

The following lead precipitation process is useful with highly pigmented extracts, which are not readily purified by the process described above. Dissolve the ethereal extract in 0·2 N sodium hydroxide and make up to a known volume. Withdraw a portion for the pea test and to the residue add 3 drops of 10% aqueous lead acetate soln., and 33% acetic acid drop by drop, until the soln. is acid to litmus. Filter through a small filter. In absence of indole this soln. may be tested directly by the perchloric acid test. This process suffers from the disadvantage that a small proportion of the auxin is co-precipitated or adsorbed in the lead ppt. Experiments on the chromatographic separation of impurities showed that the pigments could be easily removed by this means, but the technique for the recovery of auxins has not yet been worked out.

RESULTS—The figures given below show the types of results obtained and also the results of expts. in which known quantities of β-indolyl acetic acid had been added.

COLD LIME PROCESS

	Total auxins by the pea test expressed as β-indolyl acetic acid mg/100 g	β-Indolyl acetic acid mg/100 g	β-Indolyl acetic acid added mg/100 g	Added β-indolyl acetic acid recovered mg/100 g
Dried blood	0·25	0·17	0·25	0·20
*Hop manure	0·06	less than 0·03 (if any)	1·00	1·12
Peruvian guano	0·14	0·14	0·50	0·47

* A well decomposed sample.

The results given under the heading of β-indolyl acetic acid recovered in the last column were obtained by deducting the β-indolyl acetic acid naturally present in the organic manure

from the total found in the test mixture. Definite evidence of the presence of the propionic or butyric compound in the sample of dried blood was obtained.

The following results show the type of recoveries which may be expected from the Went pea test.

WENT PEA TEST

	Total auxin expressed as β-indolyl acetic acid found in the manure mg/100 g	β-Indolyl acetic acid added mg/100 g	Total auxin expressed as β-indolyl acetic acid found in mixture mg/100 g
Dried blood	0·25	1·00	1·00
Hop manure	0·06	0·50	0·62

CONCLUSIONS—It will be seen from the results given above that satisfactory recoveries of added β-indolyl acetic acid have been obtained by the cold lime process. Satisfactory recoveries of β-indolyl acetic acid have also been obtained from mixtures to which, in addition to β-indolyl acetic acid, β-indolyl propionic or β-indolyl butyric had been added.

The quantitative determination of the proportions of the propionic and butyric homologues from the number of yellow units given in the perchloric acid test is, however, not entirely satisfactory, as nearly all the results are high. As stated before, extreme caution must be exercised in the interpretation of the results for these two derivatives.

The results given by the Went pea test, while not of the same degree of accuracy as the results given by the perchloric acid test, may nevertheless be regarded as reasonable, having regard to the nature of the method and the very large multiplying factor involved.

The alcohol process has only recently been developed and it is therefore too early yet to draw any useful comparisons between the two extraction processes. It is quite certain, however, from the comparative experiments that have been made, that the extracts obtained by the cold lime process are much cleaner and freer from impurities than those obtained by the alcohol process.

It seems quite likely that with some manures the alcohol process must be used; in the same way it may be found that the alcohol process may prove successful for removing auxin which is in a combined form and not completely extractable by the lime process. In testing compound fertilisers containing ammonium sulphate and superphosphate the alcohol process is more suitable than the lime process.

The two processes described determine the free auxin present in the fertiliser, but of course do not take into account the auxin that may be produced by decomposition of the organic matter in the soil.

Soils—The cold lime water process has been found quite suitable for the determination of auxins in soils, but, owing to the very small proportions in which these substances are present, it is necessary to work on at least 500 g of the soil.

Peroxide-free Ether—The peroxide-free ether used throughout this investigation was prepared from methylated ether (0·72) by the method of Garbarini,[7] which consists in shaking the ether with an aqueous suspension of ferrous hydroxide and then distilling.

I wish to thank Mr. George Taylor, F.R.I.C., for his interest and helpful criticism throughout this investigation.

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APPENDIX—SIGNIFICANCE OF AUXINS IN RELATION TO PLANT GROWTH

Although the foregoing paper is concerned with the detection and determination of auxins in organic manures, a brief review of our present knowledge of the significance of auxins in relation to plant growth is cognate to the subject. The existence of substances affecting plant growth, or growth substances other than nitrogen, phosphoric acid and potash and the minor elements, was predicted as early as 1885 by Sachs. The work of Bottomley in England from 1914 onwards, which unfortunately was largely ignored, confirmed these predictions, but only in the last 25 years has the existence of these substances been firmly established and their composition elucidated. This big advance was mainly due to the work of Boysen-Jensen, Went, Kogl and Thimann. As a result of this work auxins may now be regarded as growth

substances which function in a manner similar to that of hormones in the human body and they are, in fact, sometimes referred to as Phytohormones. It is best to regard them as one of the many factors necessary for the ordinary growth process of plants and to postulate that without them there is no growth. The principal auxins are auxin *a* (auxentriolic acid), auxin *b* (auxenolonic acid), and hetero-auxin or β-indolyl acetic acid. These three auxins have all been isolated from urine, and urine has for some years been regarded as their richest natural source. It will be seen that they are all acid substances, and it is interesting to note that their esters are frequently inactive.

As with other natural products, a number of synthetic substances have now been made which possess some of the plant growth properties shown by the natural auxins. To give but one example of these synthetic substances, I may mention α-naphthyl acetic acid, which has the property, in common with the auxins, of accelerating the formation of roots on cuttings. Phenyl acetic acid is an example of a substance which falls into an intermediate group between the natural auxins and the synthetic substances. It occurs naturally in urine, and our work shows that it probably occurs in a number of organic manures.

A number of well-known effects in plant physiology are readily explained by auxin action. Auxins play a vital part in cell elongation and multiplication, but as yet the means by which they do this is by no means clear. The bending of a plant towards the light is due to unequal distribution of the auxin, which results in uneven growth. Auxins also furnish an explanation of why roots grow downwards in the soil. They also provide the best explanation of bud inhibition. Large quantities of auxins produce an unusual bending of the leaves. From the horticultural point of view at the present time auxins are best known by their property of accelerating the formation of roots on cuttings.

It has been shown by the American workers that not only are auxins produced naturally by the plants, but that plants also have the power of taking up auxins from the soil. A vast amount of work has been done on the rate of production of auxins, their distribution and their effect on different parts of the plant, but the problem of auxin absorption from the soil has been very largely ignored. We know that minute amounts of auxin accelerate root growth and that large amounts cause a considerable retardation. We know that auxin may have a profound influence on the plant, and that toxic effects are produced by an excess, but at the moment we have practically no knowledge of the part played by auxins in the soil or in the fertilisers which are applied to the soil. The use of seed-dressing compounds containing auxins is claimed to produce an increase in the yield of the crops to which they are applied.

It is quite clear from the work that has already been done that no dramatic effect, such as an enormous increase in plant growth, is likely to result from the application of auxins to the soil. But it is quite possible that the failure of some crops and poor yields of others on certain soils may find an explanation in the auxin balance which exists in the soil.

Hetero-auxin is produced by, and affects the growth of, a number of micro organisms which occur in the soil. Has the auxin thus produced any effect on soil fertility? Before such questions can be answered it is necessary to know the auxin content of the soil and also of the fertilisers which are applied to the soil; hence this investigation into methods of determining auxins in these materials. It seems probable that in the past this work may have been largely ignored owing to the very small proportions in which these active substances occur in the fertilisers.

In view of the somewhat unstable nature of auxins *a* and *b*, it seems unlikely that these substances will occur to any great extent in organic fertilisers. Auxin *a*, for instance, is converted into an inactive isomeric compound in a matter of weeks. Hetero-auxin, on the other hand, is far more stable, particularly in the alkaline condition, and, apart from the naturally occurring hetero-auxin, β-indolyl acetic acid and its homologues are produced by the decomposition of organic matter, particularly organic matter containing tryptophan and other amino acids. It was for this reason that our attention was mainly directed towards the estimation of hetero-auxin.

Unfortunately, at the present time no chemical tests exist for the estimation or detection of auxins *a* and *b*, and the best we can do is to obtain a rough approximation by determining the total auxins present by a biological method and to deduct from it the hetero-auxin found by the perchloric acid method.

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The Photometric Determination of Cobalt, Titanium and Iron in Unsintered Metal Carbides

BY H. COX, A.M.C.T., A.R.I.C.

THE determination of cobalt, titanium and iron in hard metal carbide powders, as carried out by the usual analytical processes, is an involved and lengthy procedure, and a simpler and speedier method of analysis is desirable.

Photometric determination appeared to offer the most promising means of effecting substantial saving in time, and a composite method has been successfully developed by which these three constituents may be determined on one initial sample with an accuracy comparable to that realised by the methods employed hitherto. In addition, the procedure for the preliminary treatment of the sample has been simplified and effects further saving of time. Advantage has been taken of the pioneer work of Vaughan[1] in connection with the general application and operation of the Spekker Photo-electric Absorptiometer and of the work of Haywood and Wood[2] on the determination of cobalt in steel.

REAGENTS—(1) *Sodium carbonate*, anhydrous, AnalaR. (2) *Potassium bisulphate*, AnalaR. (3) *Nitroso-R-Salt*, B.D.H.—2·0 g dissolved in water and made up to 1 litre. (4) *Sodium acetate trihydrate*, AnalaR—500 g dissolved in water and made up to 1 litre. (5) *Hydrogen peroxide*—30 volumes. (6) *Potassium thiocyanate*, AnalaR—200 g dissolved in water and made up to 1 litre. (7) *Nitric acid*—1·4 sp.gr. (8) *Sulphuric acid*—10% by vol.

PROCEDURE—Thoroughly oxidise in the muffle furnace 0·5 g of carbide weighed into a platinum crucible. Fuse the oxides with 5 g of sodium carbonate, extract with boiling water, filter and wash with hot water. Discard the filtrate, which contains the tungsten. Removal of tungsten is necessary because, if kept in solution with phosphoric acid this acid affects the titanium colour produced with hydrogen peroxide and also because pptn. of tungsten is not avoided when buffering with sodium acetate for the development of the cobalt colour with nitroso-R-salt. Ignite the filter-paper and contents in the original crucible and fuse with 5 g of potassium bisulphate. Transfer to a 400-ml beaker and dissolve the melt in 100 ml of water and 50 ml of conc. sulphuric acid. Cool, transfer to a 500-ml graduated flask and make up to vol. with water. This is referred to subsequently as solution A.

Cobalt—Pipette 25 ml of soln. A into a 200-ml graduated flask and make up to 200 ml with water. From this, pipette 20 ml into each of two 250-ml beakers. To one beaker add 10 ml of nitroso-R-salt solution and 10 ml of sodium acetate soln. The addition of the nitroso-R-salt must be made accurately from a burette. Heat to boiling, add 5 ml of nitric acid and boil for 1 to 2 min. Cool and dilute with water to 100 ml in a 100-ml graduated flask. Treat the contents of the second beaker, the blank, in the same manner but omit the nitroso-R-salt. Measure the extinction on the absorptiometer, using 1-cm cells and Ilford Spectrum Blue filters No. 602. Obtain the cobalt % by reference to the cobalt graph.

Titanium—Pipette 50 ml of soln. A into a 100-ml graduated flask. Add 5 to 10 ml of hydrogen peroxide and make up to volume with sulphuric acid (10%). For the blank, pipette a second 50 ml of soln. A, omit the hydrogen peroxide and make up to 100 ml in a 100-ml graduated flask with 10% sulphuric acid. Measure the extinction on the absorptiometer, using 1-cm cells and Ilford Spectrum Violet filters No. 601. Obtain the titanium % by reference to the titanium graph.

Iron—Pipette 100 ml from soln. A into a 250-ml beaker. Add accurately from a burette 5 ml of potassium thiocyanate soln. For the blank, pipette a second 100 ml of soln. A, omit the thiocyanate and add 5 ml of water to preserve equality of volume. Measure the extinction on the absorptiometer, using 1-cm cells and Ilford Spectrum Blue-Green filters No. 603. Obtain the iron % by reference to the iron graph.

It is recommended that the absorptiometer be operated with the drum aperture fully open (*i.e.*, drum reading = 0) for the coloured soln., so that the drum reading for the blank gives the extinction value directly.

CALIBRATION GRAPHS—In order to establish the graphs, two sources of the respective metal were used in each instance. For any given % of metal over the ranges covered, the max. observed difference in extinction value was 0·01 drum division.

Cobalt—Solutions were prepared from (1) AnalaR cobalt sulphate and (2) a cobalt metal powder of pre-determined cobalt content, such that 1 ml contained 0·000025 g of Co (*i.e.*, 1 ml ≡ 1·0% of Co on 0·5 g of the original sample). Amounts (from a burette) equiv. to from 4 to 14% in steps of 1·0% were made up to 100 ml after being buffered with sodium acetate and the colour was developed with nitroso-R-salt. A second series of solns. in which the nitroso-R-salt was omitted, was prepared for the respective blanks. The extinction values were obtained and a graph was prepared.

Titanium—Solutions in 10% sulphuric acid were prepared from (1) a British Chemical Standards Ferro-titanium (22·8% Ti) and (2) a sample of titanium oxide (98·75% TiO_2), such that 1 ml contained 0·00025 g of Ti (*i.e.*, 1 ml equiv. to 0·5% of Ti on 0·5 g of the original sample). From 8 to 30 ml in steps of 2 ml were run from a burette, coloured with hydrogen peroxide and made up to a final vol. of 100 ml with 10% sulphuric acid. A second series, in which the peroxide was omitted provided the respective blanks. The extinction values were obtained and a graph was prepared giving titanium % from 4 to 15.

Iron—Solutions in 10% sulphuric acid were prepared from (1) AnalaR ferrous ammonium sulphate and (2) AnalaR ferric alum, such that 1 ml contained 0·0001 g of Fe (ferric), (*i.e.*, 1 ml ≡ 0·1% on 0·5 g of the original sample). From 1 to 10 ml in steps of 1 ml were respectively made up to 100 ml with 10% sulphuric acid and then coloured with 5 ml of 20% potassium thiocyanate soln. measured accurately from a burette. A second series, in which

5 ml of distilled water replaced the thiocyanate soln., provided the respective blanks. The extinction values were obtained, and a graph prepared giving iron % up to 1·0.

COMPARISON OF RESULTS—Table I gives a comparison of the results of determinations made photometrically with those obtained by the methods employed hitherto (gravimetric iron and titanium and electrolytic cobalt).

TABLE I

	Cobalt %		Titanium %		Iron %	
Sample	Photometric	Electrolytic	Photometric	Gravimetric	Photometric	Gravimetric
A	5·70	5·56	—	—	0·30	0·32
B	10·95	10·96	—	—	0·17	0·16
C	9·02	8·98	5·25	5·27	0·22	0·24
D	11·65	11·75	13·00	13·24	0·29	0·27
E	6·58	6·52	14·35	14·31	0·20	0·20
F	10·23	10·18	9·40	9·43	0·32	0·35
G	7·45	7·43	9·80	9·86	—	—
H	9·37	9·40	5·80	5·86	—	—
I	8·94	9·00	5·35	5·61	—	—
J	6·76	6·75	14·35	14·12	—	—

NOTES—The use of nitric acid as an oxidant for ferrous iron is to be avoided. This acid produces a colour with potassium thiocyanate (in absence of iron) due to the formation of nitrous fumes. At a concentration of 5 ml of nitric acid (sp.gr. 1·4) in 100 ml this colour is rapidly produced. For this reason potassium permanganate was used to oxidise the ferrous ammonium sulphate in the preparation of the graph for iron. In the composite method described the initial oxidation of the sample avoids the need for subsequent oxidation of the iron to the ferric condition.

The addition of thiocyanate must be made accurately. The intensity of colour increases with increasing concn. of thiocyanate. For example, 0·0002 g of iron made up to a final volume of 130 ml gave drum readings ranging from 0·13 to 0·335 with thiocyanate additions ranging from 5 to 30 ml.

The colour of the iron thiocyanate is not permanent and fading is detectable photometrically after about 3 hr. Extinction measurements should be made therefore within 2 hr. of developing the colour.

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A Simplified Determination of the Alkalis in Silicates

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INTRODUCTION—In the determination of alkalis in silicates, if the silicate is decomposed by the method of Berzelius (1824), using hydrofluoric and sulphuric acids, sulphate and magnesium must be removed by special treatment with barium chloride and barium hydroxide. For that reason the method was largely replaced (in 1871) by that of J. Lawrence Smith, in which from the outset magnesium is made insoluble by excess of calcium hydroxide. That method still required, however, removal of calcium, with the consequent disadvantage of having to remove ammonium salts later.

Modern methods are now available for the precipitation of both sodium and potassium as complex triple salts (sodium magnesium uranyl acetate and potassium sodium cobaltinitrite), in presence of calcium. Hence it is possible to dispense with removal of calcium in the Lawrence Smith procedure. Modifications in this direction have been devised by Miller and Traves[1] and by Haslam and Beeley.[2] In both modifications the aqueous extracts from separate ignitions are acidified and evaporated, and sodium and potassium are pptd. as the

complex salts, to be weighed as uranyl acetate and perchlorate respectively. This avoids not only the removal of calcium, but also the conversion of alkali sulphate and carbonate into chloride and the difficulty of dissolving sodium platinichloride without loss of potassium.

Because, however, of the simplicity of the Berzelius decomposition, attempts have been made to shorten its subsequent procedure so that it may compete with the Lawrence Smith method. Two such modifications have been noted. Koenig,[3] in a method for felspars, avoids the formation of sulphates by decomposing with hydrofluoric acid alone. Fluoride and silicofluoride ions are removed by pptn. with calcium hydroxide. The filtrate of alkali hydroxides is then treated, as in the standard Lawrence Smith procedure, for removal of calcium, etc. In the second modification, given by Cantoni,[4] the standard Berzelius decomposition is employed, but the sulphates, after evaporation of free sulphuric acid, are heated to dull red heat to decompose ferric sulphate. In the hot aqueous extract the sulphate content is greatly reduced, so that the subsequent barium sulphate ppt. is small, while the fact that the extract may be slightly cloudy (presumably with colloidal basic aluminium sulphate) is considered immaterial, as in any event the remaining sulphate, together with barium, calcium, etc., is still to be removed, by the usual Berzelius procedure.

Author's experience of above modifications—In the analysis of clays I have further shortened the above-mentioned modifications[1,2] of the Lawrence Smith method by titrating the separated potassium sodium cobaltinitrite direct, instead of converting it into perchlorate. But even this shortened Lawrence Smith procedure still requires expensive platinum finger-crucibles, suitable calcium carbonate (not always easy to obtain), and tedious evaporations of large filtrates. Reverting therefore to the Berzelius method, I improved the calcium hydroxide modification of Koenig[3] by determining the alkalis as the triple salts. But with high-calcium materials, where the modification, by avoiding the formation of calcium sulphate, might have been of most use, the results were somewhat low and erratic. A modification which returns to the standard Berzelius decomposition, but introduces neither foreign matter nor bulky filtrations, was therefore explored.

New Sulphate-decomposition Modification—This analytical method was suggested by experiments with the industrial process of Turrentine, Whittaker and Fox[5] for the extraction of potash from greensands. After the method had been tested an abstract of the paper by Cantoni[4] was found. The distinction between my method and that of Cantoni is that the temperature is raised from dull redness (approx. 500° C.) to 900° C., to decompose aluminium sulphate and titanium sulphate, so that all the tervalent oxides and titanium dioxide may be rendered completely (instead of only partly) insoluble in water. The alkali sulphates still remain soluble and can be leached out quantitatively and filtered as a clear solution, neutral or barely acid to methyl red. This, unlike extracts obtained after ignition at lower temperatures, remains clear during evaporation, and yields a residue, free from insol. and gelatinous aluminium hydroxide, from which a clean separation of soda or of potash as triple salt can be effected direct.

Procedure—Decompose a 1-g sample in a 50-ml lipped platinum basin (diam. 5·6 cm) with 4 ml of 1 : 1 sulphuric acid and 8–10 ml of hydrofluoric acid on a uralite plate or, better, by the indirect heat of an asbestos hot-air bath. Avoid (particularly in this first "fuming") fine spitting of the sulphuric acid at the stage when the solid sulphates are separating out just before white fumes appear, or explosive spattering of the crusty sulphates later, from local heating. Evaporate to dryness, cool, add 2 ml of 1 : 1 sulphuric acid and "fume" again to complete dryness.

Next cover the basin with a porcelain crucible lid (internal diam. 6 cm) and place on a 9-cm triangle 1½–2 in. above the top of a No. 5 Fletcher safety-Bunsen or similar burner. Beginning with the naked flame very low, raise this carefully and gradually through at least 5 stages, pausing a few minutes at each stage until white fumes cease, and taking *ca.* 20 min. to reach full flame. Heat strongly for a further 10 min.

Cool, and if there is likely to be very little electrolyte present, as with highly aluminous clays, moisten the residue with 1 ml of 1% magnesium sulphate soln. in order to ensure clear filtration later. Rinse the under surface of the lid with hot water into the basin, and leach out the soluble sulphates from the basin with small amounts of hot water, through a 7-cm medium open paper (*e.g.*, Green's 802 or Whatman's 41) into a 100-ml measuring flask. Between decantations break up the somewhat crusty residue in the dish with a short rubber "policeman." Finally rinse and transfer the oxides on to the paper, and wash until the flask is about two-thirds full.

For a second extraction, return paper and contents to the basin, burn off the paper, heat to fuming with 4 ml of 1 : 1 sulphuric acid (note that the partly ignited oxides readily redissolve), carefully heat the covered basin in the naked flame, moisten the residue with magnesium sulphate soln. if that was previously needed, and leach the residue, now more powdery than before, with hot water through another 7-cm medium open paper until the oxides are transferred as completely as possible to the paper and the washings fill the 100-ml flask.

Determination of sodium as uranyl-acetate and of potassium as cobalti-nitrite—From the 100 ml of extract take two aliquot portions, usually 50 ml each, to give suitable amounts of sodium and potassium, *e.g.*, 1–10 mg of Na_2O and 2–20 mg of K_2O. If the sample is known to contain more than 1 or 2 % of lime, transfer the sodium aliquot portion first to a 100-ml beaker, heat, and ppt. sulphate with a slight excess of a barium chloride soln. containing 5 g of $BaCl_2.2H_2O$ and 5 ml of glacial acetic acid per 100 ml, using 3 ml—or at most 5 ml where both lime and alkali are high. Boil gently for a short time, filter through a 7-cm close paper (*e.g.*, Green's 803 or Whatman's 42), and wash with hot water. Evaporate both aliquot portions, preferably in 100-ml flat-bottomed Pyrex evaporating basins, to dryness on the water-bath. The amount and appearance of the residues—as distinct from the bulky residues obtained by other methods—may give valuable information. For example, in the rather rare event of a high calcium content not previously suspected, this is revealed here—or even during evaporation—by the many characteristic needle-crystals of calcium sulphate. It will then be necessary to redissolve the residue from the sodium aliquot portion and to remove sulphate as described above.

Determine sodium in one aliquot residue with magnesium uranyl acetate by Kahane's method, as given by Piper[6] (p. 176) or by Wright[7] (p. 106). Use tared 8-ml Jena G3 or equivalent sintered-glass crucibles for filtering off the triple salt. Wash the ppt. with alcohol and with ether, dry at 105° C. and weigh (Kahane's factor for Na_2O is 0·02022).

Precipitate potassium from the other residue with sodium cobaltinitrite strictly as described by Piper[8,9,6] (p. 162, 179) or by Wright[7] (p. 100). (It is, however, preferable to use

TABLE I

		Na_2O %		K_2O %	
Sample	CaO %	Sulphate-decomp.	L. Smith	Sulphate-decomp.	L. Smith
1	0·21	0·28, 0·27, 0·29	0·28 *a*	3·16, 3·25, 3·22	3·17 *a*
2	nil	0·17, 0·18	0·13 *b*	0·03, 0·04	0·04 *b*
3	0·73	1·51, 1·49, 1·49	1·57 *b*	3·81, 3·87, 3·90	3·72 *b*
		1·52, 1·53, 1·49		3·82, 3·85, 3·87	
4	1·13	2·72, 2·69, 2·65	2·67 *b*	2·82, 2·79, 2·82	2·73 *b*
5	37·6	0·32, 0·33	0·33 *a*	1·15, 1·17	1·15 *a*
6	60·5	0·62, 0·65	0·66 *b*	0·50, 0·50	0·46 *b*
7	24·8	1·20, 1·20, 1·20	1·32 *b*	0·56, 0·58, 0·56	0·54 *b*
8	nil	0·37, 0·36	0·44 *b*	2·12, 2·13	2·14 *b*
		0·37, 0·38 *c*		2·15, 2·14 *c*	
		0·41, 0·42		2·10, 2·12	
9	11·66	2·59, 2·61	2·66 *b*	0·72, 0·76	0·65 *b*

a—As determined by U.S. Bureau of Standards, *average* value for 8 analysts.
b—As determined by F. T. Seelye, Chief Chemist, Dominion Laboratory, Wellington.
c—As determined by 3 other analysts without previous experience of the method.

Sample 1, Plastic clay, U.S. Bur. of Stds., Standard Sample No. 98; *Sample* 2, Halloysitic clay, Kauri, N.Z.; *Sample* 3, Decomposed dacite, McLeod's Bay, N.Z.; *Sample* 4, Felspathic clay, Tadmor, N.Z.; *Sample* 5, Argillaceous limestone, U.S. Bur. of Stds., Standard Sample No. 1; *Sample* 6, Cement; *Sample* 7, Flue-ash from coal-burning furnace; *Sample* 8, Washed clay from decomposed rhyolite, Mt. Somers, N.Z.; *Sample* 9, Basalt, North Auckland, N.Z.

Note—Sample 1 contains 1·43% of TiO_2; Sample 7 contains 5·7% of SO_3 and 3·3% of B_2O_3.

ceric sulphate instead of potassium permanganate for the titration.) Filter the potassium salt through a 10-ml Gooch crucible containing asbestos previously treated with boiling dil. sulphuric acid and ceric sulphate and then washed well. Wash the salt with 35% alcohol and then with water. Put approx. 100 ml of water into a 500-ml wide-mouthed Erlenmeyer flask and add 10 ml of 1 : 1 sulphuric acid and a measured vol., judged a slight excess, of 0·05 *N* ceric sulphate. Add the crucible, and heat to slightly above 70° C., shaking the flask meanwhile to wash the asbestos pad and its ppt. out of the crucible. If the yellow colour of ceric sulphate appears likely to be discharged, remove the flask temporarily from the flame, and add a further measured vol. of ceric sulphate soln. to ensure slight excess. Heat to 85° C., keep at that temp. for *ca.* 1 min. and cool completely under the tap. Then back-titrate the small excess of ceric sulphate, either by adding excess of potassium iodide and

titrating liberated iodine with 0·05 N thiosulphate, or by titration with 0·05 N ferrous ammonium sulphate, using "ferroin" or N-phenyl-anthranilic acid as indicator, or by potentiometric titration. Determine the factor—mg of $K_2O \equiv$ ml of 0·05 N ceric sulphate—from the formula or the table given by Piper[6] (p. 180) for 0·05 N potassium permanganate, assuming that the same values hold good also for 0·05 N ceric sulphate so long as the cobaltinitrite is pptd. strictly according to Piper's standardised conditions (this empirical factor increases approx. 1 % for each 10 ml increase in the titration). Determine the normality of the ceric sulphate as suggested by Vogel[10] (p. 381, 382). The method of Wilcox[11] for giving a potassium sodium cobaltinitrite ppt. of constant composition has not been investigated here.

Discussion of the Sulphate-decomposition Modification—A comparison of determinations of sodium and potassium by the above method with those obtained by the Lawrence Smith method is given in Table I. The accuracy seems satisfactory, although not yet checked against synthetic standard samples.

The completeness of extraction of the alkalis from the ignited sulphates was tested spectrographically. The amounts of potassium and of sodium retained after the second extraction in the residue from 1-g samples of plastic clay, decomposed dacite, and argillaceous limestone (samples 1, 3, 5 of Table I) ranged from 0·004 to 0·008% of the residues—*i.e.*, were under 0·01% of the original samples. It appears that extraction is sufficiently complete.

Iron and titanium oxides, on the other hand, when fully ignited as described, are insoluble, much less than 1 mg being recoverable from the aqueous extract. Thus the residues from leaching can be used, after stronger ignition, for accurate supplementary determinations of iron and titanium oxides, as well as in many instances for an approx. estimation of alumina. With clays especially this information, taken in conjunction with determinations of alkali and combined water, may give all the facts required for "works" practice.

The residues retain, in addition to R_2O_3, most of the magnesium of the sample, through thermal decomposition of magnesium sulphate; for example, the residue from Sample 1 of Table I (Bur. of Stds. Plastic clay) retains 0·60% of a total of 0·72% of MgO.

Calcium, however, if present in only moderate amounts, passes completely into the aqueous extract as calcium sulphate. This, when the sample contains above 1 or 2% of lime, so nearly saturates the filtrate that it would not be completely soluble in the small vol. of water and uranyl acetate reagent (itself 50% of alcohol) in which the evaporated sodium aliquot portion is to be taken up. Hence the need to remove sulphate from the sodium portion with such samples as Nos. 5, 6, 7 and 9 of Table I, high in calcium. The method appears, on the other hand, to be particularly suited to materials, such as Sample 7, high in sulphate or boron.

The times required for the analysis of a typical 1-g sample decomposed on a 4 in. diam. asbestos hot-air bath are—Decomposition to first extraction, inclusive, 2½ hr.; to second extraction, 1¼ hr.; to evaporation, 1¼ hr.; total, 5 hr., although the need to convert calcium sulphate into calcium chloride may delay evaporation of the sodium aliquot portion for nearly 1 hr. longer. The sodium ppt. may be weighed 1½ hr., and the potassium ppt. titrated 3 hr. later. Expts. to develop a rapid yet sufficiently accurate "works" method for clays by omitting the second extraction gave results about 5% low. This could perhaps be allowed for in a quick routine method, although it must not be forgotten that the second extraction is also an insurance against possible faulty leaching in the first.

Conclusion—The main advantages of the sulphate-decomposition method are its simplicity, its rapidity, and the fact that it introduces no reagents between decomposition of the sample and separation of the alkalis, and such reagents as are required may be readily obtained free from alkalis. It obviates the weighing of "mixed chlorides" liable to contain impurities for which it is difficult to make allowance, and so avoids the throwing of errors on either the sodium or the potassium determination.

Summary—By taking advantage of rapid modern methods of determining alkalis as complex triple salts in presence of calcium, magnesium and sulphate, it has been possible to modify the Berzelius method so as to make it preferable to the Lawrence Smith method. In the "Sulphate-decomposition" method described the sulphates of iron, aluminium and titanium are completely decomposed by ignition, and then the alkalis are leached out quantitatively with water as unchanged sulphates. In the extract, sodium is determined as the uranyl acetate gravimetrically, and potassium from the cobaltinitrite by titration with ceric sulphate soln. A slight modification makes provision, where necessary, against the interference of calcium sulphate in the sodium determination.

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Notes

USE OF A MIXED INDICATOR IN THE TITRATION OF FATTY ACIDS

The indicator most widely used for the titration of fatty acids is phenolphthalein. It has the disadvantage of a wide range of colour change (*p*H 8·0–9·0). Consequently other indicators have been suggested; *e.g.*, Lovern[1] recommends dibromothymoltetrachlorophthalein—an indicator with a small range of colour change.

We have had success with the mixed indicator first used by Simpson[2] and recommended by Kolthoff.[3] It consists of a mixture of 1 part of a 0·1% aq. soln. of cresol red neutralised with sodium hydroxide and 3 parts of 0·1% thymol blue soln. neutralised with sodium hydroxide. The colour of the indicator changes from pink at *p*H 8·2 through grey to purple at *p*H 8·4. With the use of this indicator accurate micro-titrations of fatty acids are possible.

The following procedure was adopted. Dissolve the fatty acids in boiling ethanol and titrate with alcoholic sodium hydroxide soln., air free from CO_2 being passed through the solution during the titration. Make the usual blank titration of the reagents. Results obtained in the titration of stearic acid (after deduction of the blank, 0·04 ml) were as follows.

Stearic acid	*N*/100 sodium hydroxide	
	Used	Calculated
mg	ml	ml
2·16	0·76	0·765
3·24	1·13	1·15
4·32	1·53; 1·53; 1·52	1·53
6·48	2·32	2·30

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A MICRO METHOD FOR THE ESTIMATION OF ELLAGITANNINS

From previous work of Nierenstein *et al.*[1] it is evident that at least two, if not three, types of ellagitannins have to be considered, namely, (i) tannins of the myrobalani-type, (ii) tannins of the Knopper type and (iii) galloyl derivatives of ellagic acid. So far only the chemistry of myrobalani-tannins has been satisfactorily established.[2] All these types of ellagitannins have, however, one thing in common: *they yield ellagic acid on hydrolysis*. The estimation of ellagitannins thus depends on the amount of ellagic acid they produce.

The tannin in myrobalans, the fruit of *Terminalia chebula* Retz, was first isolated by Nierenstein[3] as a well-crytallising substance and its constitution was established; since then myrobalani-tannin has been found by Edwards and Nierenstein[4] in the bark of *Hamamelis virginica* L. and Nierenstein and Potter[5] have isolated it from a large number of plants and confirmed the original formula assigned to it by Nierenstein; *i.e.*, they have shown that myrobalani-tannin is a monoglycoside and not a diglycoside.

As already mentioned, ellagitannins give on hydrolysis ellagic acid, a substance already known to Braconnot.[6] Later Palin[7] reported that on adding to dry ellagic acid a few drops of conc. nitric acid and diluting the solution a beautiful red colour is produced. This was confirmed by Griessmayer[8] and, more

recently, by Schmidt and Beckurts[9] and by Nierenstein.[10] This colour reaction was finally perfected as a test by Gnamm,[11] and we have used his method.

METHOD—The following stock solutions are required: (i) a soln. of 2 g of tetraacetylellagic acid in 1 litre of abs. alcohol; (ii) a 1% aq. soln. of potassium nitrite; (iii) $N/10$ hydrochloric acid; (iv) a soln. of 1 g of alcohol-sol. eosin in 1 litre of abs. alcohol. Preliminary expts. proved that a mixture of 5 ml of (i), 10 ml of (ii) and 5 ml of (iii) produces within 5 min. a deep red colour, which remains unchanged for 10 min., after which the colour changes to deep blue in 17 to 20 min. In the analysis the colour during the *red* period is matched against soln. (iv) in the apparatus devised in this laboratory by Nicholson and Rhind,[12] but with the difference that the blue crinkled paper is replaced by red crinkled paper. For the estimation we place 5 ml of soln. (iv) in the Nessler tube and add abs. alcohol from a burette.

RESULTS—The following table gives (A) values thus obtained, (B) the results by Nierenstein's micro method,[13] and (C) the amounts found by Nierenstein and Potter.[5] As was to be expected, the figures for (A) are higher than the other corresponding analytical data. For the analyses we used 1 g of the material dried at 100° C. and finely ground. This was extracted with a boiling mixture of chloroform and carbon tetrachloride (1:1), then freed from the adhering solvents and again extracted with abs. alcohol in a Soxhlet extractor. The figures (%), taken at random, are typical of those given by the 45 materials we investigated.

	A	B	C
Geranium maculatum L. (root)	0·97	0·93	0·81
Nupher adorata Aitk. (underground stem)	1·47	1·51	1·06
Ailanthus glandulosa Desf. (bark)	1·07	0·98	0·84
Aralia spinosa L. (bark)	1·81	1·79	1·62
Rhizophera Ikotae Nies. (bark)	2·88	2·91	2·83
Aretostaphylas glauca Sp. (leaves)	1·31	1·29	1·02
Ouroparin zhuchophylea Mats. (leaves)	0·57	0·61	0·46
Eugenia caryophyllata Thunb. (buds)	2·41	2·37	2·11
Apidosperma sessiliflora Allem. (buds)	3·21	3·29	3·16

It is apposite to mention that Hagen[14] describes a solid, giving a deep red colour with conc. nitric acid, which he found in strawberry jam and that Kunz-Krause and Schweissinger[15] have identified ellagic acid in raspberry jam. The latter author expressed the opinion that the formation of ellagic acid in jams causes cloudiness. This may prove of interest to chemists in the jam industry.

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SUPPOSED PUTREFACTION OF GOLDEN SYRUP PACKED IN METAL CANS

IT has been noticed that Public Health Authorities have in some instances condemned cans of golden syrup, the reason being given as putrefaction. Golden syrup differs from canned meat, fish, vegetables and even fruit in thin syrup, and assumptions of decomposition made before and after opening cans of these articles cannot be applied to golden syrup.

Denting of cans is of common occurrence, and more rarely hermetically sealed cans (for export) have bulged, but not as the result of spoilage by the action of micro-organisms. Mishandling of the cases of cans during transport explains the denting and very long storage, if accompanied by extensive crystallisation and expansion, has sometimes bulged or even fractured the tagger plate. Golden syrup is essentially a carbohydrate food and contains no proteins. It consists of a supersaturated solution of the three sugars, sucrose, dextrose and laevulose, together with salts and organic non-sugars which give it the characteristic flavour and aroma. Owing to its low water content and its high dissolved sugar content it exerts a very high osmotic pressure, *viz.*, 700 atmos. at 15° C. This osmotic pressure probably accounts for the fact that putrefactive and pathogenic organisms, if introduced into the syrup, rarely survive and certainly cannot multiply and cause it to become putrid or otherwise harmful.

Blackness in golden syrup, either as streaks near the cap or as patches on the interior body accompanied by an adjoining black cloud, is only to be found after long storage. No justification exists for regarding such blackened syrup as unsound. The explanation of the blackness is simply that owing to imperfectly protected plate some of the steel surface has been exposed to the action of the syrup and a little iron has dissolved locally, with the production of a dark compound. The composition and formation of this compound has been studied in this research laboratory by G. Austin, who has shown it to be a ferrosic compound of hydroxy acids produced by the action of iron and oxygen on the reducing sugars in presence of slight

acidity. Although the iron content of the syrup has been increased locally, yet after the whole of the syrup has been warmed and mixed its average iron content is found to be very little higher than usual. The blackness is closely allied to that of black treacles, which originally have a much higher iron content than golden syrup. Until the exigencies of war led to a change, golden syrup had been mainly packed in lever-lid tins composed of tin-plate. Sporadic troubles of black streak and patches were traced to defective tin coating accentuated by lack of tightness of the lever-lid. The streak trouble rarely appeared until after 6 months' storage and the black patches until after 12 months or more. Tropical conditions or even slightly elevated temperatures reduced the time needed for the troubles to appear. The contents of one tin after a year or more in West Africa resembled black treacle in appearance but not in taste.

Since lacquered black plate has been substituted for tin-plate for can-making the blackness trouble appears to have been greatly reduced, but on occasion a new trouble has been introduced. Under-stoving of the lacquer of the black plate, which was a failing prevalent during the early transitional period, caused a faint odour of lacquer which was noticeable on first opening the can of syrup. Generally this disappeared immediately and no effect on taste could be discerned, but a few cans had a more persistent odour and a flavour could be detected in the syrup.

Another change in can-manufacture resulting from the war has been the substitution of air-drying seam dope for lead-tin solder for sealing the seams of the lacquered black cans. The seam dope was made from spirit-soluble Manila copal dissolved in a mixture of industrial alcohol and acetone in such proportions as to promote very quick drying, so that the cans could be filled within an hour of manufacture. One result of this modified manufacture was a considerable reduction in the small percentage of leaky cans.

Unfortunately, the supply position required the finding of a substitute for Manila copal. A synthetic phenol resin was developed in this laboratory by J. Barrett and was found to be even more satisfactory than copal. As there was no suitable phenol resin on the market, the new resin was specially manufactured for our use. Some time later the supply position necessitated the use of cresol resins and, although it was feared that there might be an odour of cresol, they proved satisfactory unless minute splashes of dope entered the can, which caused a cresol odour and gave rise to complaints.

One other trouble with packed golden syrup is crystallisation; it is due to long storage and has always occurred to a greater or less degree. Crystals of sucrose have a sparkling appearance, but more rarely groups or clusters of needle-like dextrose crystals appear which make the product appear unattractive. Even when the whole can contains a mixture of crystals of sugar tinged perhaps with some blackness, the contents are still a wholesome article of food, although no longer to the taste or fancy of the epicure. If the grocer had disposed of his stocks in proper rotation such long storage would not have occurred and, although few grocers are to-day in the happy position of having much stock, yet stocks exist owing to the national policy, and long storage of these may give rise to such occurrences as have been outlined.

It is submitted that when they do occur, Public Health Authorities should give the manufacturer of the product an opportunity of examination and salvage before ordering the destruction of what is, or what can be made again, an attractive and wholesome food.

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Determination of Fluorine in Foods

Report of a Sub-Committee of the Analytical Methods Committee: Published by the Authority of the Council as a Standard Method of the Society

IN view of the fact that limits for fluorine in certain foods have been recommended by the Public Analysts and Official Agricultural Analysts Committee,* the Analytical Methods Committee appointed a Sub-Committee to consider methods for the determination of fluorine in foods in amounts of the order of 5 parts or less per million, and to devise for the purpose a procedure which can be recommended for general adoption. The Sub-Committee appointed consisted of the following members: D. C. Garratt, B.Sc., Ph.D., F.R.I.C. (Chairman); N. L. Allport, F.R.I.C.; J. G. A. Griffiths, B.A., Ph.D., F.R.I.C.; D. W. Kent-Jones, B.Sc., Ph.D., F.R.I.C.; G. W. Monier-Williams, O.B.E., M.C., M.A., Ph.D., F.R.I.C.; D. D. Moir, M.Sc., F.R.I.C. (Hon. Secretary).

The Sub-Committee has recommended a method which will, provided the details of technique are followed exactly, determine small proportions of fluorine of the order of 1 part per million to within 0·15 part per million of the material (*i.e.*, 1μg per gram to within 0·15μg per gram).

The method chosen for detailed examination by the Sub-Committee was published by H. H. Willard and O. B. Winter (*Ind. Eng. Chem.*, *Anal. Ed.*, 1933, 5, 7; Abst., ANALYST, 1933, 58, 242) and has since been modified and improved by various workers. The complete method involves preliminary preparation of the sample by ashing with lime, distillation of the fluorine as hydrofluosilicic acid in presence of excess of perchloric acid and titration of

* ANALYST, 1943, 68, 233.

the fluorine ion in the distillate, the titration depending on the bleaching action of fluoride on a thorium alizarin S lake.

The Sub-Committee is of opinion that, with the procedure it has recommended, the method may be considered specific.

The work carried out by the Sub-Committee can be divided into three parts, comprising the examination of (*a*) details of technique in the titration for the accurate measurement of the fluorine present in solution after distillation, (*b*) distillation control, and (*c*) preparation of the food material before it is submitted to the distillation.

Titration—Good agreement was obtained between members of the Sub-Committee on a test solution circulated for trial. Later in the investigation the necessity for accurate adjustment of the acidity before titration was demonstrated, because a considerable amount of carbonate is obtained when foodstuffs are ashed with lime; the carbon dioxide liberated from this and found in the distillate has a considerable effect on the titration of the acidity, the end-point being indefinite if alizarin S is used. By altering the indicator for the preliminary titration to methyl orange and titrating against a comparison cylinder, comparable acidities in the test solution are obtainable. Other dyestuffs were tried unsuccessfully in place of alizarin S.

For titrations, burettes of 10 ml capacity graduated in 0·02 ml divisions are recommended.

Distillation—Early collaborative work showed the ubiquitous nature of fluorine. The water used for generating steam should be alkalised to prevent any fluorine being carried over in the steam supply; traces of fluorine in the perchloric acid must be removed, and for this purpose a preliminary distillation is necessary. Significant amounts of fluorine are often present in new apparatus; most of this is removed in the preliminary distillation, but a small amount is retained and is only diminished by subsequent use of the apparatus; hence there is usually a small "apparatus blank," which must be deducted in cases where a high degree of precision is required, but may be neglected for routine examinations. Treatment of a distillation flask with hot sulphuric acid increases the apparatus blank considerably, but the flask should be boiled out from time to time with 10 per cent. caustic soda solution to free it from gelatinous silica, which may retain traces of fluorine..

Close temperature control in distillation was shown to be an important factor, abnormally high acidities being obtained with high temperatures of distillation and slow evolution of fluorine with low temperatures. The Sub-Committee finds that steam distillation permits a more accurate control of distillation temperatures than does the dropping of water on the boiling perchloric acid. For all practical purposes the fluorine in the quantities likely to be present in foods is fully recovered from 150 ml of distillate.

The Sub-Committee is of the opinion that, provided the "apparatus blank" is normally less than 1·5μg of fluorine per 150 ml distilled, it can be neglected except where the fluorine content is less than 1 part per million or for other very precise work.

Preparation of Food Material for Distillation—From theoretical considerations, use of calcium hydroxide or barium hydroxide for retention of fluorine on ashing is preferable to the use of the sodium or potassium compound, since the fluorides of sodium and potassium are more volatile. Work on the use of lime was not possible until a fluorine-free product was obtainable; the purest commercial material that was available contained considerable amounts of the impurity. The following method was devised to produce a lime essentially free from fluorine:

> Prepare an ammonium carbonate reagent by dissolving 110 g of ammonium carbonate of Analytical Reagent purity and 55 ml of ammonia (sp.gr. 0·880) in water and diluting to 600 ml.
>
> Dissolve 200 g of calcium chloride (dry) of Analytical Reagent purity in about 600 ml of warm distilled water. Stir into this solution 20 ml of the ammonium carbonate reagent, bring the mixture just to boiling point, allow the precipitate to settle for a few minutes and then filter on a Buchner funnel with suction and reject the precipitate. Repeat the precipitation and filtration three times with 20 ml portions of the ammonium carbonate reagent. Finally treat the clear filtrate from the last precipitation with the remainder of the ammonium carbonate reagent, stir the mixture well and bring it to the boiling point. Allow the precipitate to settle, filter, wash several times with hot water until free from chloride and dry at 100° C. Ignite to oxide in a platinum dish in small quantities of 1 g to 2 g, as required.

Experiments were conducted on the retention of fluorine by lime water, baryta water, and a considerable excess of lime moistened with water. The results tended to show losses with the smaller quantities of alkali available in lime water and baryta water, and in the opinion of the Sub-Committee the use of the excess of lime is necessary.

The ashing temperature must be controlled; high temperatures such as uncontrolled red heat over Bunsen flames will cause loss of fluorine. A small quantity of carbon in the ash does not appear to affect the results; further, its presence is considered to be free from risk, many experiments having been made by each member of the Sub-Committee in the course of the work with carbon present during distillation, but with no instance of any trouble. Particular care is required to guard against contamination with fluorine derived from dust or from the muffle lining during ashing.

Chlorides in foodstuffs may affect the determination by producing in the distillate an excessive amount of free acid; silver sulphate added before distillation will eliminate this source of trouble. The technique recommended specifies the inclusion of 0·2 g of silver sulphate as an excess, but for certain commodities containing considerable proportions of chloride, *e.g.*, fish products, dried milk, and where salt has been added, an excess of silver sulphate must be assured and the amount of chloride previously determined if the approximate content is not known.

Many instances of the need for exactitude in technique have been demonstrated in the work of the Sub-Committee, who emphasise the importance, for accurate results, of exactly following the details of the method proposed.

During the later stages of the investigation a number of foods were distributed to the members of the Sub-Committee. The results given below show the quantities of fluorine found to be present by individual members. A few high results, widely divergent from the mean, have not been included, since the yields were obviously due to fortuitous contamination.

Fluorine in μg per gram (parts per million)

	White flour	National flour	Self-raising flour	National Milk powder	Tea
Member A ..	0·45	1·05	5·4	—	126
	0·6	0·9	5·7		129
	0·3				
Member B ..	0·30	0·66	5·3	0·48	108
	0·30	0·66	4·9	0·48	112
	0·35				
Member C ..	0·29	0·85	6·6	0·57	126
		0·88	6·7	0·56	120
Member D ..	0·53	0·83	6·0	0·68	111
	0·60	0·83	5·8	0·79	113
	0·38	0·75	6·0		107
	0·42	0·86	5·9		113
Member E ..	0·48	0·80	7·4	0·52	118
	0·30	0·68	7·1	0·55	110
	0·39	0·71			121
Member F ..	0·49	0·86	6·5	0·68	119
	0·45	0·98	6·3	0·83	112
	0·53	0·87			
	0·46	0·83			

Consideration of these results suggested the possibility that some of the deviations were partly due to segregation. This view receives support from the greater uniformity of the following results obtained with a liquid sample.

Evaporated Milk containing added Fluorine

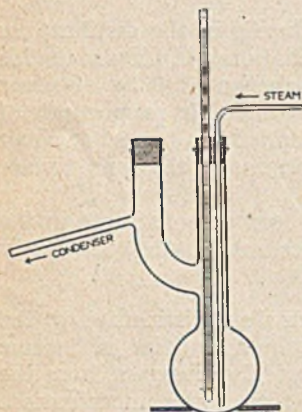
	Fluorine in μg per gram		Fluorine in μg per gram
Member A ..	6·4	Member D ..	5·8
	6·5		5·8
	6·2		5·8
			5·8
Member B ..	6·6	Member E ..	6·8
	6·3		6·5
Member C ..	6·0	Member F ..	6·5
	6·3		6·4
	6·3		6·2
	6·3		6·3

The amount of fluorine present in the circulated sample was 6·7μg per gram, of which 6·5μg per gram had been added.

In addition to the above the following individual samples of food have been examined by various members of the Sub-Committee; the fluorine contents found are given in parts per million (μg per gram):—beer 0·45, 0·25; canned pilchards 5·8; canned shrimps 2·4; corned beef 0·4; dried egg 0·3; evaporated milk 0·15; milk 0·17; rolled oats 0·6; soya grits 0·6; spinach powder 70·0; split peas 0·4; whisky 0·03.

Method

The distillation apparatus consists of a Claissen flask (see Figure) of 100–150 ml capacity, a large glass flask for generating steam and an efficient condenser. The main neck of the Claissen flask is fitted with a two-holed rubber stopper through which pass a thermometer and a glass tube for connecting with the steam supply, both the thermometer and the tube extending almost to the bottom of the flask. The side neck of the Claissen flask is closed with a solid rubber stopper and the side arm connected with the condenser. The steam is generated from water made alkaline with sodium hydroxide. Local overheating of the Claissen flask is avoided by use of an asbestos board with a hole which must fit closely to the lower surface of the flask or by use of an asbestos gauze.



Mix a suitable quantity of the sample (10 g or less according to the amount of fluorine expected) in a platinum dish with about 1 g of fluorine-free lime and 50 ml of water, evaporate on a water-bath and thoroughly char at a temperature below visible red heat. Transfer to a muffle at about 600° C. (dull red heat) and ignite for 1½ to 2 hours.

Assemble the apparatus, introduce into the flask a number of fragments of Pyrex glass, 0·2 g of silver sulphate or sufficient to precipitate all the chloride in the portion of sample being treated, 7 ml of water and 15 ml of 60% perchloric acid. Heat the flask until the temperature reaches 120–125° C., connect the steam supply, regulate the gas and steam supplies so that the temperature of the distillation is maintained at 137–140° C. and distil 150 ml in 25–35 minutes, steaming out the condenser towards the end of the distillation. Discard the distillate. Distil a further 150 ml and titrate an aliquot part by the method given below. Calculate the amount of fluorine in the whole fraction. (This figure, which should not exceed 1·5μg, may be termed the "apparatus blank" and should be approximately constant for any further 150 ml fractions.)

Cool the flask, transfer the acid contents to a suitable receptacle and rinse the flask and glass fragments with distilled water, rejecting the rinsings. Introduce the bulk of the dry ash into the flask and wash in the remainder with about 5 ml of water containing a few drops of the acid. Add the remainder of the acid, whilst cooling the flask, and rinse down the neck of the flask with 1–2 ml of water. Connect up the apparatus and distil 150 ml as before.

Titrate 50 ml of the well mixed distillate with 0·05 *N* sodium hydroxide in a Nessler tube, using methyl orange as indicator, until the colour matches that of a comparator tube containing distilled water and the same amount of methyl orange.

Transfer the remaining 100 ml of distillate to a Nessler cylinder and add an amount of 0·05 *N* hydrochloric acid to make the total acidity equal to 5·0 ml of 0·05 *N* acid. Prepare a "control" cylinder containing 5·0 ml of 0·05 *N* hydrochloric acid and distilled water and add to both "test" and "control" cylinders 2 ml of 0·01% alizarin S solution. From a burette which can be read to 0·02 ml add to the "test" cylinder a solution of thorium nitrate (approximately 0·25 g per litre) until a slight pink colour persists as compared with the yellow of the "control" cylinder. Add an exactly similar volume of the thorium nitrate solution to the "control" cylinder, which then becomes more pink than the "test" solution. Then add slowly, from a suitable burette, standard solution of sodium fluoride (0·0221 g of NaF per litre; 1 ml ≡ 10μg of fluorine) until the tints of "test" and "control" solutions match exactly. The volume of standard fluoride solution added corresponds to the amount present in the "test" portion of the distillate. Calculate the amount of fluorine present in the 150 ml of distillate and subtract the "apparatus blank." Express the result as parts per million of the food.

June, 1944.

Official Appointments

FERTILISERS AND FEEDING STUFFS ACT, 1926

THE Ministry of Agriculture and Fisheries has notified the Society of the following changes in the appointments of Agricultural Analysts which have taken place since July 15th, 1943 (ANALYST, 1943, 68, 249).

W. GORDON CAREY, F.R.I.C., Agricultural Analyst for the County Borough of Gateshead, *vice* H. C. L. Bloxam, deceased.

A. O. JONES, M.A., F.R.I.C., Deputy Agricultural Analyst for the County of Cardigan.

J. GRAHAM SHERRATT; B.Sc., F.R.I.C., Deputy Agricultural Analyst for the County Borough of St. Helens and Wigan.

July 11th, 1944

Ministry of Food

STATUTORY RULES AND ORDERS

1944. No. 654. Order, dated June 1, 1944, amending the Food Standards (General Provisions) Order, 1944. Price 1d.*

This amending Order is intended to make it clear that where standards are applied they shall apply only—(*a*) where the food sold is so described as to lead an intending purchaser to believe that he is getting food of a kind for which a standard is prescribed; (*b*) where the food is sold in response to a request for food of a kind for which a standard is prescribed.

Whereas the first Order (*S.R. & O.*, 1944, *No.* 42) *referred to any* description *of food, the new Order refers to any* kind *of food.*

— **No. 660. The Cereal Fillers (Control and Maximum Prices) Order, 1944. Dated June 5, 1944.** Price 1d.

This Order revokes and replaces the Cereal Fillers (Control and Maximum Prices) Order, 1943, and the amending Order, dated April 23, 1943. The principal alterations are as follows:—(1) The word "baked" is inserted in definition of "Cereal Filler." (2) It is now permitted to use cereal filler as a bait or mixture for destroying rats or mice. (3) A certificate of a Public Analyst or the Government Chemist proves itself unless challenged.

Legal Notes

The Editor would be glad to receive particulars of cases with points of special legal or chemical interest

SALT IN CURRY POWDER

ON April 24th a manufacturer and two tradesmen were summoned at Bow Street Police Court by the Westminster City Council for selling "Genuine Indian Curry Powder" containing 24% of salt, whereas in the opinion of the Public Analyst it should have contained at most 10%. The manufacturer was fined £10 with £15 15s. costs; no order was made as to the costs of the other defendants.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determination of Sulphur Dioxide in Dehydrated Foods. A. N. Prater, C. M. Johnson, M. F. Pool and G. Mackinney (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 153–157)—The method is an adaptation of the direct titration procedures of Iokhel'son and Nevstrueva (*Voprosy Pitaniya*, 1940, **9**, 25) and Bennett and Donovan (ANALYST, 1943, **68**, 140). Representative samples must be subdivided so that the solns. used will leach out all the sulphur dioxide. A Wiley mill is recommended and practically all the sample should pass through the 2-mm screen. A food grinder designed for preparing vegetable purées may be used if almost all the material passes a 10-mesh screen and at least 60% a 20-mesh screen. Dried fruits may be passed through a kitchen food chopper and reduced by soaking and grinding to a finely divided suspension. Suspend two 8-g samples of the prepared material in 400-ml portions of water, add 5 ml of 5 *N* sodium hydroxide to each and leave for 20 min. Acidify the mixtures with 5 *N* hydrochloric acid (7·5 ml for cabbage, carrots or potatoes and 6 ml for dried apricots, apples and peaches). To one mixture add 40 ml of acetone and titrate the other immediately with 0·05 *N* iodine, using 10 ml of 1% starch soln. as indicator. The end-point is reached when a blue colour flashes through the mixture and persists for a few sec. Treat the mixture containing acetone similarly after it has stood for 10 min. The difference between the two titres (corrected by blank determinations in which the food material is omitted) × 200 gives the sulphur dioxide content in p.p.m. Combination of sulphur dioxide with acetone occurs most easily with cabbage and carrots at *p*H 2–3, whereas with potatoes the range extends to *p*H 4, so that for general applicability the range 2–3 is chosen and affords a good end-point. Buffer curves in which the *p*H of the mixture was plotted against successive increments of 5 *N* hydrochloric acid established the vol. of acid to be added to bring the *p*H of the alkaline digestion mixture within this range for each type of food, and the prescribed vol. of alkali and acid (*supra*) should be measured accurately

* Obtainable from H.M. Stationery Office. Italics signify changed wording.

with burettes. For foods other than those mentioned, the required vol. of acid should be determined. High concns. of acetone affect the end-point. Quantitative addition of sulphur dioxide occurs with 10% of acetone (v/v), and with the amount of starch recommended the end-point is satisfactory. With starchy material (*e.g.*, potato) slight adsorption of iodine on starch particles imparts a grey cast to the mixture. This must not be mistaken for the true end-point, which is indicated by a deep blue colour. The accuracy of the method was checked against results obtained by the methods of Monier-Williams (*Brit. Min. Health Rept.*, 1927, No. 43) and Nichols and Reed (*Ind. Eng. Chem.*, *Anal. Ed.*, 1932, **4**, 79) and by measurements with the dropping mercury electrode. When known amounts of sodium metabisulphite were added to the food material results were not consistent unless time was allowed for the inhibiting action of certain plant constituents upon the oxidation of sulphur dioxide to disappear, or unless 0·1% of sodium pyrophosphate (10 H_2O) was added to the sodium metabisulphite soln. The method has an accuracy comparable with that of distillation methods, especially in the range of 500-4000 p.p.m. of sulphur dioxide. A. O. J.

Quantitative Determination of Nicotine and Nornicotine in Mixtures. P. S. Larson and H. B. Haag (*Ind. Eng. Chem.*, *Anal. Ed.*, 1944, **16**, 86–90)—The method is based on the reaction of cyanogen bromide with nicotine to form a yellowish-green colour, and with nornicotine to give a red colour. The reagent is prepared fresh each day by titrating 25 ml of cold saturated bromine water with fresh 2 *M* sodium cyanide soln. almost to disappearance of colour, and then to complete decolorisation with fresh 0·2 *M* sodium cyanide, avoiding excess; it is adjusted to *p*H 7·0 at room temp. with 0·02 *M* sodium cyanide, using a glass titration electrode, and then to *p*H 4·1–4·2 with 0·2 *N* sulphuric acid. The procedure is as follows. *Preparation of tobacco distillate*—Place 0·5–2·0 g of finely ground tobacco, 10 g of sodium chloride, 10 ml of 10 *M* sodium hydroxide soln. and a little paraffin in a 300-ml Kjeldahl flask, and steam-distil to give a distillate of about 800 ml in 1 hr., placing in the receiver 3 ml of 0·2 *N* sulphuric acid and enough water to cover the condenser opening, and keeping the vol. in the Kjeldahl flask at 50–75 ml. Adjust the distillate to *p*H 4·1–4·2 with 0·2 *N* sulphuric acid or potassium hydroxide and make up to 1 litre. When little material is available, the distillation method of Bowen and Barthel is preferable (*Ind. Eng. Chem.*, *Anal. Ed.*, 1943, **15**, 596). *Colour development*—Pipette two aliquot portions of 6 ml or less (containing not more than 80μg of nicotine and 150μg of nornicotine) into 20 × 150-mm test-tubes, and make up to 6 ml with water. Add to each 2 ml of 1·67 *M* potassium dihydrogen phosphate soln. Place one tube in a water bath at 80° C. for exactly 5 min. Remove, add 2 ml of the cyanogen bromide reagent, shake and return to the water bath in exactly 15 sec. After 2 min. 45 sec. remove the tube and place in an ice and water bath for 1 min. Transfer to a spectrophotometer cuvette and read the intensity of the colour developed by nornicotine at 540 $m\mu$ and that developed by nicotine at 375 $m\mu$. Complete this operation during 4 min. after the tube is removed from the ice-water, and meanwhile give the second tube the 5-min. preheating. Since tobacco distillates are often slightly coloured, balance the spectrophotometer to 100% transmission against a soln. containing a vol. of distillate equal to that used for colour development diluted to 10 ml with water. *Preparation of calibration curves*—Treat 1-, 3- and 5-ml aliquot portions of nicotine standard, containing about 20μg per ml and adjusted to *p*H 4·1–4·2 with 0·2 *N* sulphuric acid, as above, for colour development, reading the intensity at 375 $m\mu$. Treat similarly one tube containing 6 ml of water and the phosphate soln. to provide a blank on the cyanogen bromide reagent. Plot the data on semilog paper, plotting % transmittance on the logarithmic axis; this gives a straight line. Develop the colour similarly for 1-, 3- and 5-ml aliquot portions of nornicotine standard, containing about 60μg per ml and adjusted to *p*H 4·1–4·2 with 0·2 *N* sulphuric acid, and read the intensities at 540 and 375 $m\mu$. The cyanogen bromide reagent gives no blank at this wave-length. Prepare the calibration curve for nornicotine at 540$m\mu$. The values obtained from it for nornicotine are true, but the nornicotine has an additive effect on the nicotine colour at 375$m\mu$. To determine the degree of this, convert the spectrophotometer readings obtained with the nornicotine standard at 375 $m\mu$ into μg of apparent nicotine by using the nicotine calibration curve. Plot graphically the nornicotine values at 540$m\mu$ against their apparent nicotine equivalent at 375$m\mu$. The true amount of nornicotine in the mixtures is thus obtained from the nornicotine calibration curve at 540$m\mu$, and the nicotine content is found by subtracting from the apparent nicotine content at 375$m\mu$ the correction due to nornicotine. The calibration curves need not be re-determined each day, but should be checked when a new source of bromine water, sodium cyanide or potassium dihydrogen phosphate is used. The intensity of colour is affected by inorganic salts, which should be excluded. The quantity of cyanide is also important. The colour is affected also by temp., quantity of potassium dihydrogen phosphate and *p*H. A table shows the colour formation observed with other pyridine derivatives. E. M. P.

Determination of Camphor and Alcohol in Spirit of Camphor by Refractive Index and Specific Gravity. E. M. Plein and C. F. Poe (*Ind. Eng. Chem.*, *Anal. Ed.*, 1944, **16**, 168–169)—Samples of camphor were obtained from widely different sources during 10 years. Each sample was purified at least twice by sublimation, with rejection of initial and final fractions in each sublimation. Solns. containing from 7·5 to 11·5% w/v of camphor and from 70 to 90% v/v of alcohol were prepared from each sample as previously described (*J. Amer. Pharm. Assoc.*, 1943, **32**, 89). The sp.gr. of each soln. was determined with a 25-ml pycnometer at 20°/20° C. and n_D^{20} was determined for each soln. with a Valentine, Abbe-type refractometer. Data from *ca.* 100 solns. were plotted with sp.gr. as abscissae and n_D^{20} as ordinates. By connecting the points corresponding with the same proportions of camphor and those corresponding with the same proportions of alcohol several four-sided figures were formed which were divided into smaller units to facilitate reading. By this means a curvilinear system of co-ordinates with % alcohol as abscissae and % camphor as ordinates was superimposed on the rectilinear system with sp.gr. as abscissae and n_D^{20} as ordinates. To ascertain the composition of a sample of spirit of camphor, the sp.gr. and n_D^{20} were determined and the corresponding point on the system of rectilinear co-ordinates

was located. The co-ordinates of this point referred to the curvilinear system then gave the % of alcohol and camphor in the sample of spirit. Analyses of different samples of spirit of camphor (either from natural or synthetic camphor) by this method agreed closely with the known composition. A. O. J.

Determination of Sesamin. M. Jacobson, F. Acree, Jr., and H. L. Haller (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 166–167)—The toxicity to houseflies of a soln. of pyrethrins in kerosene is considerably increased by addition of a small amount of sesame oil (Eagleson, *Soap*, 1940, **16**, 96, 117), and Haller *et al.* (*J. Org. Chem.*, 1942, **7**, 183) showed that this synergistic effect is due to the substituted bicyclodihydrofuran sesamin. Addition of 5% of sesame oil to a soln. of pyrethrins in kerosene (Eagleson, *Soap*, 1942, **18**, 125) or dichlorodifluoromethane (Goodhue, *Ind. Eng. Chem.*, 1942, **34**, 1456) saves *ca.* 50% of pyrethrum, but the min. quantity needed has yet to be determined because the sesamin content of sesame oil varies. A mixture of perchloric acid and hydrogen peroxide gives a greenish-yellow colour, persisting for *ca.* 6 min., with any vegetable oil containing sesamin. By measuring the colour given by standard solns. of sesamin in sesamin-free sesame oil a curve is established from which the sesamin content of unknown solns. may be read. For measuring the colour an Aminco photoelectric photometer is recommended with a No. 46 blue filter (460$m\mu$) and water as blank material to balance the photometer at 100% transmission. Formation of oxygen bubbles in kerosene precludes its repeated use as blank material. To prepare the reagent, which is very corrosive, add 2 ml of 30% hydrogen peroxide to 4 ml of 70–72% perchloric acid maintained at 15°–20° C. Mixtures prepared below 10° C. give erratic transmission values. The reagent must be freshly prepared for each reading not more than 10 min before use. To prepare sesamin-free sesame oil, dissolve 100 g of oil in 100 ml of light petroleum (b.p. 30°–60° C.) and extract with *ca.* six 20-ml portions of 90% acetic acid until a test portion shows no colour with the reagent. Wash the petroleum soln. of the oil free from acid with water, dry over sodium sulphate and finally remove the solvent under reduced pressure. To obtain pure sesamin for the standard solns., wash the combined acetic acid extracts once with light petroleum and remove almost all the acetic acid under reduced pressure (60°–80° C. at 15–20 mm). Add 5-10 ml of hot 95% ethanol to induce crystallisation, collect the sesamin by filtration of the cold liquid and purify it by repeated re-crystallisation from ethanol (m.p. 121°–122° C.). Refined sesame oil contains *ca.* 1% of sesamin. Grind 100 mg of sesamin to a fine powder, make up to 10 g with sesamin-free oil and dissolve by slight warming. Dilute aliquot portions of 1000, 750, 500, 250 and 100 mg of this soln. to 10 ml with refined kerosene (Deobase) to obtain standard solns. containing 1·00, 0·75 mg, etc., of sesamin per ml respectively. Treat 1 ml of the standard soln. in a dry rubber-stoppered centrifuge tube with 6 ml of the reagent, shake for 30 sec., centrifuge for 2 min. and pour the liquid into a dry photometer tube. Measure the faint yellow to dark greenish-yellow colour of the aq. layer in the photometer exactly 5 min. after adding the reagent. Treat 1-ml portions of the other standard solns. similarly and plot the results on semi-logarithmic paper as % transmission (logarithmic ordinate) against mg of sesamin per ml of kerosene soln. To determine the sesamin content of an unknown oil, dilute 500 mg of oil to 10 ml with refined kerosene, adjusting the size of sample, if necessary, to keep the transmission readings between 80 and 28%. Treat an aliquot portion of this soln. as previously described and obtain the concn. of sesamin from the standard graph. The accuracy of the method is *ca.* ±0·05% of sesamin. A. O. J.

Biochemical

Activity of Phytase in Different Cereals and its Resistance to Dry Heat. R. A. McCance and E. M. Widdowson (*Nature*, 1944, **153**, 650)—Whereas wheat, oats, barley and rye all contain between 0·18 and 0·26% of phytic acid phosphorus, wheatmeal and flours also contain an active phytase. Since phytic acid is now considered to have anticalcifying properties, the interaction of the enzyme with its substrate during the preparation of wheat for the table (Widdowson, *Nature*, 1941, **148**, 219; Pringle and Moran, *J. Soc. Chem. Ind.*, 1942, **61**, 108) may account for Mellanby's observations (*Spec. Rep. Ser., Med. Research Council*, 1925, No. 93; 1929, No. 140) that oatmeal is more rachitogenic than wheatmeal and that hydrolysis with hydrochloric acid or germination and malting can remove the anticalcifying properties of the former. The phytase activity of different cereals was investigated by incubating the ground sample with 10 times its wt. of water at 50° C. and *p*H 4·5 and determining the time required for the destruction of 50% of the phytic acid present. The following results were obtained.

Nature of cereal	Phytic acid P in cereal mg per 100 g	Hydrolysing agent	Time required for hydrolysis of 50% of the phytic acid
Rye, 1	242	Phytase in cereal	5 min.
„ 2	217	„	8 min.
Wheat, English	242	„	12 min.
„ Manitoba	233	„	14 min.
Barley	260	„	43 min.
Oats, green ..	233	„	11 hr.
„ „ ..	210	„	11 hr.
„ „ ..	182	„	13·5 hr.
„ „ ..	198	„	15 hr.
Oats, kilned ..	220	„	30 hr.
„ „ ..	218	„	46 hr.
„ „ ..	223	„	46 hr.
„ „ ..	206	„	40 hr.
Wheat, English	242	Boiling 2*N*HCl	7·1 hr.
Oats, green ..	182	„	7·1 hr.

It is pointed out that rye phytase is the most active, whence the destruction of phytic acid in rye bread may be very high: a sample baked according to the German process contained 48% less than the whole rye flour. The identical rate of hydrolysis of wheat phytic acid and that in oats by boiling 2 *N* hydrochloric acid indicates that the apparent lack of phytase activity in oats is unlikely to be due to the latter cereal containing a more resistant form of the acid and, although various samples of oatmeal contained 44–72 mg of calcium per 100 g compared with 29–41 mg for the other cereals, at least 75% of the phytic acid was present as the potassium and magnesium salts, both of which would be in solution at *p*H 4·5. The resistance of the enzyme to dry

heat was tested by drying wheat-bran over phosphorus pentoxide and heating at 90° C. for varying periods of time. Other samples were heated in water at 90° C. for the same times. The phytase activity was then measured as described above, but incubating for 50 min. in each instance and determining the percentage of phytic acid destroyed. The results were as follows.

Time of heating at 90° C., hr.	0	0·5	1	2	5
Destruction of phytic acid (%) during incubation:					
after dry heat	87	87	85	82	78
after wet heat	87	0	0	0	0

J. A.

Estimation of Vitamin A and Carotenoids in Butter Fat. Comparison of Direct Spectrophotometry with Filter Photometry and Use of the Antimony Trichloride Reaction. F. P. Zscheile, H. A. Nash, R. L. Henry and L. F. Green (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 83–85)—The following is the method recommended by the Technical Committee on Vitamin A Researches: Weigh a 10-g sample of filtered butter-fat or butter into a 300-ml flask and add 5 ml of saturated aq. potassium hydroxide soln. and 20 ml of aldehyde-free methanol. Boil under reflux for 10 min., dilute immediately with 40 ml of water, and cool to room temp. Transfer the mixture to a separating funnel, using 50 ml of water to rinse out the flask. Shake with 100 ml of peroxide-free ether and re-extract the aqueous phase with three 50-ml portions of ether. Wash the combined ethereal extracts with water until free from alkali, concentrate to about 80 ml, dry over anhydrous sodium sulphate and dilute to 100 ml. Where direct spectrophotometry is to be used, make observations (on the same day if possible), using wavelengths of 324 and 437$m\mu$ for vitamin A and total carotenoids respectively. Pure vitamin A in ether has an absorption max. at 324$m\mu$, at which point $E^{1\%}_{1\,cm}$ is 1825; for β-carotene, $E^{1\%}_{1\,cm}$ is 2040 at 437$m\mu$. Use the same sample of ether in the blank cell as was employed for the extraction. Where the antimony trichloride method is to be employed for estimating vitamin A and where it is necessary to estimate carotene free from other carotenoids, divide the ethereal extract into two parts for the estimation of carotene and vitamin A respectively. Evaporate one part to dryness, dissolve the residue in 50 ml of Skellysolve B, and extract with four 25-ml portions of 92% aq. methanol or 94% diacetone alcohol. Dry over sodium sulphate and measure the transmission at 440$m\mu$ with a photoelectric filter photometer. Evaporate the other part to dryness, dissolve the residue in chloroform, add antimony trichloride reagent, and measure the transmission at 620$m\mu$ with the same photoelectric filter photometer. Apply a correction for the carotene present. Aqueous diacetone alcohol is better than aq. methanol for removing carotenols, *e.g.*, from butters produced from cows fed on acid or molasses silage. The method for carotenoids was examined in 7 laboratories; of a total of 42 determinations, only 5 deviated from their corresponding means by more than 7%, the max. deviation being 13·3%. In the estimation of vitamin A, four of the collaborators used crystalline vitamin A as standard, and another four collaborators the U.S.P. reference cod-liver oil; the results of the first group agreed better among themselves than those of the second group. Only one laboratory used direct spectrophotometry. Of the 18 results obtained with the colorimetric method, the max. deviation from the mean was 8% and the over-all mean abs. deviation was 3·34%. The corrected results from direct spectrophotometry had mean abs. and max. deviations two-thirds as great as the over-all averages. Attempts to improve the agreement by correction for the amounts of carotenoids present were unsuccessful. Annatto does not interfere with the estimation, as it remains in the aq. methanol soln. after extraction with ether. Two azo dyes, however, were extracted, but, as their absorption curves differed materially from those of β-carotene, it was possible to apply a correction, except when appreciable quantities of other carotenoids were present. In such instances the azo dyes could be removed with the carotenols by extraction with aq. methanol or diacetone alcohol, enabling the β-carotene to be accurately estimated. The azo dyes interfered with the direct photometric estimation of vitamin A, but not with the antimony trichloride reaction. F. A. R.

Determination of Vitamin A and Carotene in Milk. A Rapid Extraction Procedure. P. D. Boyer, R. Spitzer, C. Jensen and P. H. Phillips (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 101–102)—To a 20-ml sample of milk in a separating funnel add 30 ml of alcoholic potassium hydroxide soln. (20 g of potassium hydroxide in 10 ml of water shaken with 90 ml of abs. alcohol). Shake and leave for 3 hr. Add 25 ml of ether, shake and allow to separate, re-extract with a further 18 to 20 ml of ether, and discard the lower layer. Wash the extracts in succession first with 75 ml of water, and then with about 10 ml of acidified alcohol (1 ml of hydrochloric acid in 100 ml of ethyl alcohol diluted to 1 litre with water), and discard the washings. Add 3 ml of light petroleum to each ethereal extract, and wash the mixtures in succession with two 10-ml portions of acidified alcohol. Finally, evaporate the combined extracts to dryness by warming at 30–40° C. under reduced pressure, removing the last traces by heating at 60–70° C. Cool and add exactly 5·0 ml of ether. When the residue is dissolved, shake with 5 ml of sat. sodium chloride soln., add exactly 10 ml of light petroleum, and shake. Transfer a 10-ml aliquot portion of the clear upper phase to an Evelyn colorimeter tube, and measure the total carotenoids, using the 440 filter. Attach the colorimeter tube to a vacuum pump and evaporate the solvent. Dissolve the residue in 1 ml of chloroform, add one drop of acetic anhydride and then 9 ml of antimony trichloride soln. (20 g of antimony trichloride in 100 ml of chloroform; filter immediately before use). Measure the max. deflection, using the 620 filter. Calculate the carotene content from a standard curve prepared from solns. of crystalline carotene, and the vitamin A content from a curve prepared with a standardised fish oil or, better, with pure vitamin A. The recovery of vitamin A alcohol added to the original milk was at least 95% of the theoretical. Although normally the carotenoids of cow's milk consist principally of β-carotene, other carotenoids may be present, and for accurate determinations the light petroleum extract may be freed from these interfering pigments by extraction with diacetone alcohol; xanthophylls may be removed by extraction with 85% phosphoric acid, or by phase separation with 95% methyl alcohol. F. A. R.

Extraction of Oil and Vitamin A in Shark Liver Analysis. The Xylene Centrifuge Method. V. M. Sycheff (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 126–127)—Homogenise a 0·2 to

0·5-g sample of the liver for 5 to 8 min. (in a Waring Blendor) and transfer quickly to a tared 15-ml graduated conical centrifuge tube. Weigh to the nearest mg and then direct 10 ml of xylene (purified by washing with conc. sulphuric acid and then with water, followed by treatment with 10–20% sodium bisulphite soln., washing, drying over sodium sulphate and distillation in an all-glass still) in a sharp stream on to the material in the tube. Shake vigorously, centrifuge for 5 min. at 2000 r.p.m., invert once or twice, and re-centrifuge for another 5 min. Record the vols. of liver, solvent and xylene-insol. material, and evaporate a 5-ml aliquot portion of the supernatant extract to dryness at 65–85° C. under atmos. pressure to determine the oil content. Remove a 1-ml aliquot part of the supernatant liquid and dilute with xylene, as required for the colorimetric assay. To 2 ml of the resulting soln. in a Coleman tube add 2 ml of 0·5% guaiacol-chloroform soln. and 6 ml of 30% antimony trichloride-chloroform reagent. Immerse the corked tube in water at 62° C. for 45 sec., and cool to room temp.; the colour is stable for 20–30 min. Measure % transmission, referred to a blank consisting of the reagents and the appropriate solvent instead of the oil soln., with a Klett-Summerson photoelectric colorimeter, using a No. 54 filter, and calculate the results from the following formulae:

$$\% \text{ of oil in liver} = \frac{20\,w\,(V - v)}{W}$$

where W is the wt. of liver, w the wt. of oil in the 5-ml aliquot portion, V the total vol. of liver and solvent, and v the vol. of the xylene-insoluble residue

$$\text{I.U. per g of oil} = \frac{R}{V} \times \frac{F}{p}$$

where R is the colorimeter reading, F the conversion factor to convert readings to I.U., V the vol. of undiluted extract and p the % of oil in the undiluted extract. The value of F, as determined on various concentrates, was 42·6. F. A. R.

Aneurine in Beef Muscle. A Comparison of Values by the Thiochrome Reaction Applied with and without Adsorption. W. F. Hinman, E. G. Halliday and M. H. Brookes (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 116–120)—Considerable discrepancies were encountered when the method of Harris and Wang (*Biochem. J.*, 1941, **35**, 1050; *Chem. and Ind.*, 1942, **61**, 27; ANALYST, 1942, **67**, 106, 107) was compared with that of Hennessy and Cerecedo (*J. Amer. Chem. Soc.*, 1939, **61**, 179), using the same beef muscle digests. Expts. were made to ascertain whether these discrepancies were due to the presence of fluorescent compounds which would be eliminated in the adsorption method, or to failure to adsorb aneurine completely from the digests. The results indicated that the discrepancy is due to both factors, and the following recommendations, whilst not eliminating the discrepancies completely, improved the accuracy of the two methods. In preparing the extracts a Waring Blendor should be used at 70°–75° C. and the ratio of sample to solvent should be about 1 to 10. Digestion with Clarase is best carried out overnight at 45–47° C., and the digest can be clarified by shaking vigorously with 5 ml of chloroform to remove fat. In applying the method of Hennessy and Cerecedo, the activity of the Decalso should first be tested by adsorbing 5μg of synthetic aneurine hydrochloride on a 7- to 8-cm column; the recovery should not be less than 95%. In applying the Harris and Wang thiochrome method to beef muscle, particular care should be taken to avoid excess of ferricyanide; whereas the original authors observed that excess of ferricyanide destroyed thiochrome, it has now been found with beef muscle that excess of ferricyanide leads to an increase, not a decrease, in the thiochrome value. The Harris and Wang method gave satisfactory results when these were calculated from standards made with solns. of pure aneurine, but with the Hennessy and Cerecedo method it was found better to calculate the results from readings given by the unknown eluates to which known amounts of pure aneurine had been added. F. A. R.

Effect of Commercial Canning on the Aneurine Content of Vegetables. L. E. Clifcorn and W. G. Heberlein (*Ind. Eng. Chem.*, 1944, **36**, 168–171)—The results of tests, described in detail, showed that the retention of aneurine during the commercial blanching of asparagus, green beans, Lima beans, and peas (Alaska and Sweet varieties) ranges from 64 to 100%, and during commercial processing from 58 to 79%. The over-all aneurine retention, for the 9 products studied, ranged from 31 to 89%; average 57%. The aneurine content of canned vegetables is significantly affected by the grade and size of some of the raw products, and, with asparagus, by the segment of the stalk canned. Expts. showed a decrease in the aneurine content on storage. The liquid portion of the canned products contained from 27 to 45% of the total aneurine in the can. The importance of using this portion of the canned product is therefore emphasised. Stress is also laid on the importance of sampling technique for the validity of results. To even out the variations in cans packed at the same time, more than one can should be included in the final sampling, and the number of cans should be stated in the report.

Vitamin C in Plants. Nasturtium (*Tropaeolum majus*). M. D. Sutherland (*Nature*, 1944, **153**, 683)—The leaves of the nasturtium, *Tropaeolum majus*, are very rich in vitamin C, the concn. ranging from 200 to 465 mg per 100 g in the samples tested. The leaves of any particular plant usually show less variation than this, although small leaves tend to have higher values than large ones. The stalks contain 100–160 mg per 100 g. Little or no dehydroascorbic acid is present, but ascorbic acid oxidase is very active in disintegrated tissues. The vitamin was determined by titration of a 1% metaphosphoric acid extract with dichlorophenolindophenol soln., the reducing action of the extracts being proved due to ascorbic acid by isolation of pure dehydroascorbic acid dinitrophenylosazone from a boiling-water extract previously oxidised with iodine and also by the fact that rapid enzymatic hydrolysis takes place in water extracts unless prevented by boiling, etc. A suitable extract for the fortification of the diets of infants can be made by adding the leaves to boiling water until no more can be immersed, boiling for 3 mins. longer and draining. This extract normally contains more than 150 mg of ascorbic acid per 100 ml and is fairly stable if stored in a sealed container, while the pungent taste of the fresh leaves is absent. J. A.

Vitamin C in Plants. Iris (*Iris germanica*). E. J. Baumann (*Nature*, 1944, **153**, 683)—The undried leaves of the common iris, *Iris germanica*, contain in the spring 0·6% of ascorbic acid, which rarely falls below 0·3% as the season advances. The leaves are heavy and can be cut with little injury to the root stalks, while the vitamin can be

separated from press juice much more easily than that from any other source used (*cf.* Baumann and Metzger, *Proc. Soc. Exp. Biol. Med.*, 1933, **30**, 1268). J. A.

Vitamin C in Plants. Indian Gooseberry (*Phyllanthus emblica*). M. Srinivasan (*Nature*, 1944, **153**, 684)—Attention is directed to the report by Damodaran and Srinivasan (*Curr. Sci.*, 1935, **3**, 953; *Proc. Indian Acad. Sci.*, 1935, **2B**, 377) that the Indian gooseberry, *Phyllanthus emblica*, contains 290–468 mg of vitamin C per 100 g, and it is stated that higher values have been reported by later workers, *viz.*, 540 mg per 100 g (*Arch. Neerl. Physiol.*, 1938, **23**, 433), 720 mg per 100 g of the fresh pulp (*Indian J. Med. Res.*, 1938, **26**, 165) and 921 mg per 100 ml of the juice (*Nature*, 1943, **152**, 596). Further, Damodaran and Srinivasan (*loc. cit.*) pointed out that the fruit has a mechanism capable of protecting the vitamin from oxidation, so that it remains largely intact even in the desiccated fruit. This source of ascorbic acid is being utilised for making edible preparations intended to meet in some measure the vitamin C requirements of the Indian troops, while the fruit has proved useful in treating cases of human scurvy. J. A.

Water

New Index for Determining the Amount of Calcium Carbonate Scale formed by a Water. J. W. Ryznar (*J. Amer. Water Works Assoc.*, 1944, **36**, 472–486)—The Langelier Index (*cf. J. Amer. Water Works Assoc.*, 1936, **28**, 1500) gives only a qualitative indication of the tendency of a water to dissolve or to deposit calcium carbonate scale when heated. The proposed "stability index" is a quantitative inverse measure of this tendency. It is given by $2pH_s - pH$. $pH_s = \log K_s/K_2 - \log (Ca^{++}) - \log (\text{total alkalinity}) + 9{\cdot}30 + 2{\cdot}5\sqrt{u}/(1 + 5{\cdot}3\sqrt{u} + 5{\cdot}5)$ (*cf.* Larson and Buswell, *Id.*, 1942, **34**, 1667). K_s is the activity product of calcium carbonate, K_2 is the second dissociation constant of carbonic acid, and u is the total ionic strength (= residue on trying at 105° C. in p.p.m. × 25 × 10^{-6}). The dotal alkalinity is calculated to p.p.m. as $CaCO_3$. To determine the amount of scale deposited from a water in the laboratory, the sample was passed through a weighed glass coil immersed in a constant temperature bath. The rate of flow and total vol. of water were kept constant. The results were reproducible to within 10%. Good correlations were obtained between the stability index and the quantity of scale deposited from both raw and treated waters (including some treated with polyphosphates), and also between the stability index and field observations. [*Note*—The Langelier Index is $pH - pH_s$. This last quantity is the pH which the water would have if it were just saturated with calcium carbonate. When the actual pH of a water is greater than the calculated value of pH_s, it will deposit scale on heating; when it is less, the water will dissolve any calcium carbonate scale with which it comes into contact until equilibrium is established—ABSTRACTOR.] D. D.

Agricultural

Chromatographic Determination of Carotene in Alfalfa. L. W. Charkey and H. S. Wilgus, Jr. (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 184–187)—Collect representative samples (*ca.* 1000 g) by cutting off within 1 in. of the ground small handfulls of alfalfa at random positions throughout the plot. Wrap the sample immediately in paper with enough cracked "dry ice" (solid CO_2) to freeze it quickly and maintain it in the frozen condition in an insulated double plywood box. On the same day run the frozen material with any remaining "dry ice" twice through a Russwin food chopper (not a meat grinder) and mix thoroughly by hand. Weigh all required 5-g samples immediately and directly into 125-ml Erlenmeyer flasks containing 25–30 ml of ethanol containing 0·5 g per litre of potassium hydroxide in excess of that required for neutralisation and, after shaking, leave the mixture to simmer on a hot plate under reflux for 10 min. Fill the cooled flasks completely with light petroleum (Skellysolve B), stopper tightly with rubber stoppers and store (even for 30 days) in a refrigerator at −1° C. Transfer the entire contents of each flask to a Waring Blendor by means of a stream of light petroleum, blend for 2 min., and filter in one operation into a separating funnel through closely woven cotton cloth. Wash and squeeze out the filter repeatedly with light petroleum. Discard the lower layer and wash the soln. rapidly as follows. Add 25–30 ml of 10% (w/v) potassium hydroxide in 80% ethanol, shake vigorously, discard the lower layer and repeat the operation; add *ca.* 100 ml of water, shake and discard the lower layer; shake with *ca.* 100 ml of *ca.* 4% sulphuric acid and discard the lower layer; without undue shaking wash the liquid twice with 200 ml of water; finally, immediately before chromatographing, draw off and discard the small amount of water that settles out. Pass the liquid through a drying tube containing anhydrous sodium sulphate placed above an adsorption tube, packed with 1 part by wt. of magnesia to 8 parts of soda ash, which in turn is connected with a graduated cylinder so fitted with a rubber stopper and tube that suction can be applied to the entire system. The drying tube and adsorption column should be wetted with light petroleum before introduction of the sample. As soon as all the liquid in the separating funnel has passed into the drying tube rinse the funnel with light petroleum, passing it also through the system, and continue the percolation with light petroleum containing 1% of ethanol until the upper pigment bands begin to move down; note the final vol. of percolate in the cylinder. Measure the carotene content of the percolate in a photometer previously standardised against pure β-carotene (an Aminco type-F photoelectric photometer with a No. 42 filter is suitable). From the data thus obtained calculate the carotene content of the alfalfa sample. Merck's technical soda ash and Micron Brand magnesia are recommended for the adsorption column, which should be supported on a cotton wool or glass wool plug and packed so that the surface is not broken when the adsorption tube is held horizontally. The adsorbent may be reclaimed for further use by placing it in porcelain crucibles in a cold muffle furnace, heating at low red heat for 1 or 2 hr. and allowing the material to cool in the furnace. A. O. J.

Organic

Furfural Solution Temperatures of Hydrocarbons. H. T. Rice and E. Lieber (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 107–110)—For the determination of miscibility soln. temps. of petroleum fractions furfural has the advantages over aniline of non-toxicity, stability under proper conditions of storage, and applicability to a wide range of petroleum fractions. The miscibility point is

easily determined even with dark-coloured petroleums. A furfural point is given by petroleums containing even more than 50% of aromatic hydrocarbons. The apparatus and procedure recommended are those described by Williams and Dean for determining aniline points (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 63), using equal vols. of furfural and petroleum. Furfural miscibility temps. below 30° C. are determined with the same apparatus, except that cooling with *iso*propyl alcohol and "dry ice" (solid CO_2) is used instead of heating; the "dry ice" is added slowly to a point slightly below miscibility and the bath is allowed to warm spontaneously to the miscibility point. The furfural was purified by the method of Adams and Vorhees ("*Organic Syntheses*," Vol. I, p. 49, New York, 1921) and within 1 hr. of distillation the miscibility point with the selected petroleum solvent was determined; this determination was repeated each day, and the sample was re-distilled and re-standardised when the variation was more than 0·5° C. Tables show the effect on the furfural point of the aromatic content of the petroleum and of the relative vol. ratio of furfural to oil. Comparative aniline and furfural points of 10 petroleum solvents, benzene and toluene are also tabulated. The average difference between the aniline and furfural miscibility temperatures was 32·1 to 32·3; for certain petroleum fractions the range of difference was much wider. E. M. P.

Determination of Total Phthalic Anhydride in Oil-modified Alkyd Resins. A. I. Goldberg (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 198–200)—The Kappelmeier method for determining phthalic anhydride in alkyd resins (*Farb.-Ztg.*, 1935, **40**, 1141; 1936, **41**, 161) involves hydrolysis of the resin with alcoholic potassium hydroxide, potassium phthalate with 1 mol. of alcohol of crystallisation being pptd. Drying of the alcoholate to constant wt. in a sulphuric acid vacuum desiccator is inconvenient for routine work, but alcohol can be quantitatively removed by heating at 150° C. for 3 or 4 hr., the residue being anhydrous potassium phthalate. The preliminary heating and prolonged refluxing of Kappelmeier's method are unnecessary with the easily hydrolysed alkyd resins, and direct saponification at 55° C. for 4 to 6 hr. is recommended. With resins that are saponified with difficulty it may be necessary to heat overnight. A study of the effect of known amounts of water upon pptn. of phthalic anhydride showed that an error of 1% is introduced in presence of 2% of water (in addition to *ca.* 0·5% introduced by commercially pure potassium hydroxide). Alcoholic potassium hydroxide prepared as follows yields the max. value for the phthalic anhydride content of the resin. For each determination add 4·5 g of potassium hydroxide to 125 ml of abs. alcohol, add 10 g of calcium oxide to remove 2·5% of water and heat the soln. under reflux for 20 min. Filter the warm soln. rapidly with suction, avoiding absorption of atmospheric carbon dioxide. A volumetric procedure was designed to include the advantages of Kappelmeier's method of isolating phthalic anhydride as the dipotassium salt. Hall and Werner (*J. Amer. Chem. Soc.*, 1928, **50**, 2367) have shown that perchloric acid is the most suitable of the common acids for titrimetric purposes in a glacial acetic acid medium. Removal of water by addition of acetic anhydride is necessary, excess of this reagent having no effect upon the titre. To prepare standard perchloric acid (*ca.* 0·1 *N*), add 9 ml of 60% perchloric acid, drop by drop, to 36 ml of acetic anhydride in a chilled glass-stoppered flask. Dilute the soln. with 720 ml of glacial acetic acid and 24 ml of acetic anhydride and leave the soln. for 2 weeks until reaction with water is complete. To standardise the soln., dissolve a known amount of sodium carbonate with gentle warming in a mixture of 20 ml of glacial acetic acid and 2 ml of acetic anhydride and heat to b.p. under a hood to promote reaction with water. Titrate the cold soln. with the perchloric acid soln., using as indicator 2 drops per 10 ml of a 0·2% soln. of methyl violet in glacial acetic acid. The results obtained by titrating dipotassium phthalate isolated by the modified Kappelmeier procedure were *ca.* 0·8% too high. Potassium perchlorate is quantitatively pptd. during the titration, and may be collected, dried and weighed. A. O. J.

Determination of Total Phthalic Anhydride in Modified Alkyd Resins. C. D. Doyle (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 200)—This modification of the Kappelmeier method is similar to that described in the preceding abstract. The tedious drying of the residual alcoholate of potassium phthalate is avoided by heating the crystals at 210° C. for 1 hr. to convert them into anhydrous dipotassium phthalate. The standard Kappelmeier procedure is followed but, instead of the filtration residues being dried over sulphuric acid, the crucibles containing the residues are transferred from the low-temp. oven to an oven at 210° C. for 1 hr. They are then cooled over Dehydrite and weighed. This modification is applicable also to resins containing maleic anhydride or fumaric acid as well as phthalic anhydride. A. O. J.

Determining Plasticiser Content of Cellulose Esters. B. S. Biggs and R. H. Erickson (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 93–94)—Plasticiser content is determined by the vacuum distillation of the plasticiser from the sample. Dry samples of 1 g or less are placed, with acetonyl-acetone as solvent, in weighing bottles on the floor of a special vacuum still maintained at 256° C. with boiling Dowtherm. The construction of the still is described. Heating is continued for 1½ hr.; the loss in weight is due to plasticiser content plus a slight decomposition of the cellulose ester. The latter is determined on a blank of unplasticised ester, an approx. normal amount of plasticiser being added to make the conditions comparable. The blank value is usually sufficiently small and uniform to enable a fixed value to be assumed for it. Plasticiser content is given within about 0·3%. The method has been used with success on cellulose acetate and acetate-butyrate containing as plasticisers diethyl phthalate, dibutyl phthalate, dibutyl sebacate and butyl stearate. E. M. P.

New Test for Estimating Small Amounts of Mechanical Modification in Wool Materials. C. S. Whewell and H. J. Woods (*J. Soc. Dyers and Col.*, 1944, **60**, 148–151)—The method is based on mild chlorination of the wool followed by staining with methylene blue and microscopical examination of the fibres, the final appearance of which will depend on the previous history of the wool and the time of chlorination. The following standardised method was finally adopted. Purify a sample of about 0·1 g of the wool by extraction with alcohol and ether, and immerse it for 15 min. at 20° C. in 100 ml of sodium hydrochlorite soln. containing 0·12% of chlorine and having *p*H 10, agitating the wool during the treatment. Remove, wash for

1 min. with 5 changes of 200 ml of tap water, transfer immediately to 100 ml of methylene blue soln. (0·4 g of microscopic stain per litre) for 5 min., wash in running water for 5 min., and dry between filter-paper. Cut the stained fibres into short lengths (about 1 mm), spread a sample of some thousands of fragments on a microscope slide, cover with liquid paraffin and examine at *ca.* × 200, using in the eyepiece a stop to limit the field of view to a band about 50μ wide and counting only fibres which lie across this band and make an angle of more than 45° with the length of the band; these conditions are necessary because various parts of the same fibre are often stained differently, making it impossible to grade the fibre. The fibres are graded into the following classes: (0) no sign of attack; (1) scale edges only attacked; stained area extending down the scale for not more than ½ of the max. scale length; (2) any signs of damage other than those in class (1), but not extending over the whole surface, *e.g.*, blotchiness; (3) complete staining of scales, but scale edges still well marked; (4) heavy general damage; scales not clearly visible. The test is highly sensitive, revealing the small amount of mechanical damage occurring during carding. The precision is increased because the judgment is based on the area which is stained rather than upon depth of staining, colour or shade. Agreement between different observers is reasonably good, and the results appear to be reproducible. The test can be used to evaluate the condition of a particular sample or to estimate the amount of damage due to some treatment or process. Some methods of treatment cause sufficient damage for the majority of the fibres to be in classes (3) and (4); the test should then be repeated, using modified conditions of chlorination and staining. Occasionally a fibre of untreated wool may be placed in class (4), since a few rare types of fibre appear to be stained by methylene blue without preliminary chlorination. E. M. P.

Determination of Lignin in Wood. Anon. (*Pulp and Paper Mag. Canada*, 1944, **45**, 312–313)—The following is T.A.P.P.I. Tentative Method, T 13-m 43. Weigh out accurately, in duplicate, *ca.* 1-g samples of wood in the form of sawdust, and determine their water contents by drying for 2 hr. and then 1-hr. periods until the wt. is constant. Weigh similar samples into medium- to fine-porosity fritted glass or alundum extraction crucibles, and extract for 4 hr. in a Soxhlet apparatus with 95% alcohol (unnecessary if catechol tannins are known to be absent, *e.g.*, with spruce, pine, fir, hemlock, poplar, birch, beech and maple). Then extract the residue with a mixture of 95% alcohol (1 vol.) and benzene (2 vols.) to remove resins, oils, fats and waxes (*cf.* ANALYST, 1943, **68**, 62), remove as much solvent as possible by suction, wash with 50 ml of alcohol by suction, and digest the residue with 400 ml of water at *ca.* 100° C. for 3 hr. (to remove any residual water-sol. substances). Filter, wash successively with 100 ml of hot water and 50 ml of alcohol (to facilitate removal of the residue from the crucible), and allow the residue to dry in air. Transfer the residue to a small beaker, and add slowly, with stirring, 15 ml of 72 ± 0·1% sulphuric acid at 12–15° C. Stir continuously for 1 min., and then at frequent intervals at 18–20° C. Wash the mixture into a litre flask, dilute with 560 ml of water, and boil for 4 hr. under reflux. Allow the mixture to settle, filter it in a crucible which has previously been dried at 100–105° C. and weighed in a stoppered weighing-bottle. Wash the residual lignin free from acid with 500 ml of hot water, dry the residue at 100-105° C., first for 2 hr., and then for 1-hr. periods until the wt. is constant. If a correction for ash is desired the residual lignin may be ashed in the crucible, provided that this is not made of fritted glass and that it was ignited until constant in wt. before the filtration. Report the result as % by wt. of the water-free unextracted wood, and state whether the wood was extracted with alcohol and if the result is corrected for the ash content of the lignin. J. G.

Stain Reaction of Unbleached and Bleached Groundwood Pulps. J. H. Graff (*Paper Trade J.*, 1944, **118**, Apr. 20, *T.A.P.P.I. Sect.*, 133)—Mechanical wood pulps which have been commercially bleached by the peroxide process cannot be distinguished from the original unbleached pulps by the Bright, pholoroglucinol, aniline sulphate or Herzberg stains, by the "W" stain or by Texchrome. The ultra-violet fluorescence was light "vinaceous-grey" and dark, drab purple to dark "vinaceous-grey," respectively, but this differentiation is too uncertain for use with mixtures, especially in presence of lightly-bleached mechanical wood. The Loften-Merritt stain may, however, be adapted to this determination as follows. Prepare the fibres on a microscope slide in the usual way, add the stain (a mixture of 10 vol. of 2% malachite green and 20 vol. of 1% magenta AB solns., and 3 vol. of 0·05 *N* hydrochloric acid), drain off the excess after 2 min., and rinse the fibres well with distilled water. Place 2 drops of water on the slide, put on the cover slip, and drain off excess of water. The colour reactions are:—unbleached mechanical wood pulp, deep blue-violet to pale violet; the peroxide bleached mechanical pulp, amethyst-violet to light violet. The standard deviation of analyses of mixtures of such pulps is consistent with that of accurate fibre analysis. Both the fluorescence effects in the unstained state, and the above stain reactions remain unchanged when 16 months have elapsed since bleaching. J. G.

Inorganic

Simultaneous Determination of Copper and Iodate, Bromate, or Permanganate. D. N. Hume and I. M. Kolthoff (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 103–104)—Citrate quantitatively reverses the pptn. reaction of cuprous iodide, with formation of a cupric complex. *Copper and iodate*—Treat the neutral soln. (20–50 ml; 0·5–3 mg-equiv.) with 2 ml of 6 *N* acetic acid, 6 g of potassium iodide and, after 6 min., 20 ml of 1·0 *M* sodium citrate soln. Swirl until the soln. becomes clear, dilute to 200 ml and titrate with 0·1 *N* thiosulphate; at the starch end-point the deep murky blue soln. sharply becomes light blue. For the copper determination add 12 ml of 6·0 *N* hydrochloric or sulphuric acid to the titrated liquid, set aside for 2 min., and again titrate with thiosulphate Addition of 3 g of solid potassium thiocyanate just before the end-point is recommended. *Copper and bromate*—Determine bromate like iodate, but using 6 *N* hydrochloric acid (2 ml per 30 ml of assay soln.) instead of acetic acid, and subtrating the amount added from that required in the copper determination. *Copper and permanganate*—In the permanganate assay the original soln. is acidified with 6 *N* sulphuric acid, with a corresponding reduction in the acid added in the copper determination. The permanganate results are low, the

copper results high, by 0·3 to 0·5%. It is, therefore, better to determine copper by the usual process after reducing the permanganate with ferrous salt and oxidising any excess of the latter by boiling with a little bromine water, interference of ferric salt being prevented by addition of fluoride. The sum of copper and permanganate is determined separately by the method of Swift and Lee (ANALYST, 1942, 67, 341). W. R. S.

Spot Test for Cadmium. F. Feigl and L. I. Miranda (*Ind. Eng. Chem., Anal. Ed.*, 1944, 16, 141–142)—Dissolve 0·25 g of α,α'-dipyridyl and 0·146 g of ferrous sulphate crystals in 50 ml of water, add 10 g of potassium iodide, shake well for 30 min. and filter. The filtered reagent is stable. Place a drop of weakly acid, neutral, or ammoniacal soln. on thick filter-paper, add a drop of reagent before the drop is absorbed; cadmium at once forms a red ppt., which forms a red fleck or ring on the paper. A blank test is necessary only for minute amounts of cadmium. The identification limit is 0·05μg. Most of the members of the hydrogen sulphide group, which give iodide ppts., interfere. The unknown soln. should therefore be treated with hydrochloric acid, which ppts. the silver group; the filtrate is made ammoniacal, filtered if necessary, and tested as described. In ammoniacal soln. copper and zinc are inert. W. R. S.

Colorimetric Determination of Iron. J. H. Yoe and A. L. Jones (*Ind. Eng. Chem., Anal. Ed.*, 1944, 16, 111–115)—Aqueous solns. of disodium 1, 2-dihydroxybenzene-3, 5-disulphonate react with ferric iron giving a red colour at *p*H 9–10 (sensitivity 1 : 200,000,000) and a blue colour at *p*H 3·5–4·5 (1 : 30,000,000). The coloured solns. obey Beer's law. Fluoride, phosphate, tartrate, citrate and oxalate do not interfere. The ferric complex is very soluble in water, and the reagent is colourless. The visual or spectrophotometric method may be used. The buffer solns. used are 68 g of sodium acetate crystals and 33·3 ml of hydrochloric acid (12 *N*) per litre for the blue, and 71·6 g of disodium phosphate crystals and 4 ml of *N* sodium hydroxide per litre for the red, compound. Only a few metals interfere: titanium gives an intense yellow, copper a greenish-yellow, molybdate and uranium a yellow colour. The amount of sample taken should contain not more than 1 mg of iron. Transfer the soln. to a 100-ml flask, pipette a 5-ml aliquot portion into a 50-ml Nessler tube, add 1 ml of reagent (2·7 g of the salt per litre), and dilute to the mark with the acid or alkaline buffer. Prepare a scale of standards under the same conditions. W. R. S.

Colorimetric Determination of Germanium. R. E. Kitson and M. G. Mellon (*Ind. Eng. Chem., Anal. Ed.*, 1944, 16, 128–130)—Germanomolybdic acid, $H_4[Ge(Mo_3O_{10})_4].xH_2O$, which has an intense yellow colour, may serve for colorimetric work. The authors' measurements were made with a General Electric spectrophotometer at 440μμ with 5-cm cells, under which conditions Beer's law is valid up to 30 p.p.m. Arsenate, silicate, and phosphate interfere. Transfer a suitable volume of germanate soln. to a 50-ml flask, add 15 ml of glacial acetic acid and dilute to 40 ml; add 5 ml of 2·5% ammonium molybdate soln., make up to volume, measure the transmission without delay, as the colour fades slowly (not more than 2% within 15 min.). Greater stability is secured by adding the molybdate reagent to the acetic acid and adding the mixture to the germanate soln. Picric acid, or solns. of potassium chromate or dichromate buffered to *p*H 9, are suitable permanent colour standards. A soln. of 4 mg of picric acid per litre ≡ 10 p.p.m. of germanium. If separated by distillation, germanium is pptd. as sulphide from 6 *N* sulphuric acid soln. Dissolve the washed sulphide ppt. in a minimum of re-distilled ammonia soln., receive the yellow soln. in a platinum dish, decolorise with hydrogen peroxide in slight excess, and destroy the excess by gentle boiling. Neutralise the cooled soln. with dil. sulphuric acid, dilute to a suitable vol, and treat an aliquot portion representing 1–3 mg of Ge as described. W. R. S.

Volumetric Determination of Zinc as Oxalate. P. J. Elving and J. C. Lamkin (*Ind. Eng. Chem., Anal. Ed.*, 1944, 16, 194–198)—Zinc is quantitatively pptd. as oxalate from solns. containing 70% of acetic acid, and can then be titrated with permanganate. The process is applicable to alloys and ores. *Brass*—Dissolve 0·5 g in 10 ml of nitric acid in a porcelain dish, evaporate to dryness and bake for a few min. Take up in 10 ml of strong nitric acid, dilute with boiling water to 50 ml, digest hot for 1 hr., filter into a 200-ml beaker and electrolyse for copper and lead, adding 1 drop of 0·1 *N* hydrochloric acid 10 min. after beginning. Add 3 ml of strong sulphuric acid after 30 min. and increase the current to 0·4–0·5 amp. Remove and wash the electrodes, ppt. any manganese with persulphate, neutralise the filtrate with ammonia, evaporate to 50 ml, transfer to a 100-ml graduated flask and dissolve any ppt. in a minimum of nitric acid. Dilute to 100 ml, neutralise 25 ml with ammonia, add 70 ml of glacial acetic acid and 5 ml of sat. ammonium oxalate soln., and digest on a water-bath for 1–1½ hr. The ppt. may be centrifuged or collected, either on paper or in a filtering crucible. Wash with 70% acetic acid. Dissolve the ppt. in warm 5% sulphuric acid and titrate with 0·025 *N* permanganate. *Bronze* is treated like brass, but the whole of the electrolyte is taken. *Zinc concentrates and ores*—Treat 0·2 g with 5 ml of nitric acid and heat on the water-bath. When the soln. is down to 3 ml add 7 ml of hydrochloric acid and evaporate to 2 ml. Add 4 ml of strong sulphuric acid and heat until dense white fumes are freely evolved. Cool, dilute to 25 ml, filter off silica, wash with hot water, and collect the filtrate in a 100-ml graduated flask. Make faintly alkaline to methyl red, and slightly acidify with sulphuric acid. Dilute to 100 ml and treat 25 ml as described above under *Brass*. Chlorides, which impede the pptn. of the oxalate, must not be present. Metals which form insoluble oxalates under the above conditions (nickel, lead, copper, manganese, calcium, magnesium) interfere. W. R. S.

Determination of Zinc in Cyanide Brass-plating Baths. A. S. Miceli and I. O. Larson (*Ind. Eng. Chem., Anal. Ed.*, 1944, 16, 165–166)—Centrifuge the soln. until clear, pipette 10 ml into a 180-ml beaker, add 10 ml of strong hydrochloric acid, drop by drop, and heat until the pptd. ferrocyanides dissolve. Cool, slowly add 7 ml of acid mixture (3 : 2 nitric-sulphuric) and heat until copious white fumes are given off. Dissolve the cold residue in water, add 1 ml of 1 : 1 nitric acid,

and electrolyse for copper. Add 3 drops of 5% ammonium persulphate soln. to oxidise iron, evaporate to 25 ml, cool, rinse down, and add 2 g of sodium pyrophosphate to form the ferric complex and 5 ml of strong ammonia. If the soln. does not become alkaline (phenolphthalein) add a little more ammonia, neutralise with sulphuric acid (1 : 1) and add 6 ml in excess. Vigorously agitate the soln. at 45° C. with a mechanical stirrer and add 3 drops of 1% diphenylbenzidine soln. in strong phosphoric acid and 3 drops of 0·2% potassium ferricyanide soln., which should produce a violet colour. Titrate with 0·025 *M* potassium ferrocyanide soln. containing 0·3 g of ferricyanide per litre. The soln. becomes blue, then light-blue, blue-green, yellow-green, and finally light violet. If less than 0·02 g of zinc are present, the addition of a known amount of standard zinc (chloride) soln. may be necessary. Make a few practice titrations with solns. of known zinc content. The operation takes *ca.* 1½ hr. Nickel interferes. W. R. S.

Determination of Zinc in Magnesium Alloys. L. G. Miller, A. J. Boyle and R. B. Neill (*Ind. Eng. Chem., Anal. Ed.*, 1944, 16, 256–257)—Dissolve 2 g in 75 ml of water and 25 ml of strong hydrochloric acid in a 400-ml beaker. If copper is present add *ca.* 3 g of test lead and boil for 5 min. Filter into a 600-ml beaker, wash 3 times with 10-ml portions of water, add 2 ml of 0·5% potassium ferricyanide soln., and, at *ca.* 45° C., a measured excess of reagent (11·2 g of potassium ferrocyanide and 0·2 g of sodium carbonate per litre) drop by drop while stirring vigorously. After 5 min. filter by suction through a pulp pad covered with a thin asbestos layer, wash twice with 15-ml portions of hydrochloric acid (1 : 10) and titrate the excess ferrocyanide with 0·025 *N* ceric sulphate. Internal indicator: 1·485 g of *o*-phenanthrolin dissolved in 100 ml of 0·025 *M* ferrous sulphate soln. The cerium soln. contains 22 g of ceric ammonium sulphate dihydrate and 28 ml of strong sulphuric acid in 1000 ml. If the alloy contains tin, add an excess of saturated mercuric chloride soln. before adding the ferricyanide, and titrate potentiometrically with the calomel half-cell and a platinum electrode. A routine assay may be carried out in 30 min. with an accuracy of 1% for high zinc to 6% for low zinc contents. W. R. S.

Physical Methods, Apparatus, etc.

Determination of the Wet Tensile Breaking Strength of Paper and Paperboard. Anon. (*Paper Trade J.*, 1944, 118, Apr. 27, *T.A.P.P.I. Sect.*, 143–144)—This revision of the T.A.P.P.I. Tentative Standard T 456 m–42 is of special use in evaluating food wrappings, paper towels, blueprint and photographic papers, etc. It is concerned with a standard method of wetting the test-strip; the tensile-strength is determined in the usual way. When the test-strip is easily handled in the wet state, immerse it in a shallow dish of water at 23 ± 2° C. for an appropriate time (*e.g.*, from 15 ± 3 sec. for paper towels, to 20 ± 2 min. for blueprint papers, *vide infra*); remove it, lay it flat and straight on a pad of blotting paper, cover it with a sheet of blotting paper, and roll a cylindrical brass weight (diam. *ca.* 40 mm; wt., 500 g) over it, taking *ca.* 1 sec. The suitable immersion time is determined by making the test with various immersion times, and finding the time after which there is no significant change in tensile strength (ultimate wet strength); use 5 strips cut in the cross-direction of the sheet for this preliminary test. The Finch attachment is used for papers which cannot easily be handled when wet (*e.g.*, tissues). It is a stirrup (length, 3 in.; width, *ca.* 1·5 in.) made of 2 metal straps, which supports horizontally a cylindrical metal rod (diam., 5 mm; length, just over 1 in.); between the straps is a small vessel (containing water) which can be moved vertically and locked in the upper position so that the horizontal rod is immersed in the liquid to a depth of at least 0·75 in. A metal tongue at the base of the stirrup enables it to be clamped in the lower jaw of an ordinary tensile strength tester. Fill the container almost full with the water, bring it to its lowest position, loop the dry test-strip under the dry horizontal rod, and clamp the 2 ends of the strip in the upper jaw of the tester. Raise the container, lock it in its upper position, and at the end of the immersion period make the test with the container still in the same position; halve the result to obtain the wet strength of a single strip. Report results for both directions of the sheet, in kg per mm of width, to the nearest 2% of the total reading, stating the max., min. and average values obtained, the immersion time, and the wet tensile strength as a percentage of the dry tensile strength. J. G.

Reviews

"AnalaR" Standards for Laboratory Chemicals. The British Drug Houses, Ltd., and Hopkin & Williams, Ltd. 3rd Edn. Pp. xvi + 230. London, 1944. Price 5s.

Analysts will welcome the publication of this new edition of "AnalaR" standards, for it is 10 years since the previous volume appeared. The companies concerned are to be congratulated on their ability to issue such a book in the fifth year of war, and such minor criticisms as may be offered should be tempered by this fact.

The main changes since the last edition are indicated in the preface; 11 substances have been added, 4 are displaced. Improvements have been effected in some of the tests, although many of these remain limit tests and their interpretation is open to subjective error.

The value of this book is by now well established, so that one tends to look only for minor faults and omissions. There is no lowering of the limit for alkali in calcium carbonate, although the new method of stating the maximum impurity might at first suggest it. The glass analyst would have wished for a lower limit for non-volatile matter in hydrofluoric acid

and a boron specification for calcium chloride (dried). Analysts will be inclined to look for improvements in those reagents in which they are specially interested, but there is another aspect of the matter to which perhaps the publishers may not have given so much thought. Although the book is essentially concerned with analytical reagents, research workers have come to look upon it as a compendium of chemicals of known purity that can be used for other purposes. Viewed from this angle the book has obvious omissions; for example, there is no strontium chemical listed and there are other "laboratory chemicals" not mentioned. To the reviewer this seems the more important as there is, apart from B.P., no established second grade by which the purity of these other chemicals may be judged. "AnalaR" has come to have a meaning of which the two firms concerned may be justly proud, but "Pure" is a term which has very little meaning, and for which it is suggested the chemical suppliers might find some new basis.

The book is well produced and every laboratory will wish to have a copy of the new edition.

R. C. CHIRNSIDE

THE PHARMACEUTICAL POCKET BOOK. 14th Edn. Editor: C. E. CORFIELD, B.Sc., F.R.I.C., Ph.C. Pp. x + 423. London: The Pharmaceutical Press. 1944. Price 10s.

The last edition of this work appeared in 1938 and, while the present volume is produced on the same plan, it has been considerably revised and extended and includes commentaries upon the contents of all the B.P. Addenda. While primarily for the pharmacist, there is much information of value to the analyst, and in this connection the section on Forensic Pharmacy is particularly worthy of mention, since it contains most useful summaries of the Pharmacy and Poisons Act, 1933; the Poisons Rules, 1935; the Pharmacy and Medicines Act, 1941; the Therapeutic Substances Act, 1925; the Dangerous Drugs Acts; the Sale of Food and Drugs Act, 1938, and much other legislation associated with the sale of medicinal substances. There is a valuable section of nearly 20 pages on Poisoning, in which methods for treatment in cases of emergency are described. Useful tabulated data include the solubilities in water, alcohol, chloroform, glycerin and oils of the officially recognised substances used in pharmacy; Alcoholic Strengths of B.P. Preparations; Doses of Official Medicaments; Materia Medica of Vegetable and Animal Origin indicating the geographical sources and pharmacopoeial requirements; and a Veterinary Posological Table.

In the section on Dispensing one notices the statement that keratin may be used for producing an enteric pill coating notwithstanding that during the last fifteen years investigators, too numerous to quote, have shown it to be unsatisfactory; again, although a process for preparing enteric gelatin capsules by dipping in dilute solution of formaldehyde is described, no warning to the effect that they must be used within a few days of manufacture is included (*cf.* Cooper, *Pharm. J.*, 1943, **150**, 101). In the handy summary of information concerning Food and Diet it is remarked with regard to vitamin E that "attempts are being made to devise spectroscopic or chemical tests," an observation that is doubtless true but ignores the useful colorimetric method originated by Emmerie and Engel (*Rec. trav. chim*, 1938, **57**, 1351; 1939, **58**, 283, 895).

In the introduction to a section on Biochemical Analysis it is suggested that the examination of body fluids and excreta "is work for which the pharmacist is peculiarly fitted by his training," and a somewhat superficial account of certain aspects of this extensive and highly specialised subject follows. It is true that the reader is referred to specified authoritative books for more detailed information, but it is regrettable that the necessarily brief descriptions of the methods employed in pathological chemistry which follow should be offered as working instructions for conducting such important and intricate procedures. Thus, although the microscopical examination of urinary sediments is discussed, no diagrams are presented, yet even the expert will occasionally need their help to assist in the identification of casts and cellular tissue of pathological origin. Again, brief instructions are given for conducting the difficult colloidal gold reaction of Lange on cerebrospinal fluid without specifying that one must not use "gold chloride" but gold sodium chloride, $AuCl_3.NaCl.2H_2O$. As an introductory survey calculated to stimulate the interest of the pharmaceutical student this section is admirable, but as a guide to actual practice it is inadequate.

However, these are but minor blemishes on an otherwise excellent work of reference which concludes with an exceptionally useful Dictionary of Synonyms and Trade Names, extending over 77 pages; herein one learns that Philosophers' Wool is, in fact, zinc oxide.

N. L. ALLPORT

THE CONSTITUENTS OF WHEAT AND WHEAT PRODUCTS. By C. H. BAILEY, Professor of Agricultural Biochemistry, University of Minnesota. Pp. 332. New York: Reinhold Publishing Corporation. London: Chapman & Hall. Price $6.50.

This book, so packed with facts and references to published literature, is issued as No. 96 of the American Chemical Society's Monographs. In 1925, Dr. Bailey wrote No. 26 of this series, entitled "The Chemistry of Wheat Flour." Dr. Bailey has now produced this second book after many years of preparation and cereal chemists the world over will be thankful to him for his labours.

The present volume has a definitely restricted scope and no attempt is made to deal with the processing of wheat and its products. The book is a description of the biochemistry of wheats and wheat products, leaving dynamic biochemistry for treatment in a later volume which will be awaited with particular interest, especially after the book under review. Dr. Bailey's present book contains sixteen chapters, which cover wheat, proteins, starch, lipids, minerals, etc., while the final one deals with the vitamins of wheat and wheat products. Each subject is treated historically and very full references are given, even though this often means describing work now known to have been of little value. In such a book it is easy to note failure to record work which might be regarded as of historical importance. On the other hand, even the most experienced workers in this field will be surprised to find references to interesting work with which they were probably only slightly acquainted. In passing, one omission may be noted—the failure to record in the starch chapter any reference to the recent work of Lampitt and his co-workers.

The book is obviously not intended for millers or bakers but for chemists and especially for research workers in this field. If it is permissible to venture any criticism of this valuable work, it is to regret that, after the recording of all investigations on any particular subject, the result of which is sometimes to leave the reader rather perplexed, there is insufficient summary and guidance from such a distinguished author.

As a survey of an intricate subject, this book is welcome and we can only record our appreciation of the valuable contribution which Dr. Bailey has again made to this subject.

D. W. KENT-JONES

INAUGURAL MEETING OF THE MICROCHEMICAL GROUP

IN view of the present uncertain conditions and of the extreme difficulties of travelling long distances, the Council has decided to postpone the inaugural meeting of the Microchemical Group until the day of the Ordinary Meeting of the Society in October. Members of the Group will receive notification of the meeting in due course.

PROPOSED FORMATION OF A GROUP DEALING WITH PHYSICAL METHODS OF ANALYSIS

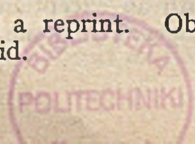
IN pursuance of the policy for the formation of Groups for special branches of analytical chemistry proposed by the Council and approved by a meeting of the Society on November 3rd, 1943, the Council has had under consideration a proposal to form a Group dealing with physical methods of analysis. The Group would deal with such methods as for example:

1. Spectrographic methods
   (*a*) Emission spectrograph; (*b*) U.V. and visible absorption spectrograph; (*c*) Infra-red absorption spectrograph; (*d*) Mass spectrograph.
2. Quantitative photometric methods by means other than spectrophotometry.
3. Polarographic methods.
4. X-Ray diffraction.

The Council's decision as to the formation of the Group will depend on the number of members of the Society desirous of joining it. Members of the Society who wish to become members of the Group are asked to notify the Hon. Secretary of the Society, 7/8, Idol Lane, London, E.C.3.

DETERMINATION OF FLUORINE IN FOODS

THE Report (pp. 243–246) will shortly be available as a reprint. Obtainable from the Editor. Price: Members 1/6; Non-Members 2/-; Prepaid.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