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Furfural and Diastase in Heated Honey.

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The presence of furfural and its derivatives in honey is generally stated in the literature to be indicative of the addition of commercial invert sugar to the natural product, but the claim is often made that a slight positive reaction (to the Fiehe test or to the furfuraldehyde test) may be obtained if the honey has been previously subjected to heat. Honey, in practice, is heated during the "vatting" process for mixing or blending, and it is therefore apparent that the whole question of the reaction of honey to the tests for furfural is of some importance.

The scheme of the work naturally falls under the following:—

(*a*) Critical examination of the tests used hitherto for the detection of furfural and its derivatives in honey, with the development of the technique used in this work.

(*b*) The literature referring to the interpretation of positive tests.

(*c*) A study of the action of heat on honey with reference to the production of furfural.

To this has been added a short note on the effect of heat on the diastatic activity of honey, a point which also appeared of some interest.

Tests for Hydroxy-methyl Furfural and for Furfuraldehyde in Honey.

Hydroxy-methyl Furfural.—(*a*) *Fiehe's Test.*—The test, as originally described by Fiehe,[1] was as follows:—Ten grms. of honey are rubbed for 5 minutes

with sufficient ether in a mortar; the ether, which is coloured a pale yellow, is decanted off and evaporated to dryness at room temperature. To the residue so obtained are added 2 drops of 1 per cent. resorcinol in fuming hydrochloric acid. The production of an immediate cherry-red colour (sometimes violet tinted) is a positive indication of furfural derivative.

This method of procedure does not result in satisfactory extraction, and, moreover, the colour immediately formed is not a reliable indication because it changes rapidly.

(*b*) *Caillas's Modification.*—Caillas[2] criticises the method and suggests the following modification:—Extract 5 grms. of the honey in a test-tube with 5 c.c. of ether by shaking for one or two minutes, decant off the ethereal extract, to this add 2 c.c. of a freshly prepared 1 per cent. solution of resorcinol in pure hydrochloric acid, noting the immediate colour produced in the acid portion and the colour after standing for 20 minutes.

Here again the method of extraction is unsatisfactory.

(*c*) *A.O.A.C.* (*Tentative*) *Method.*—The A.O.A.C.[3] specifies the following conditions:—Ten c.c. of a 50 per cent. solution of honey are extracted with 5 c.c. of ether; for the test a large drop of fresh 1 per cent. resorcinol in concentrated hydrochloric acid is added to 2 c.c. of the ethereal solution and shaken. It is directed that a cherry-red colour appearing immediately indicates commercial invert sugar, but that yellow or salmon shades have no significance.

Criticisms of this method are that only a small amount of honey is used and the size of the drop of reagent makes an appreciable difference in the results. We find that by this test it is not easy to detect 5 per cent. of commercial invert sugar added to a dark honey, though 10 per cent. can be detected fairly readily.

(*d*) *Roux and Muttelet's Modification.*—Further modifications described by Roux and Muttelet[4] are as follows:—Twenty grms. of honey are dissolved in 20 c.c. of cold water, and the solution extracted with 20 c.c. of ether, which is then decanted and allowed to evaporate at room temperature, a few drops of resorcinol reagent being added to the residue.

The objection here is that, in the case of dark-coloured honey, the yellow residue, containing wax from the ethereal extract, changes the colour given by the reagent.

(*e*) *Method adopted for this work.*—We have found that most satisfactory results and most sensitive reactions can be obtained by employing a modified Roux and Muttelet method, using 40 c.c. of ether for the extraction and taking up the residue after the evaporation of the ether in a small quantity of the solvent, in order to minimise the effect of the coloured residue, but at the same time to retain the furfural material in a concentrated solution. The following are the details of the method we apply:—

Dissolve 20 grms. of honey in 20 c.c. of cold water and extract with 40 c.c. of ether, with gentle mixing; decant off the ether and evaporate at room temperature; dissolve the residue in 10 c.c. of ether, using 2 c.c. of this for the Fiehe test

and reserving the remainder for the aniline acetate test to be described later. To this 2 c.c. of ethereal extract add 2 c.c. of 1 per cent. solution of resorcinol in concentrated hydrochloric acid. A positive test is indicated by the immediate appearance of a pink colour in the acid layer; this rapidly darkens, until after 20 minutes there is a deep cherry-red colour at the junction of the acid and the ethereal layers.

It should be noted that the resorcinol reagent must be prepared freshly, as required, because the colour of the reagent changes to pinkish on keeping for even a short time, and darkens on further standing. It is also important that, in the test, the colour produced should be noted at the 20 minutes' interval; if the colour appears after that period, it should be disregarded. Only a definite cherry-red colour is of significance, brownish colours being disregarded.

FURFURALDEHYDE.—(*a*) *A.O.A.C. (Tentative) Method* (not applicable to dark coloured honey).—For this test the A.O.A.C.[3] prescribes the following:—A reagent containing 100 c.c. of aniline and 30 c.c. of 25 per cent. hydrochloric acid (by weight) is used; 2·5 c.c. of this reagent are stirred directly into 5 grms. of honey. An orange to a dark red colour indicates the presence of commercial invert sugar, but yellow to salmon shades have no significance.

We have found it not easy by this method to detect 5 per cent. of added invert sugar, especially if the latter has a dark colour, although 10 per cent. gives a fairly definite result.

(*b*) *Leach Method (Browne's Test)*[5].—The reagent is made by suspending 5 c.c. of aniline in 5 c.c. of water and adding glacial acetic acid to clear the emulsion (about 2 c.c. required). From 1 to 2 c.c. of this fresh reagent is poured carefully down the side of a test-tube containing 5 c.c. of a 50 per cent. solution of the honey, so as to form a layer on the surface of the honey solution. If, when the tube is gently agitated, a red ring forms beneath the aniline solution, this colour becoming gradually imparted to the whole layer, artificial invert sugar is present.

This method is preferable to the A.O.A.C. method, as the effect of the colour of the honey is not so great, but the colours obtained are not very definite.

(*c*) *Method used in this work.*—We prefer to carry out the test using the well-known aniline acetate reaction as commonly employed in testing for furfuraldehyde (the significance of which had already been studied by us),[6] as follows:—

The ethereal solution put aside from the Fiehe test (*q.v.*) is evaporated at room temperature in a porcelain dish, and to the residue so obtained is added 2 c.c. of fresh aniline acetate solution made by dissolving 1 c.c. of redistilled aniline in 4 c.c. of glacial acetic acid. In positive tests a pink to orange colour appears within 15 minutes.

Both the Fiehe test and the aniline test as described will give distinct positive reactions with honey containing 5 per cent. of commercial invert sugar, strong colours in both cases being obtained when 10 per cent. of invert sugar is present.

Commercial invert sugar is considered to be present only when positive reactions are given by both the aniline acetate and Fiehe tests.

It is important that reagents (ether and acetic acid) should be free from furfural bodies, as ascertained by control tests.

LITERATURE REFERRING TO FURFURAL TESTS ON HEATED HONEY.—The following are the most important references to the interpretation of the results of furfural tests in connection with the effect of heat on honey:—

Leach[4] states that boiled honey will give a positive aniline test for furfural, but adds that such treatment impairs the flavour and is probably never practised. In the Fiehe test an immediate orange to rose colour disappearing quickly may be due to heated honey.

Woodman[7] considers that positive aniline chloride and Fiehe reactions are sometimes given by heated honey, but, usually in the case of honey heated to the temperature prevailing in commercial blending, the tests, if used with discrimination, will be found reliable.

Cox,[8] referring to the application of Fiehe's test to heated honey, states that it may give a momentary pink colour if the heating has been sufficiently prolonged.

Muttelet[9] found that even after honey had been heated at 105–110° C. for three hours, yielding a caramelised product very much unlike honey in appearance and flavour, only very faint colours were produced in Fiehe's test.

Caillas[10] claimed that the colour produced in the Fiehe test with honey containing 1 per cent. of added invert sugar could easily be distinguished from that given by heated honey. He found that heated honey gives no colour immediately, but on prolonged standing a cherry colour may be produced, though after 20 minutes the colour is very much lighter than would be obtained with 1 per cent. of invert sugar.

We have not been able to obtain such delicacy of reaction; probably this author used very light-coloured honey, and his invert sugar must have contained much furfural.

Voermans and Bakker[11] heated genuine honey for 6 hours in a boiling water-bath and also for 3 hours at 105° C., rendering the honey quite unpalatable, but the heated samples gave only transient and very slight rose colours in the Fiehe tests.

The A.O.A.C.[12] conducted some experiments on heated honey, but the results are not very consistent. The conclusion was that honey heated to 72° C. for 1 hour, 80° C. for ½ hour, and 98° C. for 20 minutes gave negative Fiehe and aniline chloride tests, but the results may not be reliable, as three of the collaborators obtained negative results even on a honey containing 20 per cent. added invert sugar.

THE EFFECT OF HEAT ON HONEY.—The general procedure adopted by us in these tests was as follows:—

Samples of honey were placed in glass vessels, in thermostats at temperatures between 60° C. and 100° C. for periods from ½ hour to 11 hours, with occasional stirring, small samples of about 30 grms. being withdrawn at intervals for examination.

The results of the application of the furfural tests to the various samples are shown in Table I (brown and white commercial honey) and Table II (English honey centrifuged from the comb). In all cases the unheated honey gave negative tests by both the aniline acetate and Fiehe tests.

TABLE I.

Temperature.	Time.	Aniline acetate test.	Fiehe test.
60° C.	Up to 1 hour.	—	—
65° C.	After 6 hours.	+	—
	After 8 hours.*	++	++
70° C.	After 5 hours.	++	—
	After 7 hours.*	++	+
80° C.	After 2 hours.	+	—
	After 4 hours.*	++	+
	After $5\frac{3}{4}$ hours.*	++	++
90° C.	After 1 hour.	+	—
	After $2\frac{1}{4}$ hours.*	++	++
100° C.	After $\frac{1}{2}$ hour.*	+	+
	After 1 hour.*	++	++

* Indicates that the honey had become partly caramelised, the colour being decidedly darker than originally and the flavour also affected.

TABLE II.

Temperature.	Time.	Aniline acetate test.	Fiehe test.
60° C.	Up to 12 hours.	—	—
70° C.	After 6 hours.	+	—
	After 9 hours.	+	— (Very slight colour.)
	After 12 hours.	++	— (Very slight colour.)
80° C.	After 2 hours.	+	— (Very slight colour.)
	After 4 hours.*	+	+
	After 6 hours.*	++	++
90° C.	After 1 hour.	+	—
	After 2 hours.	+	++
	After 3 hours.*	++	++
100° C.	After $\frac{1}{2}$ hour.*	+	—
	After 1 hour.*	++	++

* Indicates that the honey had become partly caramelised, the colour being decidedly darker than originally and the flavour also affected.

Reference to the tables will show that, in general, definitely positive results were only obtained in those cases where the colour and flavour had been affected and caramelisation had commenced. In other words, where the honey has been cooked sufficiently to give definitely positive furfural tests, it would be of little value from a commercial point of view on account of its flavour.

An explanation of the cause of the production of furfural in honey after prolonged heating may be found in the fact that the honey is slightly acid and that laevulose (but not dextrose) forms furfural compounds when treated with acids. The samples of honey used in these tests had an acidity equivalent to about 10–15 c.c. of $N/10$ caustic soda per 100 grms., corresponding to about 0·05 per cent. of formic acid. The P_H of 10 per cent. solutions ranged from 3·9 to 4·2. Proof of this explanation was obtained as follows:—

Samples of honey neutralised with caustic soda, and control samples of the same honey, were heated in a boiling water-bath for $1\frac{1}{2}$ hours. The control samples then gave strongly positive aniline acetate and Fiehe tests, but the neutralised samples gave no reaction (P_H of 10 per cent. solutions of control samples 4·0; of the neutralised honey 6·3).

The relative importance of laevulose and dextrose in this connection was also demonstrated. (In the following tests samples were first of all brought to a P_H of 4·0 for a 10 per cent. solution.)

(*a*) Eighty grms. of laevulose + 20 c.c. of water heated to 100° C. for 15 minutes gave positive aniline acetate and Fiehe tests.

(*b*) Seventy grms. of dextrose in 20 c.c. of water (this is a saturated solution) on heating at 100° C. for 1 hour gave negative aniline acetate and Fiehe tests, and on heating for a further hour, only a very slight pink colour was obtained in each test.

(*c*) A 74 per cent. (saturated) solution of equal amounts of laevulose and dextrose gave positive results in both tests after heating for 15 minutes at 100° C.

INTERPRETATION OF POSITIVE FURFURAL TESTS.—As a result of our work we are of the opinion that if any sample of honey gives positive aniline acetate and Fiehe tests, it is adulterated with commercial invert sugar, unless there is marked evidence that strong heating has occurred.

It may be of interest to note that a sample of honey containing sufficient added commercial invert sugar to give positive furfural tests may still possess a ratio of laevulose: dextrose high enough for it to be considered genuine according to the results of Auerbach and Bodlander.[13] These authors state that in genuine honey the ratio of laevulose: dextrose should not be less than 106: 100, and that for commercial invert sugar it is generally about 90: 100.

We would draw attention to the fact that there is on the market commercial invert sugar prepared by invertase in which the ratio is 99 or 100: 100 (this, of course, gives no furfural reaction).

We have determined this ratio on 310 samples of commercial honey of various origins and have obtained the following results:—

Fifty-two samples (17 per cent.) had a ratio of below 106: 100, 29 of them giving definite furfural tests; 94 samples (30 per cent.) had a ratio of from 106 to 110: 100, 8 of these giving positive furfural tests; 119 samples (38 per cent.)

had ratios between 110 and 120: 100, 5 of these giving positive furfural reactions; the remaining 46 samples (15 per cent.) had ratios between 120 and 169 to 100.

Samples of Californian honey extracted direct from the comb without heating had ratios of 107: 100, 115: 100, and 118: 100; two samples of English honey extracted direct from the comb had ratios of 114: 100 and 136: 100, respectively.

It is thus evident that the ratio of sugars in honey varies within wide limits, although it generally does not fall below 106: 100. It is therefore possible to add invert sugar to honey of high ratio and still obtain a product which has a ratio above the minimum limit; the above figures confirm this.

Auerbach and Bodlander[13] further found that the ratio of laevulose to dextrose in honey increased with the time of storage, but determinations we have carried out have failed to reveal any such change.

THE EFFECT OF STORAGE ON HEATED HONEY.—In the J.A.O.A.C.[12] it is stated that honey heated to 72° C. for 1 hour, 82° C. for 30 minutes, and 98° C. for 20 minutes gave negative furfural tests immediately after this treatment, but after keeping the treated samples at air temperature for 11 months, positive furfural tests were obtained in each case.

We have carried out similar tests, though so far samples have only been kept 8½ months, but they have not yet shown any development of furfural due to storing after heating. Details of some of the tests carried out in this connection are as follows:—

(*a*) Two samples of honey heated at 50° C. for 6 hours gave, immediately after heating, a slight positive aniline acetate test, but negative Fiehe test, and similar results were obtained after 8½ months' storage.

(*b*) Another honey gave negative tests by both methods after heating to 60° C. for 5 hours and to 70° C. for 4 hours, respectively, and also negative results in each case after the heated honey had been stored for 5 months.

(*c*) Three other samples of honey which initially gave negative tests were heated as follows:—

Sample No. 1 ..	60° C. for 4 hours.	70° C. for 3 hours.
	80° C. for 1½ hours.	90° C. for ¾ hour.
Sample No. 2 ..	80° C. for 2 hours.	90° C. for 1 hour.
Sample No. 3 ..	60° C. for 4 hours.	70° C. for 4 hours.

All these samples gave negative tests immediately after heating, except in case 3, where slightly positive aniline acetate tests were obtained. After 6 months' storage in each case the results were still negative. Further tests will be made after longer periods of storing.

EFFECT OF HEAT ON THE DIASTATIC ACTIVITY OF HONEY.

LITERATURE REFERRING TO THE ENZYME ACTIVITY OF HONEY.—Several investigators have studied honey qualitatively from the standpoint of the enzymes present, but the determination of the activity of the enzymes in heated honey

has received little attention. Gothe[14] reported the presence in honey of invertase, catalase, and diastase, and a few lesser known enzymes. He found that the invertase present had a maximum activity at 40° C., but was rendered inactive by heating the honey at 60° C. for one hour; that the catalase activity was strong in dirty honey, but that a low value did not necessarily mean a low grade or a heated honey; and that heating honey at 60° C. for one hour considerably reduced the catalase activity. From subsequent work on honey diastase[15] he concluded that a high diastatic power indicates a pure honey, and that in the case of a honey of low diastatic power Fiehe's test must be applied before condemning the honey as adulterated. The diastatic activity was lost by heating above 70° C., but such treatment caused loss of aroma, and a honey so treated was considered a denatured product.

Invertase in honey was studied by Nelson and Cohn,[16] who prepared an invertase solution from honey by alcohol precipitation followed by dialysis of an aqueous extract of the precipitate. The activity of this extract was determined on a sucrose solution.

Determination of the Diastatic Activity of Honey.—Two methods for the determination of the diastatic power of honey have been investigated by us, namely, Fiehe's modification of Gothe's method[17] and Ohlsson's malt extract method.[18]

Auzinger[19] studied diastase, catalase and peroxidase in various kinds of honey, but he carried out no quantitative work on heated honey. Qualitative tests showed that the diastase was destroyed at temperatures above 75° C.

The former method was used for several different samples of honey, and typical results obtained are given in Table III. Fiehe considered that honey

Table III.

Gothe Numbers of Honey Samples.

	Gothe number.
(1) Genuine honey	18·0
(2) Genuine honey	23·5
(3) Genuine honey	16·0
(4) Genuine honey	18·0
(5) Genuine honey	15·0
(6) Genuine honey	10·3
(7) Genuine honey	13·9
(8) Adulterated honey	13·0
(9) Containing 50 per cent. of added invert sugar	3·0

giving a number below 17·9 was to be regarded as suspicious, and below 10·9 as definitely adulterated. On this basis only samples 2 and 4 are satisfactory, yet only 8 and 9 gave positive furfural tests.

It appears, therefore, that Fiehe's standards require modification, but too much reliance cannot be placed on diastase tests, as heat considerably affects the diastatic power. This method was not further employed by us, as it did not prove suitable for the detection of small changes in diastatic power.

Ohlsson recognised the presence in malt extract of two enzymes: one he called "Saccharogen-amylase," which gives products in which reducing sugars (especially maltose) predominate, and the other "Dextrinogen-amylase," which gives predominantly dextrin when the malt extract is allowed to react with starch. The following method used by us for honey is a modification of that described by Ohlsson.

(*a*) *Dextrinogen-amylase.*—A series of tubes was prepared containing 1 c.c. of 0·2 per cent. starch solution, 8 c.c. of a phosphate buffer solution of P_H 5·6, and 1 c.c. of solutions of honey of various concentrations. The amounts of honey were arranged so that the quantity in one tube was 1·25 times that in the previous tube, convenient quantities being:—

10 per cent. solution, 1·0 c.c. 0·8 c.c. down to 0·26 c.c., made up to 1 c.c.
2 per cent. solution, — 0·85 c.c. down to 0·45 c.c., made up to 1 c.c.

(The P_H of the final mixture is about 5·7.)

The tubes were heated to 38° C. for 30 minutes for the diastatic action to take place, cooled in ice water, and 2 drops of $N/50$ iodine solution added. The colours of tubes then ranged from pale brown through purple to blue. The last pale brown and the first purple tubes, and the last purple and first blue tubes, were noted, the former colour boundary giving a value X for the diastatic power (for dextrin and reducing sugars), and the latter a value Y (for starch and dextrin).

The diastatic activity X or Y $= \frac{1}{2}\ (2/W + 2/W_1)$, where W and W_1 are the weights of honey in the two boundary tubes for each case.

Table IV gives the results on several samples of commercial honey.

TABLE IV.

	Dextrinogen-amylase.		Saccharogen-amylase.		
	X.	Y.	½ hour.	1 hour.	16 hours.
1. Californian honey direct from comb	30·8	39·2	1·0	1·5	33·9
2. English honey taken direct from comb	15·9	25·0	Nil	Nil	12·9
3. English honey	20·0	28·2	Nil	Nil	20·0
4. West Indian honey	36·4	48·8	3·15	7·2	36·3
5. West Indian honey	39·2	48·8	2·4	7·2	39·0
6. West Indian honey	39·2	54·7	2·6	6·6	30·9
7. Jamaica honey	44·0	60·6	—	—	—
8. Commercial blended honey, No. 1	39·2	48·8	4·7	5·2	28·4
9. Commercial blended honey, No. 2	25·0	35·2	1·6	2·5	27·3
10. Commercial blended honey, No. 3	40·0	54·7	2·6	2·6	30·7
11. Containing 50 per cent. invert sugar added	Nil	12·5	—	—	—

(*b*) *Saccharogen-amylase.*—Two 4 per cent. solutions of the honey were made, one with cold water, the other being boiled for about 15 minutes and serving as a control. Twenty-five c.c. of a 2 per cent. solution of soluble starch was placed in each of two flasks, 10 c.c. of a phosphate buffer (P_H 5·6) added to each and then warmed to 38° C. Ten c.c. of the honey solution were added to one flask, and the same quantity of the control honey solution added to the other flask, the mixtures heated at 38° C. for 30 minutes, also for 1 hour and for 16 hours. The flasks were cooled, the contents washed into 100 c.c. flasks, 10 c.c. alumina cream added, and the solutions made up to volume, filtered, and sugar determinations made by the Bertrand copper method, the results being expressed in equivalent of maltose. The diastatic power was taken as the number of mgrms. of maltose formed in the procedure as described above.

Ohlsson's method for malt extract specified 30 minutes' contact between the enzyme solution and the starch. With honey it was found that the amounts of maltose formed in that period were very small, and the reactions were therefore allowed to proceed overnight (16 hours). Table IV gives the values obtained with commercial honey.

It will be seen that where the honey has a high dextrinogen-amylase activity, the saccharogen-amylase activity is also high, and *vice versa*, and the orders of the activities are about the same in each case.

There is therefore definite evidence that honey diastase will act on starch, forming both dextrin and reducing sugars. The amounts of reducing sugars found for honey are very much lower than Ohlsson found for malt extract; in fact, they are of the order of those he obtained with pure dextrinogen-amylase solutions prepared from malt extracts, whence he concluded that dextrinogen-amylase can form small quantities of reducing sugars in addition to the dextrins. It is here suggested that a similar effect is obtained in honey and that saccharogen-amylase is not present, the sugars formed when honey reacts with starch being produced by the dextrinogen-amylase alone.

APPLICATION TO HEATED HONEY.—The method of heating was as described in the furfural section, in some cases both series of tests being carried out on the same sample of honey.

(*a*) *Dextrinogen-amylase.*—Some typical figures obtained on heated honey are given in Tables V and VI.

TABLE V.

Dextrinogen-amylase in Commercial Honey.

Temperature.	Time.	X.	Y.
Sample I.			
Original honey ..	—	44·0	68·8
90° C.	5 minutes	Nil	Nil
80° C.	10 minutes	25·0	35·2
	30 minutes	12·5	20·0

TABLE V.—*continued.*

Dextrinogen-amylase in Commercial Honey.

Temperature.	Time.	X.	Y.
Sample II.			
Original honey	—	44·0	60·6
75° C.	2½ hours	8·0	12·5
	4 hours	Nil	5·1
70° C.	1 hour	31·3	44·0
	4 hours	15·9	25·0
	8 hours	6·4	11·5
	12 hours	Nil	Nil
65° C.	4 hours	31·3	45·0
	8 hours	25·0	31·3
	16 hours	12·5	20·0
	24 hours	10·6	12·5
60° C.	4 hours	39·2	48·8
	12 hours	31·3	44·0
	24 hours	25·0	31·3

TABLE VI.

On English honey direct from comb.

Temperature.	Time.	X.	Y.
Original honey ..	—	20·0	28·2
100° C.	5 minutes	Nil	Nil
80° C.	10 minutes	18·0	22·5
	1 hour	10·0	15·9
	2 hours	5·8	10·0
75° C.	1 hour	14·2	25·0
	4 hours	10·0	12·5
	8 hours	5·1	8·0
70° C.	1 hour	25·0	31·0
	6 hours	15·9	25·0
	12 hours	9·0	12·5
	18 hours	6·4	8·0
65° C.	4 hours	18·0	25·0
	12 hours	12·5	15·9
	24 hours	8·0	10·0
60° C.	4 hours	20·0	25·0
	12 hours	15·9	25·0
	24 hours	14·2	20·0

Table VII gives the time required for the destruction of half of the diastase as obtained from graphs plotted from the figures given in the preceding two tables.

These results show that the diastatic activity of honey (Dextrinogen-amylase) is considerably affected by heating, and at temperatures above 70° C. it is destroyed fairly readily.

TABLE VII.

Temperature.	Commercial honey.		English honey.	
	Halving period.	Complete destruction.	Halving period.	Complete destruction.
85° C.	Less than 10 minutes	15–20 minutes	20 minutes	·1 hour
80° C.	12 minutes	80 minutes	1¼ hours	3 hours
75° C.	Less than 2½ hours	4½ hours	3½ hours	More than 8 hours
70° C.	3 hours	12 hours	11 hours	More than 18 hours
65° C.	8 hours	More than 24 hours	15 hours	More than 24 hours
60° C.	20 hours	More than 24 hours	More than 24 hours	More than 24 hours

(*b*) *Saccharogen-amylase.*—Table VIII gives the saccharogen-amylase activity of the heated commercial honey used in the previous section.

TABLE VIII.

Temperature.	Time.	1 hour.	16 hours.
Original honey ..	—	2·6	37·9
70° C.	4 hours	0·5	13·6
	8 hours	0·5	9·0
	12 hours	0·4	3·4
65° C.	3 hours	2·0	28·2
	8 hours	1·5	20·6
	16 hours	0·4	11·7
	20 hours	0·5	8·1
	24 hours	Nil	7·1
60° C.	4 hours	2·0	36·7
	8 hours	1·0	27·1
	12 hours	1·0	25·9
	16 hours	0·5	22·3
	20 hours	0·5	21·8
	24 hours	Nil	21·2

The loss in saccharogen-amylase activity proceeds at approximately the same rate as the loss in dextrinogen-amylase activity noted previously. This is to be expected if the sugars formed by the action of honey diastase on starch are due to the dextrinogen-amylase alone, as was suggested above.

GENERAL SUMMARY.—(1) The methods described in the literature for the detection of furfural and hydroxy-methyl furfural in honey have been critically reviewed, and a technique developed for carrying out tests for these compounds in a satisfactory manner.

(2) It has been shown that heated honey may give positive reactions in these tests, but when this occurs the honey has been over-heated, so that the colour and flavour have been adversely affected.

(3) It is concluded that the presence of furfural and hydroxyl-methyl furfural in honey indicates that it has been adulterated with commercial invert sugar, or that it has been over-heated.

(4) Storage of heated honey has been shown to produce no development of furfural in a period of about 8 months.

(5) A technique has been evolved for the study of the diastatic activity of honey.

(6) Heating of honey causes a considerable loss in its diastatic activity, as would be expected, but the full significance of this has not yet been worked out.

The above work has been carried out in the Laboratories of Messrs. J. Lyons & Co., Ltd., to whom our thanks are due for permission to publish this paper.

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DISCUSSION.

The PRESIDENT remarked that very often papers were given which had a large title, but dealt with a small point. This was an example of the contrary; it was a paper with a small title, but dealing fully with a complicated matter, starting with a test which was widely used and but little understood, and then dealing with the factors influencing and underlying the test. He congratulated the authors on their valuable investigation.

Dr. H. E. Cox said that he would like to congratulate the authors on the thoroughness of their investigation; he envied the opportunity they had of examining so large a number of samples (320), which enabled them to draw conclusions with more certainty than most analysts who had a comparatively small number of such samples. He asked why there appeared a constant slight increase in diastatic activity in certain honeys on heating; also what was the approximate quantity of furfuraldehyde shown by the tests; how many parts per million did "a slight reaction" imply? Were there any pentoses or pentosans present which could give rise to furfural in small quantity? One reason why Fiehe's test was so

popular was perhaps that it was included in the German official regulations regarding artificial honey. Many data on the effect of heat on the enzymes of honey were contained in a paper by Auzinger (*Z. Unters. Nahr. Genussm.*, 1910, **19**, 65, 353), and they agreed, to some extent, with the authors' observations. Was there any evidence from the manufacturing side that honey was ever heated?

Mr. NORMAN EVERS mentioned the statement made by the authors that commercial invert sugar had been prepared with invertase. Had the Fiehe reaction been applied to this product? He stated that honey was sometimes heated for pharmaceutical purposes in order to get a darker colour, as some users of medicinal products liked this darker colour.

Mr. RENDLE asked if he might answer Dr. Cox's query: So far as he was aware, honey for edible purposes was heated only to facilitate blending, and therefore to as low a temperature and for as short a time as possible. With regard to the variation of diastase, had the authors determined any of the conditions which might influence this—for example, acidity?

The PRESIDENT here raised a point with regard to the diastatic power and asked whether, if one found a normal diastatic power, did this show that the honey had not been heated sufficiently to bring about the Fiehe reaction?

Mr. ROOKE, replying, said that with regard to the President's query about the normal diastatic power and the Fiehe reaction, sufficient work on this subject had, as yet, not been done by the authors of this paper, but they hoped to do it later. He would like to point out that where they had obtained definitely positive furfural reactions by the aniline acetate and Fiehe tests the honey was pretty well caramelised and not suitable for commercial use. Replying to Dr. Cox's query regarding the apparent increase of the diastatic activity, he said that they had not gone sufficiently into the question to answer this. He had no data on the subject of the quantity of furfural present in heated honey, but amounts of furfural and methylhydroxy-furfural were extremely small; for instance, the aniline acetate test would detect one part in ten millions of furfural. He mentioned a German (Troje) who worked out methods for the determination of methylhydroxy-furfural; his results were found to be erroneous by Fiehe himself, but were of the order of a few parts per million. With regard to the presence of any other substances, such as pentoses, in honey which might give furfural on heating, he pointed out that the acidity of honey was extremely small—0·05 per cent. as formic acid; he had also shown that the P_H of 10 per cent. solutions of honey was 4, so that, even if pentoses were present, there was little likelihood of furfural being formed. Referring to the paper mentioned by Dr. Cox, he said that the literature on the subject was very contradictory and very difficult to follow up, because various authors had rather different criteria; for instance, Fiehe himself specified that the immediate colour must be noted; in the tests recorded in the present paper the authors had found that the immediate colours varied rather considerably and varied with the colour of the honey; thus, a dark-coloured honey might give a rather yellowish colour which might develop into a cherry-red, whereas a white honey might give an almost pure pink. With regard to the heating, honey was heated in some parts of the world before it was despatched to England in casks in what was known as the "vatting process," and heating might be as high as 70° C. Replying to Mr. Norman Evers's question regarding the presence of furfural in the invert sugar, he said that such invert sugar did not contain furfural, and with reference to the heating of honey for medicinal purposes, he had come across some very dark honeys which apparently were natural and,

he should imagine, quite suitable for the use mentioned. The authors had not yet gone into the effect of the acidity on the diastatic activity of the honey. He would like to make one other suggestion: apparently the diastase present in honey came from the flowers visited by the bees, and he thought it quite likely that flowers which grew in the tropics, for instance, might contain more diastase than those which grew in England.

Mr. HAIGH JOHNSON referred to the statement that the acidity was due to formic acid. He would like to know if this had been confirmed.

Mr. ROOKE replied that, personally, he had not made any tests on honey, but in the literature it was stated that the acidity was generally due to formic acid with, perhaps, some malic acid.

Some Analytical Applications of Sodium Hydrosulphite.*

(Antimony, Bismuth, Lead, Cadmium.)

BY B. S. EVANS, M.C., Ph.D., F.I.C.

SODIUM hydrosulphite ($Na_2S_2O_4$), though extensively used by organic chemists, appears to have found very little application in inorganic analytical chemistry, and in particular one very curious group of reactions seems to have been overlooked altogether. In 1903 J. Meyer published a review of the chemistry of "hyposulphurous" acid (Meyer, *Z. anorg. Chem.*, 1903, **34**, 43–61), in which he made the statement that sodium hydrosulphite reduces salts of copper, silver, mercury, bismuth and selenium to the metallic state. The significance of this observation, from the analytical standpoint, lies in the fact that, unlike most other reductions to the state of metal, it takes place in alkaline solution, and therefore is not interfered with by many oxidising agents which would be fatal in an acid medium, notably nitric acid which is such a convenient solvent for most metals. An investigation of the reaction showed that not only the metals mentioned by Meyer, but others, notably lead, arsenic and antimony, could be precipitated; that potassium cyanide, whilst absolutely preventing the precipitation of copper, improves that of antimony (in fact it appears to be necessary for its quantitative separation); and that, at any rate for lead, antimony and bismuth, the separation is rapid and complete, the metal being obtained in the form of a dense black powder.

* Communication from the Research Department, Woolwich.

Many attempts were made to apply the reaction to the determination of arsenic, but so far without success, as it seemed impossible to precipitate the arsenic completely, at any rate in the presence of a large excess of, say, copper cyanide. The behaviour of the reagent towards cadmium was peculiar; it was not to be expected that metallic cadmium would be precipitated, and yet the early attempts made to separate cadmium from lead, antimony and bismuth always gave low results. This was eventually traced to the slow formation of cadmium sulphide which precipitated along with the reduced metals.

The ease and clean nature of the separations and the fact that, taking place in alkaline solution, they are not affected by the acid radicles present, and that consequently certain acids, *e.g.* nitric acid, do not have to be eliminated, suggested the application of the reaction to various determinations which are either difficult or very tedious. The following processes were worked out:—

(A) Determination of Antimony in High-antimony Copper Alloys.

Certain acid-resisting commercial metals (*e.g.* "tant-copper") contain 4 or 5 per cent. of antimony alloyed with a large proportion of copper. In this case direct titration, such as might be carried out on a lead-antimony alloy, is entirely out of the question, and one is apt to fall back on a hydrogen sulphide separation, which is very cumbersome and tedious. The separation of antimony and copper by the alkaline sulphide method is not by any means complete in one precipitation, and it does not appear to be generally known that passage of hydrogen sulphide into an ammoniacal solution of antimony will actually cause precipitation of antimony sulphide which only very slowly re-dissolves as the liquid becomes saturated with the gas. The hydrosulphite separation of antimony from copper is clean, rapid and complete; details of the process are as follows:—

A weight of 1·0 grm., or a convenient amount, is dissolved in a mixture of 15 c.c. of hydrochloric acid, 5 c.c. of nitric acid, and 20 c.c. of water; 20 c.c. of a solution of 100 grms. of citric acid in 200 c.c. of water are added, and the liquid is made slightly alkaline with ammonia. A saturated solution of potassium cyanide is treated with bromine water until a drop removed gives no violet colour with a solution of sodium nitroprusside; the resulting solution is run into the ammoniacal solution of the sample until the blue colour is just discharged, and 20 c.c. in excess is added, followed by 50 c.c. of 20 per cent. ammonium chloride and 7 grms. of sodium hydrosulphite. The liquid is heated just to boiling point and allowed to stand on the steam-bath for 1 hour; about 2 grms. more of sodium hydrosulphite are then added, and the flask is placed in running water until completely cool.

The solution is next filtered through a pulp filter, and the precipitated antimony washed with a cold solution containing 20 c.c. of saturated potassium cyanide solution, 4 grms. of ammonium chloride, and 2 grms. of sodium hydrosulphite in 400 c.c.; filtration and washing should be carried out as quickly as possible to avoid any oxidation and re-solution of the finely divided antimony. It is desirable to heat the filtrate to boiling to make sure that no antimony has escaped precipitation; the solution should remain clear or contain only a cloud of

sulphur, and in this case it is rejected; should it, however, contain further antimony, the presence of this will be shown by the black precipitate; in these circumstances more hydrosulphite must be added, boiling continued for about a minute, and the liquid then allowed to stand, cooled, filtered and washed as before.

The filter or filters containing the antimony are transferred to a beaker and covered with a solution of bromine in dilute (1: 1) hydrochloric acid, the pulp is thoroughly broken up and stirred till all the antimony has dissolved, and the solution is then filtered off through a small pulp filter into a tall 800 c.c. beaker, and the pulp washed with dilute (1: 1) hydrochloric acid.

The antimony in the solution is determined by the method of Györy (*Z. anal. Chem.*, 1893, **32**, 415), carried out as follows:—The bromine colour of the solution is discharged with sulphurous acid, and 25 c.c. excess added; the liquid is then boiled down to about 10 c.c. with a cover on the beaker; it is diluted with a mixture of 10 c.c. of hydrochloric acid and 120 c.c. of water, which is used to rinse down the cover glass and sides of the beaker; it is again heated to boiling, 3 drops of methyl orange solution (0·1 per cent.) are added, and it is cautiously, drop by drop, titrated with standard potassium bromate solution until the colour is just discharged, the temperature not being allowed to fall below 80° C. If the amount of antimony is approximately known it is preferable to add the bromate solution to within about 3 c.c. of the required amount before adding the methyl orange; it is then again heated to boiling, and the titration cautiously finished, drop by drop.

The following results were obtained in a series of trials on electrolytic copper to which varying amounts of antimony had been added:—

Copper taken. Grm.	Antimony added. Grm.	Titration.		Antimony.	
		Theoretical.	Actual.	Added. Per Cent.	Found. Per Cent.
1·0	0·0898	29·35	29·25	8·16	8·14
1·0	0·0719	23·50	23·30	6·72	6·66
1·0	0·0539	17·60	17·65	5·13	5·14
1·0	0·0360	11·75	11·65	3·46	3·43
1·0	0·0180	5·90	5·90	1·76	1·76
1·0	0·0090	2·95	3·00	0·89	0·91

(B) Determination of Small Amounts of Bismuth in Tin-zinc Alloys.

The difficulty of this determination lies in the fact that, tin and zinc both being present, it is not possible to carry out an ordinary hydrogen sulphide separation without getting a bulky and unwashable precipitate of either tin or zinc sulphide, unless the acid content is so high as to interfere with the precipitation of the bismuth. On the other hand, precipitation of the bismuth as hydroxide also precipitates the tin, whilst precipitation of the tin as metastannic acid drags down the bismuth almost quantitatively. The following method proved to be rapid and accurate:—

A 5·0 grm. portion of the alloy is dissolved in 30 c.c. of hydrochloric acid, diluted with 20 c.c. of water, 5 c.c. of nitric acid being added after the first violent

action is over; 15 grms. of tartaric acid and about 5 c.c. $N/10$ arsenious oxide* are added, and the solution is made alkaline with ammonia. Five grms. of ammonium chloride are added, followed by 40 c.c. of saturated potassium cyanide solution which has been treated with bromine water in the manner described in the preceding method, and, finally, 7 grms. of sodium hydrosulphite.

The resulting solution is heated to boiling, allowed to stand on the steam-bath for 1 hour, about 2 grms. of sodium hydrosulphite are added, and the flask is cooled in running water until quite cold. The mixed bismuth and arsenic precipitate is filtered off through a pulp filter and washed with the solution described for washing the antimony precipitate in the preceding method; the filtrate and washings are discarded. Re-heating of the filtrate will always show a precipitate, but this is arsenic, and not bismuth, which is completely precipitated.

The filter is transferred to a beaker and covered with a solution of bromine in dilute (1: 1) hydrochloric acid, the pulp is thoroughly broken up with a glass rod and stirred until the precipitate is completely dissolved, after which the liquid is filtered through a small pulp filter into a beaker, and the pulp is washed 2 or 3 times with dilute (1: 1) hydrochloric acid and 2 or 3 times with water. The filtrate is boiled down, 20 c.c. of dilute (1: 3) sulphuric acid are added, and it is heated till all hydrochloric acid is driven off, after which a few drops of nitric acid are added to destroy the brown colour due to the charring of fibres from the filter, and it is again heated until the sulphuric acid fumes strongly. The beaker is allowed to cool, the acid liquid is diluted with 15 c.c. of water, boiled, cooled and rinsed into a Nessler glass and the bismuth determined colorimetrically by the bismuth iodide process.

To carry out this process, 10 c.c. of a saturated solution of sulphur dioxide diluted ten times are added, followed by 10 c.c. of 4 per cent. potassium iodide solution, and the liquid is made up to the mark with water; in the standard glass are placed 20 c.c. of dilute (1: 3) sulphuric acid and 10 c.c. each of the two reagents; standard bismuth sulphate solution (1 c.c. = 0·0001 grm. Bi.) is run into the standard glass until the colours match.

Tests of the process were made on mixtures of tin and zinc to which varying amounts of bismuth had been added. The following results were obtained:—

Tin taken. Grms.	Zinc taken. Grms.	Bismuth added. Grm.	No. of c.c. reqd. Total.	No. of c.c. reqd. Net.	Bismuth recovered. Grm.
3·0	2·0	Blank on Sn and Zn	2·0	—	—
3·0	2·0	0·0001	3·0	1·0	0·0001
3·0	2·0	0·0002	4·0	2·0	0·0002
3·0	2·0	0·0003	5·0	3·0	0·0003

(C) Determination of Small Amounts (0·01–0·20 per cent.) of Antimony in Lead and Lead Alloys.

High amounts of antimony alloyed with lead are easily determined by direct solution of a small sample in a solution of bromine in hydrochloric acid and

* Arsenic is added in the process to produce a filterable precipitate, as the amount of bismuth is so very small.

determination by Györy's volumetric potassium bromate method. Minute amounts (up to, say, 0·05 per cent.) may be determined by the method published by the author (ANALYST, 1927, **52**, 565), or, better, by this method finished by S. G. Clarke's colorimetric determination (ANALYST, 1928, **53**, 373). Between these two ranges, however, is a short gap which does not lend itself readily to either method of determination; on the one hand, the quantity (say 0·1 per cent.) is rather too large for accurate colour work; on the other, the bromate titration is not very accurate for small amounts (say under 0·007 grm., representing 0·14 per cent. on a 5 grm. sample), and one is precluded from using a larger sample to get a higher titration value by the impossibility of getting it to dissolve directly in bromine and hydrochloric acid. The method described by the author (ANALYST, 1927, **52**, 568), whilst working perfectly for the determination of tin, is inadmissible in the case of antimony, owing to the fact that antimony would undoubtedly be extracted from the red rubber stoppers, connections, etc. The following process was devised to cover the above-mentioned gap:—

A sample weight of 20 grms. (which need not be finely divided) is dissolved in 100 c.c. of dilute nitric acid (sp. gr. 1·2) to which 5 grms. of tartaric acid have been added. The crystallised lead nitrate is dissolved by the addition of hot water, the solution is heated to boiling, 80 c.c. of dilute (1: 3) sulphuric acid is added, and the liquid is cooled, filtered, and the precipitate washed with 2 per cent. sulphuric acid. The filtrate is made slightly alkaline with ammonia, 20 c.c. of a saturated solution of potassium cyanide which has been treated with bromine water, as described in the process for determining antimony in copper-antimony alloys, is added, followed by 7 grms. of sodium hydrosulphite, and the whole is heated to boiling, allowed to stand for 1 hour on the steam-bath, and finished as described in the process for determination of antimony in copper antimony alloys.

The following results were obtained with a sample of lead to which varying amounts of antimony had been added; some results are also shown for a sample of lead to which 2 per cent. of tin, in addition to the antimony, had been added:—

Lead taken. Grms.	Tin taken. Grm.	Antimony added. Grm.	No. of c.c. $KBrO_3$ soln.			Antimony.	
			Total.	Net.	Calc.	Added. Per Cent.	Recovered. Per Cent.
20·0	—	Blank on lead	0·70	—	—	—	—
20·0	—	0·0045	2·10	1·40	1·45	0·022	0·021
20·0	—	0·0090	3·40	2·70	2·90	0·045	0·041
20·0	—	0·0179	6·20	5·50	5·80	0·089	0·084
20·0	—	0·0268	9·10	8·40	8·70	0·134	0·129
20·0	0·40	Blank on lead and tin*	0·25	—	—	—	—
20·0	0·40	0·0092	3·15	2·90	3·00	0·046	0·044
20·0	0·40	0·0184	6·20	5·95	6·00	0·092	0·091

* A different sample of lead.

Subsequent to obtaining the above figures it was found that, contrary to expectations, cadmium exercised a retarding effect on the precipitation of antimony, being itself precipitated as sulphide; in the presence of cadmium, therefore, a slight modification of the process is required. The method is followed exactly up to the point where the potassium cyanide has been added, the solution is then heated to boiling, 7 grms. of sodium hydrosulphite are cautiously dropped in, boiling is continued for ten minutes, followed by a ten minutes' stand on the steam-bath; from this point on, the original process is followed. The method was tested on 5 grm. samples of lead, to each of which 0·04545 grm. of antimony and varying amounts of cadmium had been added, with the following results:—

Lead taken. Grms.	Antimony added. Grm.	Cadmium added. Grm.	Titration.		Antimony.	
			Actual c.c.	Calculated c.c.	Added. Per Cent.	Found. Per Cent.
5·0	0·04545	0·010	15·15	15·15	0·909	0·909
5·0	0·04545	0·020	15·20	15·15	0·909	0·912
5·0	0·04545	0·030	15·15	15·15	0·909	0·909
5·0	0·04545	0·040	15·10	15·15	0·909	0·906

(D) Separation of Cadmium from Bismuth and Lead.

It seemed likely that hydrosulphite precipitation would prove a ready method of separating cadmium from bismuth, antimony, and the traces of lead that are so much trouble to eliminate in the determination of cadmium in lead and lead-base alloys. As stated above, the separation of antimony from cadmium by the methods given so far is quite out of the question; in the case of lead and bismuth, trials showed that approximate, but low, results were obtained.

Quantities of 5 grms. of lead, 0·05 grm. of bismuth, and 0·1 grm. of antimony were taken, and varying amounts of cadmium were added. The samples were dissolved in nitric acid (sp. gr. 1·2), the bulk of the lead thrown out as sulphate, citric acid added, the antimony removed by the sodium sulphide method and the precipitated sulphides of lead, bismuth and cadmium, after washing with 5 per cent. potassium nitrate solution, dissolved in hot dilute *aqua regia*. The solution was made alkaline with ammonia, 1 or 2 grms. of potassium cyanide (A.R) and 2 grms. of sodium hydrosulphite added, and the liquid heated to boiling, allowed to stand for 10 minutes on the steam-bath, cooled, filtered, and the precipitate washed with the solution described for washing the antimony precipitate in method (A) of this paper. Hydrogen sulphide was passed into the filtrate, and it was allowed to stand on the steam-bath for ½ hour (a clear yellow precipitate was obtained in three out of the four determinations); it was then filtered, washed with 2 per cent. ammonium nitrate solution, dissolved in hot dilute *aqua regia*, evaporated with addition of 5 c.c. of dilute (1 : 3) sulphuric acid until it fumes, transferred to a weighed platinum dish, evaporated, ignited at a temperature of

about 500° C., and weighed. These experiments gave the following results:—

	Lead taken. Grms.	Bismuth taken. Grm.	Antimony taken. Grm.	Cadmium added. Grm.	Cadmium found. Grm.	Weight of $CdSO_4$ obtained. Grm.
(*a*)	5·0	0·05	0·10	0·0100	0·0082	0·0168
(*b*)	5·0	0·05	0·10	0·0200	0·0181	0·0352
(*c*)	5·0	0·05	0·10	0·0300	0·0308	0·0588
(*d*)	5·0	0·05	0·10	0·0400	0·0370	0·0702

Experiment (*c*) was the one which gave a dark-coloured sulphide, which explains the higher result. This may have been due to incomplete separation of the antimony in the preliminary treatment, as the antimony determined on the sulphide filtrate gave a lower result than in the other experiments. It was noted, however, in trials made on cadmium alone that no precipitation of cadmium sulphide took place until the solution was actually, or at any rate nearly, boiling; on the other hand, lead and bismuth both precipitate at temperatures far below boiling point, and the two precipitates being totally unlike in character, on the one hand a direct precipitation of colloidal sulphide (owing, presumably, to the formation of sulphide in the solution), on the other, a reduction to, probably crystalline, metal, there seemed little likelihood of an adsorptive dragging-down of the cadmium, such as would almost certainly have taken place if the two reactions had been similar.

It seemed worth while, therefore, to try and effect a separation at, say, 60° C. Quantities of 0·05 grm. of lead,* 0·05 grm. of bismuth, and varying amounts of cadmium were brought into solution with nitric acid, 10 c.c. of citric acid solution (100 grms. of citric acid to 200 c.c. water) were added, and the solutions made alkaline with ammonia; about 1 grm. of potassium cyanide (free from sulphide) was added to each, followed by 7 grms. of sodium hydrosulphite. A thermometer was placed in the solution, which was heated on the steam-bath to a temperature of 60° C.; the flask was then removed and allowed to stand on the bench for $\frac{1}{4}$ to $\frac{1}{2}$ hour; it was then filtered through pulp, and the precipitate washed with the washing liquid described above. Hydrogen sulphide was passed through the filtrate, which was then heated to boiling and allowed to stand on the steam-bath until the cadmium sulphide had separated sufficiently to allow of filtration; it was then filtered. The precipitate was thoroughly washed with 2 per cent. ammonium nitrate solution and burnt off in a weighed porcelain crucible at a low heat.

After cooling, the residue in the crucible was treated with 9 drops of dilute (1 : 3) sulphuric acid and 2 drops of concentrated nitric acid, the liquid was evaporated off, and the crucible very gently ignited at a temperature of about 500° C. and weighed; a blank determination was made by washing a pulp filter with 2 per cent. ammonium nitrate solution, burning off in a weighed platinum dish, treating with 9 drops of dilute (1 : 3) sulphuric and 2 drops of nitric acid, and finishing as described for the experiment; the weight of this blank was deducted

* This quantity was taken as being considerably more than the amount left in solution after an ordinary sulphuric acid precipitation of lead.

from the weight of crude cadmium sulphate found, and the remainder calculated to cadmium. The following results were obtained:—

Lead taken. Grm.	Bismuth taken. Grm.	Cadmium added. Grm.	Cadmium found. Grm.	Corrected weight of cadmium sulphate. Grm.
0·05	0·05	0·0100	0·0101	0·0188
0·05	0·05	0·0100	0·0101	0·0188
0·05	0·05	0·0200	0·0197	0·0366
0·05	0·05	0·0300	0·0301	0·0558
0·05	0·05	0·0400	0·0399	0·0740

To prove that the substance obtained was really cadmium sulphate, and not merely a low amount of cadmium sulphate balanced by some adsorbed alkali salt, the residues in the crucibles were dissolved in dilute (100 c.c. of water : 7 c.c. of 1 : 3 sulphuric acid) sulphuric acid, and the solutions saturated with hydrogen sulphide; after standing overnight the cadmium sulphide (which again was pure yellow) was dissolved in hot dilute *aqua regia* and finished as described by A. T. Etheridge (ANALYST, 1924, **49**, 575). The results obtained were as follows:—

Weight of cadmium taken. Grm.	Original weight of of cadmium found. Grm.	Weight of cadmium found after acid H_2S precipitation. Grm.
0·0100	0·0101	0·0099
0·0200	0·0197	0·0195
0·0300	0·0301	0·0299
0·0400	0·0399	0·0393*

* In this experiment a minute fragment of cadmium sulphide was lost.

These experiments show conclusively that the loss of weight after acid hydrogen sulphide is within the limits of experimental error; also, that an alkaline sulphide precipitate treated in the way described gives substantially correct results, and that lead and bismuth can be separated completely from cadmium by means of sodium hydrosulphite.

SEPARATION FROM ANTIMONY.—There still remained the problem of separation from antimony, which forms a constituent of a large proportion of the lead cadmium alloys. The sodium sulphide separation, by adding another operation to be performed, increases considerably the length of time taken in the determination of the cadmium. As stated above, it is impossible to separate antimony and cadmium by boiling with hydrosulphite, and a pure antimony solution does not begin to precipitate until just below the boiling point. It will be observed, however, that we are here dealing with a mixture which is the converse of the bismuth and cadmium mixture referred to above; in that case the cadmium sulphide precipitate was of a radically different character from the metallic precipitate of bismuth, and therefore the cadmium did not show any tendency to co-precipitate with the latter at 60°; in this case, antimony, lead and bismuth all form metallic precipitates, only distinguished by the temperatures at which they come down,

and one would expect considerable co-precipitation of antimony with either lead or bismuth at 60°.

It seemed worth while to attempt a separation on these lines. Solutions containing 0·05 grm. of lead, 0·10 grm. antimony, and varying amounts of cadmium were taken; 0·2 grm. bismuth in the form of a solution of the nitrate was added to each (this with a view to providing sufficient precipitate to drag down the antimony) followed by 10 c.c. of citric acid solution (50 grms. of citric acid : 100 c.c. water); the solutions were made alkaline with ammonia and 20 c.c. of dilute (1: 1) ammonia added in excess, 10 c.c. of saturated solution of potassium cyanide treated with bromine water, as already described, were added, and, finally, 7 grms. of sodium hydrosulphite. A thermometer was placed in the liquid and it was heated to 60° C. on the steam-bath, then allowed to stand for 15 minutes, filtered through pulp and washed as described above. Hydrogen sulphide was passed through the filtrate for several minutes, and it was heated to boiling, allowed to stand for 1 hour on the steam-bath, filtered through pulp, and the precipitate washed with 2 per cent. ammonium nitrate solution. The precipitate, which in every case was of a clear golden yellow, was burnt off in a weighed porcelain crucible and finished as described under the separation of cadmium from lead and bismuth.

The following results were obtained:—

Lead taken. Grm.	Antimony taken. Grm.	Bismuth taken. Grm.	Cadmium added. Grm.	Cadmium found. Grm.	Cadmium sulphate obtained. Grm.
0·05	0·10	0·20	0·0100	0·0102	0·0190
0·05	0·10	0·20	0·0200	0·0196	0·0360
0·05	0·10	0·20	0·0300	0·0300	0·0556
0·05	0·10	0·20	0·0400	0·0395	0·0732

The above figures show that by this process cadmium can be determined in the presence of antimony, as well as of lead and bismuth. For all that, however, it must not be supposed that co-precipitation with lead or bismuth by hydrosulphite removes all antimony from solution; on the contrary, a large number of experiments have shown that a small, but appreciable, amount of antimony remains in the solution; what the hydrosulphite has done, in addition to completely removing lead and bismuth, is to bring down the antimony concentration so far that it does not precipitate in the ammonium sulphide liquid in which the cadmium is precipitated. If, however, after the cadmium has been thrown down the liquid is boiled (or even allowed to stand, hot, for any length of time), the remaining antimony precipitates as sulphide, as is shown by the darkening of the precipitate. Addition of ammonium sulphide restores the yellow colour to the precipitate, and it is a wise precaution to add it, whatever the colour, subsequent to heating to the boiling point and to allow the flask to stand on the bench and not on the steam-bath for the cadmium sulphide to settle out.

In applying the process finally to the determination of cadmium in lead base alloys an unexpected difficulty was encountered; losses persistently occurred which

were at last traced back to the sulphates derived from the sulphuric acid used in precipitating the lead. Apparently some difficultly soluble compound of cadmium is formed which is filtered off with the precipitated metals. It was found, however, quite easy, and, in fact, considerably simpler and quicker to throw down the whole of the lead with hydrosulphite, thus avoiding the use of sulphuric acid at all. The quantity of hydrosulphite required for 5 grms. of lead is only 10 grms., and the lead is completely and cleanly removed along with the bismuth and most of the antimony in one operation taking, perhaps, 20 minutes.

PROCESS OF DETERMINATION.—The complete process worked out for the determination of cadmium in lead antimony (bismuth if present) alloys is as follows:—

A sample weight of 5 grms. of the alloy is dissolved in 50 c.c. of citric acid solution (50 grms. of citric acid : 100 c.c. of water) and 50 c.c. of dilute nitric acid (sp. gr. 1·2); the solution is neutralised with ammonia, and 20 c.c. of dilute (1 : 1) ammonia added in excess, followed by 10 c.c. of saturated potassium cyanide which has been treated with a few drops of bromine water. The solution is cooled, 10 grms. of sodium hydrosulphite dropped in, and the liquid gently swirled, care being taken not to let the liquid mount high up the walls of the flask, because the white precipitate first formed adheres to the glass, and, though reduced and detached when the solution gets hot, is inaccessible in the upper parts of the flask. A thermometer is inserted in the liquid, which is then heated to 60° C. on the steam-bath, being shaken gently when the precipitate begins to blacken and fairly vigorously as the temperature gets higher. When the temperature reaches 60° the flask is removed from the bath, cooled, the liquid filtered through pulp, and the precipitate washed* with the solution described earlier for washing the antimony in section (A) of this paper.

To the filtrate are added 10 c.c. of ammonium sulphide solution (made by saturating dilute (1 : 1) ammonia with hydrogen sulphide) the liquid is heated just to boiling, removed from the plate, 10 c.c. more of ammonium sulphide solution are added, and the flask is allowed to stand on the bench for half an hour. As an alternative to the use of ammonium sulphide, hydrogen sulphide can be passed through the filtrate in a rapid stream for 10 minutes for the initial precipitation, and, after heating, 20 c.c. of dilute (1 : 1) ammonia are added, and a current of hydrogen sulphide again introduced.

The precipitate, which should be of a pure golden yellow colour, is filtered off through pulp, well washed with 2 per cent. ammonium nitrate solution, burnt off at a low temperature in a weighed porcelain crucible, cooled, treated with 9 drops dilute of (1 : 3) sulphuric acid and 2 drops of nitric acid, evaporated to dryness, gently ignited at a temperature just below red heat, cooled, and weighed. A small blank, due to the filter ash and any residue in the sulphuric and nitric acids, must be deducted from the weight of cadmium sulphate. The entire process, from start to finish, takes about five hours.

* This is best done by decantation.

The following results were obtained with samples which had already been analysed by the ordinary process:—

Lead (by diff.) Per Cent.	Antimony. Per Cent.	Copper. Per Cent.	Cadmium by old process. Per Cent.	Cadmium by hydrosulphite process. Per Cent.
97·42	1·77	—	0·81	0·82 0·82
97·99	0·50	—	1·51	1·50
98·22	0·47	—	1·31	1·32
99·16	0·50	0·11	0·23	0·22
97·04	2·00	—	0·96	0·94

If tin is present, it must be removed before the hydrosulphite treatment; this is due to the fact that tin prevents the precipitation of cadmium by ammonium sulphide. This curious reaction, which appears to have escaped notice, is being investigated further. The tin is easily removed as metastannic acid, which does not appear to adsorb cadmium; the sample is dissolved in nitric acid without the addition of citric acid, the solution is evaporated to dryness, the residue taken up with dilute nitric acid, filtered, and the residue washed with dilute nitric acid. To the filtrate are added 50 c.c. of the citric acid solution, and the liquid is made alkaline with ammonia, potassium cyanide and sodium hydrosulphite added, and the remainder of the process carried through as already described.

It would seem probable that almost all the other commoner metals could be separated from cadmium by this process. Needless to say, zinc would not be separated and would require the usual acid sulphide separation. Large amounts of sulphates must not be present.

The Volumetric Determination of Mercury.

By H. B. DUNNICLIFF, M.A., Sc.D., F.I.C., and H. D. SURI, M.Sc.

(*Read at the Meeting, December*, 1928.)

Numerous methods have been proposed for the volumetric determination of mercury.

F. M. Litterscheid (*Arch. Pharm.*, 1903, **241**, 306) precipitated mercury from mercuric chloride solution by a standard solution of potassium dichromate and ammonia and determined the excess of dichromate in the filtrate by an iodimetric method.

L. W. Andrews (*Amer. Chem. J.*, 1903, **30**, 187) analysed mercuric chloride by adding excess of a neutral solution of hydrocyanic acid and titrating the liberated hydrochloric acid against a standard alkali.

E. Rupp (i) (*Arch. Pharm.*, 1903, **241**, 328) determined the mercury in mercuric cyanide by an iodimetric method; and (ii) (*ibid.*, 1905, **243**, 300) reduced a mercury salt to metallic mercury with alkaline formaldehyde. The mercury was dissolved in excess of $N/10$ iodine solution, and the excess of the iodine was determined by thiosulphate.

E. Ebler (*Z. anorg. Chem.*, 1905, **47**, 377) reduced the mercuric salt with excess of $N/40$ hydrazine sulphate, and determined the excess of hydrazine with standard iodine.

P. W. Robertson (*Chem. News*, 1907, **95**, 253) precipitated mercury as $ZnHg(CNS)_4$ by standard ammonium thiocyanate and excess of zinc sulphate, and determined the excess of thiocyanate in the filtrate.

H. Morawitz (*Z. anorg. Chem.*, 1908, **60**, 456) titrated mercuric chloride against $N/10$ potassium cyanide, with *p*-nitrophenol as indicator.

J. Knox (*J. Chem. Soc.*, 1909, T., **95**, 1768) found that the results obtained by Rupp and Kraus's method for the determination of mercury by titrating against ammonium thiocyanate (*Ber.*, 1902, **35**, 2015) were low. This he ascribed to contamination of mercuric nitrate by mercurous nitrate.

E. Rupp and F. Lehmann (*Chem. Ztg.*, 1910, **34**, 229) determined mercury in the presence of silver by treating a suitable quantity of the nitric acid solution with an alkaline solution of potassium iodide, making up to a definite volume and filtering. The mercury in the filtrate was determined by E. Rupp's formaldehyde method (*loc. cit.*, ii).

F. Reinthaler (*Chem. Ztg.*, 1911, **35**, 593) reduced mercuric chloride or nitrate with excess of standard $N/10$ arsenious acid. The excess of arsenious acid was then determined iodimetrically.

G. S. Jamieson (*Amer. J. Sci.*, 1912, [iv], **33**, 349) determined calomel by means of standard potassium iodate.

J. E. Clennell (*Eng. and Min. J.*, 1914, 787) precipitated mercury as hydroxide, dissolved the precipitate in standard potassium cyanide solution, and titrated the excess of cyanide against silver nitrate, using potassium iodide as indicator.

W. Böttger and R. Heinze (*Z. Elekt. Chem.*, 1916, **22**, 69) suggested two methods for determining mercury in small quantities, depending on the precipitation of mercury as diphenylcarbazide.

G. Adanti (*Boll. Chim. farm.*, 1916, **55**, 553) reduced mercury salts to metallic mercury by formaldehyde in the presence of potassium hydroxide (*cf.* E. Rupp, *loc. cit.* [ii]).

A. Tagliavini (*Boll. Chim. farm.*, 1917, **56**, 297) analysed solutions of mercuric cyanide and cyanate by adding sodium chloride and methyl orange and titrating against $N/10$ hydrochloric acid.

E. Votoček (*Chem. Ztg.*, 1918, **42**, 271) suggested that his method (*ibid.*, p. 257) for the determination of chlorides, bromides and cyanides by titrating with standard mercuric nitrate, with sodium nitro-prusside as indicator, might, conversely, be applied to the determination of mercury.

G. Jamieson (*J. Ind. Eng. Chem.*, 1919, **11**, 296) suggested a modification of Robertson's method (*loc. cit.*).

G. Hinard (*Amer. Chem. anal.*, 1920, [ii], **2**, 297) determines mercury in the presence of iron and vanadium. The mercury is precipitated as mercuric sulphide, which is subsequently oxidised with bromine water. The solution is rendered alkaline with caustic potash, excess of potassium cyanide is added, and the excess titrated with silver nitrate.

E. Bilman and K. Thanlow (*Bull. Soc. Chim.*, 1921, [iv], **29**, 587) described two methods for the determination of mercury based on the fact that allyl alcohol reacts with mercuric salts to give a hydroxide which is so feebly basic that it does not redden phenolphthalein. The hydroxide reacts with potassium bromide to liberate an equivalent amount of potassium hydroxide, which is titrated against standard acid.

Jellinek and Krebs (*Z. anorg. Chem.*, 1923, **130**, 263) and Jellinek and Kühn (*id.*, 1924, **138**, 109) determined mercury in mercuric chloride by titrating it against potassium cyanide, using phenolphthalein as indicator, and obtained results which were 3 per cent. too low. E. Rupp (*Z. anorg. Chem.*, 1925, **144**, 313) showed that these low results were due to the potassium cyanide being impure.

E. J. Kraus (*Chem. Ztg.*, 1926, **50**, 281) dissolved freshly precipitated mercuric sulphide in hydrochloric acid and iodine, and titrated the excess of iodine against thiosulphate.

E. Zintll and Rienacker (*Z. anorg. Chem.*, 1926, **155**, 84) determined mercury in the presence of salts of other metals by reducing the mercuric salts to metallic mercury with titanous chloride in hot acetic acid solution containing ammonium chloride and a bismuth salt as carrier, the end-point being determined potentiometrically.

Methods are suggested for the determination of mercury in small quantity, by (1) H. S. Booth, N. E. Schreiber and K. G. Zwick (*J. Amer. Chem. Soc.*, 1926, **48**, 1815), (2) P. Dennis (*Ann. Méd. legale*, 1921, **1**, 348), (3) A. Stock and R. Heller (*Z. angew. Chem.*, 1926, **39**, 466), (4) A. Stock and E. Pohland (*Z. angew. Chem.*, 1926, **39**, 791) (colorimetrically).

No reference in literature has been found in which stannous chloride has been employed for the volumetric determination of salts of mercury. N. A. Tananaeff (*Z. anorg. Chem.*, 1924, **133**, 372) suggested a spot test for the detection of mercury and tin, but did not develop his method for a quantitative determination.

FREE MERCURY IN COMMERCIAL PRODUCTS.—When attempting to determine free mercury in commercial products by converting it into mercuric bromide, Dunnicliff and Lal (ANALYST, 1927, **52**, 329) failed to determine the mercuric bromide by any volumetric method. In connection with another problem the methods to be described were devised for the volumetric determination of mercuric chloride in neutral or hydrochloric acid solutions. They fail with mercuric bromide, but this compound in solution or suspension in water may be quantitatively converted into the chloride by passing chlorine gas through the liquid.

The excess of chlorine is removed by warming on a water-bath and introducing a current of CO_2-free air, and the resulting mercuric chloride may be determined volumetrically. Analyses carried out in this manner gave

Mercuric bromide.	Determined by the stannous chloride method described, after conversion into chloride.	Sulphide method.
Grms.	Grms.	Grms.
1·8216	1·818	1·822
	1·821	

These results indicate that the volumetric method could be applied to all the types of commercial products referred to in the previous paper (*loc. cit.*).

Any processes by which mercury is separated as sulphide may be adapted to the volumetric process by dissolving the sulphide in *aqua regia* and evaporating the solution to dryness on a water-bath with excess of hydrochloric acid, and so converting the sulphide into chloride. The results obtained are about 1 per cent. low, owing to the loss of some mercuric chloride during the evaporation.

EXPERIMENTAL.—(1) Stannous chloride solution was prepared and standardised as described by E. Knecht and E. Hibbert ("New Reduction Methods in Volumetric Analysis," p. 17). The solution was stored in an atmosphere of carbon dioxide in an apparatus similar to that used for the storage of titanous chloride (*id.*, p. 63).

(2) Titanous chloride solution was prepared and standardised as described by Knecht and Hibbert (*loc. cit.*, p. 62). The solution of titanous chloride shows a continuous fall in strength on standing, and it is necessary to standardise the solution each day before use.

(3) Iron alum solution was made by boiling 48·20 grms. of pure ferric ammonium sulphate with strong hydrochloric acid and making the solution up to 1 litre. It was standardised against titanous chloride.

METHOD.—In the earlier part of the investigation an excess of standard $N/4$ stannous chloride was added to a mixture of 10 c.c. of mercuric chloride and 10 c.c. of a 25 per cent. solution of Rochelle salt, which was used to prevent the oxidation of stannous chloride by air. The precipitate of mercury so formed was allowed to settle in a vessel, though which a slow current of washed carbon dioxide was passed continuously. The clear supernatant liquid, which contained excess of stannous chloride, was pipetted into an excess of a hot solution of standard ferric alum, and the excess of ferric alum titrated back against titanous chloride. From the amount of ferric iron so reduced the corresponding quantity of stannous chloride was calculated, and, by difference, the amount of stannous chloride used in precipitating mercury from mercuric chloride. Calculation was facilitated by stating the strength of all solutions in terms of mercuric chloride and mercury.

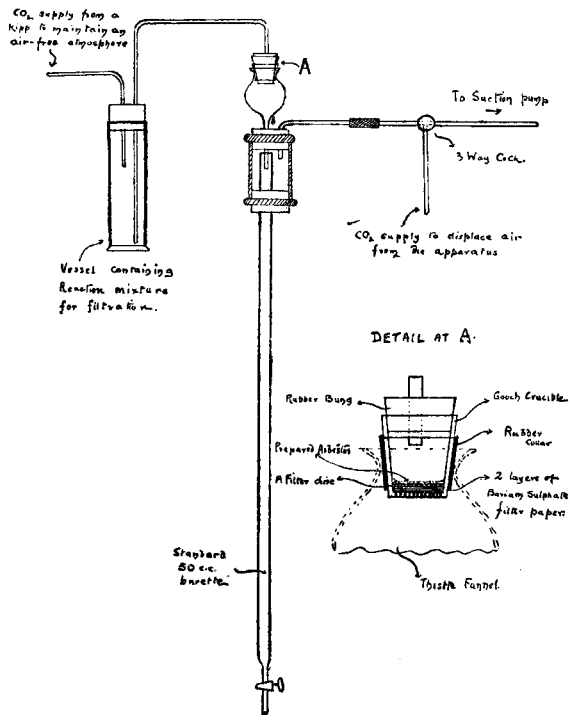
The results obtained were generally very high, and sometimes were as much as 25 per cent. in excess of theory. These high results were found to be due to the

presence of a considerable excess of free hydrochloric acid in the stannous chloride solution, which must therefore be neutralised if the process is to succeed.

A mixture of 20 c.c. of stannous chloride with 20 c.c. of a 25 per cent. solution of Rochelle salt was neutralised with sodium bicarbonate. If the solution is not neutralised, the mineral acid in the original stannous chloride or that existing in the titanous chloride causes the separation of sparingly soluble potassium hydrogen tartrate, which is liable to interfere in the titration (*cf.* Knecht and Hibbert, *loc. cit.*, footnote, p. 7).

The results obtained were more encouraging and showed an error of about +5 per cent. To avoid the error induced by the separation of the sparingly soluble potassium hydrogen tartrate, sodium tartrate was used in place of Rochelle salt, and, after the conditions favourable for the reaction to proceed quantitatively had been studied, the following method was found to give the most accurate results:—

I. A measured volume of stannous chloride is mixed with an equal volume of a 25 per cent. solution of sodium tartrate in an open-mouthed graduated cylinder, through which a constant current of carbon dioxide is passed. The hydrochloric acid is then neutralised with a calculated amount of sodium bicarbonate, and the volume made up to 80–90 c.c. The contents are vigorously stirred (still in an atmosphere of carbon dioxide), and the solution standardised by the ferric alum and titanous chloride method (*vide supra*).



II. Process I is repeated and 10 c.c. of the mercuric chloride solution added to the neutral stannous chloride, and the whole made up to the same volume as in I. The solution is then quickly filtered through a Gooch crucible provided with a double layer of barium sulphate filter paper and asbestos, into a a burette in an atmosphere of carbon dioxide, as shown in the figure. Ten c.c. of the filtrate are added to an excess of a hot solution of standard ferric alum (water-bath), acidified with hydrochloric acid (to facilitate the reduction of ferric iron to ferrous), and the excess back-titrated against titanous chloride. Table I shows the results obtained by the above method.

TABLE I.

Strength of Mercuric chloride in grms. per litre.

	Observed.	Taken.	Approx. normality.	Error. Per Cent.
I.	(*a*) 34·22	34·002	*N*/8	(*a*) +0·5
	(*b*) 34·00			(*b*) 0·0
II.	(*a*) 27·04			(*a*) −0·22
	(*b*) 27·14	27·100	*N*/10	(*b*) +0·14
	(*c*) 27·04			(*c*) −0·22
III.	(*a*) 13·60			(*a*) +0·29
	(*b*) 13·56	13·560	*N*/20	(*b*) 0·00
	(*c*) 13·55			(*c*) −0·07
IV.	(*a*) 9·01	9·032	*N*/30	(*a*) −0·21
	(*b*) 9·04			(*b*) +0·11
V.	(*a*) 6·80			(*a*) +0·44
	(*b*) 6·76	6·776	*N*/40	(*b*) −0·14
	(*c*) 6·76			(*c*) −0·14
VI.	(*a*) 5·42	5·420	*N*/50	(*a*) 0·00
	(*b*) 5·45			(*b*) +0·55

The stannous chloride and titanous chloride solutions require standardisation each day to obtain accurate results.

ALTERNATIVE METHOD.—A second method for the determination of mercuric chloride in solution, giving results of corresponding accuracy, has also been used. In this case, also, it is necessary to filter off the metallic mercury. The stannous chloride solution is neutralised as before. Mercuric chloride solution is added, and, after complete reduction has taken place, is made up to a known volume and filtered through the Gooch crucible into the burette. A known volume of the filtrate is run into standard iodine solution more than sufficient to oxidise the stannous chloride in the filtrate, and the excess of iodine titrated against *N*/20 sodium thiosulphate.

The degree of accuracy is about the same as in the first method. The two essentials for success are exclusion of air at the proper time and the separation of the metallic mercury from the solution to be titrated.

Table II gives some values obtained by this method.

TABLE II.

Strength of Mercuric Chloride in grms. per litre.

	Observed.	Taken.	Error Per Cent.
I.	27·03	27·10 = *N*/10	−0·26
II.	13·52	13·55 = *N*/20	−0·22
III.	5·45	5·42 = *N*/50	+0·55

From Tables I and II it is seen that the range of concentrations over which these methods are valid is considerable, but we suggest that they should not be relied upon for concentrations lower than *N*/40.

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Official Appointment.

Mr. HAROLD EDWARD MONK, B.Sc., F.I.C., as Public Analyst for the County Borough of Salford (to date from July 1st, 1929).

Notes.

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

THE KREIS REACTION AS A METHOD FOR THE DETECTION OF INCIPIENT RANCIDITY IN CACAO BUTTER.

CACAO butter being one of the most stable of the natural fats, rancidity seldom troubles the chocolate manufacturer. However, it would be an advantage here, as in other industries, to be able to detect incipient rancidity before the senses definitely indicate that a change has taken place.

The chemical literature was searched for a suitable test; the Kreis reaction having been fairly fully investigated, and apparently approved, seemed to have possibilities.

The Kreis test (*Verhandlungen der Naturforschenden Gesellschaft in Basel*, 1903–04, **15**, 225) consists in shaking the fat with strong hydrochloric acid and a 1 per cent. ethereal solution of phloroglucinol. A rancid fat should give a red or pink colour, the depth of which is proportional to the degree of rancidity. Kreis ascribed the reaction to the presence of aldehydes and ketones in the decomposing fat.

Winckel (*Z. Nahr. Genussm.*, 1905, **9**, 90) stated that the test was valueless; it condemned oils and fats which were apparently fresh and sweet, in which even incipient rancidity would not be expected. Moreover, the test is not specific, since the reaction is given by ketones and aldehydes which do not occur in rancid fat.

After testing many hundreds of samples at the Washington Meat Inspection Laboratory the following conclusions were reached (Kerr, *J. Ind. Eng. Chem.*, 1918, **10**, 471; ANALYST, 1918, **43**, 327):—(1) All rancid fats react to the Kreis test. (2) The intensity of the reaction is roughly proportional to the degree of rancidity. (3) Fresh sweet fats do not give the reaction, except in certain special cases, *e.g* crude. cottonseed oil (the substance causing the reaction can be removed by refining with caustic soda). (4) The Kreis test is too delicate as a criterion of rancidity. (5) The Kreis test is not specific, but is given by aldehydes and ketones other than those occurring in rancid fat, by most essential oils, by crude cottonseed, and probably other crude oils.

Kerr's method, as modified by Swanger and Marsh and used at the Meat Inspection Laboratories, is as follows:—Ten c.c. of the oil or melted fat are placed in a large test-tube (8 in. × 1 in.) and 10 c.c. of strong hydrochloric acid (sp. gr. 1·19) added. A rubber cork is inserted, and the tube shaken violently for about 30 seconds. Ten c.c. of a 0·1 per cent. ethereal solution of phloroglucinol are

added, and the tube shaken as before. On standing, to permit separation, a rancid fat gives a red or pink acid layer.

Kerr and Sorber (*J. Ind. Eng. Chem.*, 1923, 383) deal with the characteristics and causes of rancidity and give a number of more or less cumbersome methods for its detection. In reviewing the Kreis test they say "it is the most valuable and generally applicable."

In view of these favourable opinions I decided to investigate the suitability of the Kreis test as standardised for use at the Washington Meat Inspection Laboratories.

Supplies of rancid cacao butter were difficult to obtain, and all the commercial samples examined failed to give the reaction. We had, however, several old laboratory samples which were obviously very rancid and absolutely unpalatable. None of these specimens gave a positive reaction.

In the original Kreis reaction a 1 per cent. ethereal solution of phloroglucinol is recommended, as against the 0·1 per cent. of Kerr's modification. On changing the test solution to the original concentration no available genuine cacao butter reacted to the test, but a sample of cacao shell butter (which was at least 8 years old) gave a faint positive reaction. This fat was obviously very rancid. Diluted with an equal quantity of fresh sweet cacao butter the mixed fat, although still repulsive to the taste, failed to give the Kreis reaction.

In order to investigate the test in greater detail a graded series of rancid cacao butters was required. Use was made of the fact which we had known for some time, that the radiations from a mercury vapour arc lamp rapidly turn cacao butter rancid.

Quantities of 150 grms. of finest cacao butter were melted and poured into 250 c.c. glass beakers ($3\frac{1}{4}$ in. high × $2\frac{3}{4}$ in. diameter). The depth of the fat layer was 2·1 in., and the upper surface $4\frac{1}{2}$ in. below the mercury vapour arc.

The beakers of fat were exposed for periods ranging from 5 minutes to 8 hours to the radiations from the mercury vapour lamp, the uroxameter value of which for the period of the test had an average value of 32 (*cf. J. Soc. Chem. Ind.*, 1925, **44**, 453T). It seems reasonable to assume that the degree of rancidity will vary with the time of exposure to the lamp.

The Kreis test was tried on all the cacao butters prepared as above. Nine members of the Laboratory staff were asked to remark on the slightest evidence of rancidity.

The following observations were obtained:—

No.	Exposure to Mercury vapour arc. Minutes.	Temperature at start. °C.	Temperature at end. °C.	No. of the 9 observers who recorded fat as rancid.	Kreis reaction.
1.	0	32	—	2	Negative
2.	5	32	32	1	,,
3.	10	32	34	1	,,
4.	15	32	36	1	,,
5.	30	32	39	2	,,
6.	60	32	41	6	,,
7.	120	32	41·5	6	,,
8.	240	32	45·0	9	,,
9.	480	32	49·0	9	Positive

It will be observed that the Kreis reaction only differentiates one of these fats as being rancid. Taste and odour throw out four fats as rancid by large majorities, in two cases without a dissenting vote.

Whilst I am aware that a fat in which rancidity has been produced under other conditions than the above may contain different decomposition products, yet in view of these tests and the fact that none of the cacao butters which had gone rancid under ordinary conditions gave the reaction, I conclude that the Kreis reaction as a means of testing for incipient rancidity in cacao butter is useless. In sensitiveness it does not approach the faculties of smell and taste of the ordinary observer.

I wish to thank Mr. A. W. Knapp, F.I.C., for help and suggestions, and Messrs. Cadbury Bros., Ltd., Bournville, in whose laboratories the work was carried out, for permission to publish this note.

T. H. COOKE.

DYES AS AN INDICATION OF ADULTERATION IN BUTTER.

IN cases where butter is suspected to be adulterated with margarine it is usual to carry out qualitative tests for cottonseed oil and sesame oil, in addition to other tests. Where the amount of margarine added is small, say, 10 per cent., the amount of cottonseed or sesame oil contained might only be about 1 per cent. according to the percentage of oils present in the margarine used, and the tests, in consequence, are of little value. It is well known that 10 to 15 per cent. of a margarine of a certain composition can easily be added to butter without the adulteration being detected by the Reichert-Wollny and Polenske methods, and the test to be described affords a useful indication of possible adulteration, or of the necessity for further investigation.

All samples of butter and margarine which pass through this laboratory are tested for prohibited colouring matters. It was found during these tests that 97 to 98 per cent. of the margarines examined contain a dye which is extracted by ammonia, whilst, with the majority of butter samples (94 to 95 per cent.), no colour is extracted by ammonia, and in cases where colour is shown, the colour is usually slight. To carry out the test, about 10 ml. of fat are shaken in a boiling tube with about 10 ml. of petroleum spirit and 10 ml. of 3 per cent. ammonia solution. The appearance of a coloration in the aqueous layer when butter fat is examined is an indication of possible adulteration with margarine.

Recently it was necessary to examine a series of butter samples which were suspected of adulteration with small percentages of margarine. Out of ten butters examined, the only sample which from the figures could be definitely described as genuine was also the only sample to give a colourless ammoniacal layer. The test described is obviously not a definite one, either negatively or positively, but may occasionally be helpful, as the presence of 10 per cent. of a dyed margarine is easily shown. The dye used in margarine appears to be of the same nature in all these samples. Thus it is extracted with $N/100$ sodium hydroxide solution, giving a yellow solution (*cf.* Nicholls, ANALYST, 1927, **52**, 588). On applying Test A (*id.*, 589), the soda solution becomes pinkish-yellow, with a doubtful coloration of the ethereal layer. The weak alkaline solution is *not* decolorised by dilute acid.

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Notes from the Reports of Public Analysts.

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

CITY OF BIRMINGHAM.

ANNUAL REPORT OF THE CITY ANALYST FOR 1928.

IN presenting my twenty-sixth and last annual report I think that it will be of interest to review the steps which have been taken to prevent adulteration in Birmingham during the past half century.

In 1861, Dr. Alfred Hill was appointed Borough Analyst by the Town Council for one year, and was re-appointed the next year, in spite of an amendment that the office should be abolished. In 1863 he was required to undertake some sanitary work in addition to his analytical duties, and in 1872 he was appointed Medical Officer of Health, as well as Borough Analyst.

Unfortunately no records of the earlier years are extant, but from 1873 Dr. Hill's records and reports are available, and my personal knowledge of the working of the Food and Drugs Acts dates back to 1885.

In comparing the adulteration figures over a series of years, adulteration with preservatives has been dealt with separately, and, so far as possible, present-day standards have been applied to all the samples. For the last twenty years the "Comparative Adulteration Figure" (*i.e.* the number of vendors of adulterated samples for each 100 samples bought) has been used, instead of the percentage of adulteration; this figure depends much less on varying conditions of sampling. In 1908 to 1918 this figure was 3·4, and in the last decade it fell to 2·4.

In the period 1873–1878 twenty-eight per cent. of the samples were milk; in the last period they were 54 per cent. For fair comparison, the relation of the numbers of the various articles analysed should be similar for each period. A correction of the percentage of adulteration has been made by calculating the number of adulterated samples for 100 samples, made up of 52 milks, 9 butters, 2 spirits, 5 drugs, and 32 other articles. This figure gives the percentage of adulteration with standard sampling.

In the following table the figures for England and Wales and for London are calculated from data given in the annual reports of the Local Government Board from 1876 to 1913, and from those of the Ministry of Health from 1919 to 1927. These figures include a certain amount of adulteration by means of preservatives, and the only correction that can be applied to them is that to standard sampling.

	Percentage of Adulteration with Standard Sampling.			Total Number of samples per year per 100,000 persons living in Birmingham.
	England and Wales.	London.	Birmingham.	
1873— ..	—	—	40·6	30
1879— ..	13·8	15·5	20·6	127
1889— ..	9·9	13·9	12·7	216
1899— ..	8·6	10·6	7·9	276
1909— ..	8·3	8·7	7·1	438
1919–1928 ..	6·4*	4·5†	4·6	481

* 1919—27 only. † 1920—27 only.

The above table shows that the average percentage of adulteration in Birmingham during the 56 years fell from 40·6 to 4·6. During the 49 years the figure for England and Wales fell from 13·8 to 6·4, and that for London from 15·5 to 4·5.

In the decade commencing 1879 the proportion of adulteration in Birmingham was distinctly higher than the other figures, but in later years the disproportion diminished, and now Birmingham is better than the average of the country and similar to London; owing to the difference in administrative conditions, the comparison of the figures must not be pressed.

In the first six years of the table the annual average of samples taken was 30 per 100,000 persons living in Birmingham; in the last decade this number had increased to 481. The proportion in England and Wales for 1927, the last available year, was 317.

ADULTERATION WITH PRESERVATIVES.—Before 1896, samples of ale and beer were tested for excess of common salt, but apart from this no examination for preservatives was made. In that year Dr. Hill, at the request of the Public Health Committee of Birmingham, investigated the question of the use of preservatives in food.

Boric acid was detected in milk, butter, cream, bacon, pork pie, vinegar, and other foods. Salicylic acid was found to be present in jam, cream, sherry and ipecacuanha wine. Formic aldehyde was also detected in milk.

As a result of these investigations, Dr. Hill publicly advocated prohibition of the use of preservatives in food. At a conference of the Sanitary Institute held in Birmingham in 1898, he took that subject for his presidential address to one of the sections, and the next year gave a paper before the Incorporated Society of Medical Officers of Health, and a resolution was passed deprecating the use of preservatives in foods. In the same year he gave evidence as representative of that Society before the Departmental Committee which enquired into the use of preservatives and colouring matters. In 1901 that Committee made a report and recommended limitation or prohibition of preservatives in food. Unfortunately, no legislation resulted, and, although prosecutions for preservatives in milk were instituted under the Sale of Food and Drugs Acts, it was not until 1912 that the Milk and Cream Regulations definitely prohibited the use of preservatives in milk.

In 1923 another Departmental Committee was appointed, and that Committee finally reported in 1924. In the following year the Public Health Preservatives, etc., in Food Regulations were passed.

Since the passing of these Regulations various foods have been tested for the presence of sulphur dioxide; in most cases with negative results. Samples of pearl barley contained up to 120 parts of sulphur dioxide per million, and samples of ginger up to 3000 parts per million. One sample of crystal mints contained nearly 1000 parts per million.

MILK.—In 1900 no less than 11·4 per cent. of the samples examined contained either boric acid or formaldehyde. In several cases 6 to 9 grains of boric acid per pint were present, and in one case 16 grains per pint were found. In the period 1896–1902 there were 7 prosecutions for boric acid in milk, and only 2 cautions for formaldehyde. In 168 cases no action was taken, since the Public Health Committee was not well supported by the magistrates. In one instance, in which boric acid and formaldehyde were both present, as well as 14 per cent. of water, the defendant was only ordered to pay 5s. costs. The vendor of a milk containing 16 grains of boric acid per pint was only fined 2s. 6d.

In 1897, when a vendor was cautioned for preserving his milk with formaldehyde, he said that he intended to go on using the preservative, and that the

Committee could not stop him. Unfortunately that statement was correct, as at that time while formaldehyde could readily be detected in milk, there was no available means for determining it, and prosecutions could not be instituted unless the amount of adulterant was stated.

As vendors were being prosecuted for the use of boric acid and not for formic aldehyde, the latter preservative became more popular. Between 1897 and 1903 the proportion adulterated with boric acid fell from 5·5 per cent. to 1·5 per cent., whilst milks containing formaldehyde increased from 3·3 per cent. to 6·4 per cent.

In 1902 I devised a method for the determination of formic aldehyde, and in the next year there were successful prosecutions for this preservative, which were among the earliest in the country.

The results of the action taken in the second period resulted in the fall of preservative adulteration to 0·2 per cent. in the third period; since 1915 the adulteration of milk with these preservatives has been very unusual.

CREAM.—Before the issue of the Regulations of 1912 only 17 per cent. of the samples of cream were free from boric acid. Each subsequent period showed an improvement, and last year nearly all the samples of cream were free from boric acid.

Only 64 per cent. of the preserved creams bought under the 1912 Regulations were correctly labelled, but during the last three years the proportion increased to 84 per cent. When creams had been preserved with boric acid there was a decided decrease in the proportion used. In 1913–1916, 66 per cent. of the samples did not contain more than 28 grains per pound. In the next period the proportion improved to 98 per cent.; on the other hand, samples containing over 35 grains per pound decreased from 12 per cent. to 1 per cent. in the two periods. The largest amounts detected in a period fell from 70 to 7 grains per pound.

BUTTER.—Butters were first tested for boric acid in 1896, and Dr. Hill reported all samples containing this preservative as adulterated. Fourteen vendors were cautioned for samples containing from 6–25 grains of boric acid per pound. This action was, however, in advance of the times, and no further action was taken until 1898, when a vendor was fined for 70 grains per pound. Other prosecutions followed, and in the years 1898–1904, 13 vendors were prosecuted for samples of butter containing from 55–112 grains of boric acid per pound, and paid fines and costs amounting to £24. There were also 9 prosecutions for samples of butter which contained boric acid and also an excess of water, for which fines and costs amounting to £29 were paid by vendors.

During each decade 68 to 69 per cent. of the samples were free from boric acid. From 1899 to 1905 no less than 11·9 per cent. of the preserved samples contained over 35 grains of boric acid per lb., but the prosecutions during that period had a salutary effect, for in the next decade only 1·9 per cent. exceeded that figure, and after that very few samples contained more than 35 grains per lb.

SAUSAGE.—In 1908, 44 per cent. of the samples were free from boric acid, but in 1922–6 only 14 per cent. were free. Since Preservatives Regulations prohibiting boric acid came into force, in 1927, all the samples examined have been free from this preservative.

Besides the increasing use of preservative, the amount used was also greater. In 1908, 37 per cent. contained under 14 grains per pound, and 16 per cent. 35 grains and over. In 1922–6 samples containing the smaller proportion of preservative had decreased to 28 per cent., and the larger increased to 22 per cent. There have been no prosecutions in Birmingham for the presence of boric acid in sausage.

Three of the 10 samples bought as "sausage" last year were adulterated, containing from 100 to 290 parts of sulphur dioxide per million. The last one,

after frying for a quarter of an hour, contained 240 parts per million, showing that little of the preservative was dissipated by cooking.

BEER.—In the first sixteen years (1873–1888) 3 per cent. of the samples contained more than 70 grains of chlorides. In the next twenty years the proportion increased to 10 per cent., in the next decade (1909–1918) 6 per cent. were above the limit, and in the last decade the proportion fell to 1 per cent.

COLOURED AND BLEACHED FOODS.—In 1874 three vendors were fined for sweets known as "Birds' Eggs," which were coloured yellow with chromate of lead, but no similar adulteration has been detected since. In 1907, 11 samples of preserved peas and beans contained copper varying from 0·1 to 1·0 grain of metallic copper per pound. In 3 samples the presence of a "small quantity" of copper was declared on the label, but not in the case of the larger amounts. The two samples of preserved peas examined last year were found to be free from copper.

The colouring of milk has never been common in Birmingham. In the decades commencing 1888 and 1898, 1·4 per cent. of the milks were coloured. In 1904 the Committee sent out a warning circular on the matter to milk dealers, and in the next ten years only 0·3 per cent. of the samples were coloured.

In the 5 years commencing 1910, 57 per cent. of the Birmingham samples of flour analysed contained from 2 to 10 parts per million of sodium nitrite as the result of bleaching. Before 1905 flour was sold in its natural state, but gradually much "flour" was withdrawn and bleached flour sold in its place without any notice being given to the consumer of the substitution that had been made.

Last year three samples of sultanas were examined. The palest, which was the highest priced, had been bleached with sulphur dioxide, but the other two were free from it. This appears to be another case in which an article, inferior because of containing preservative, is considered superior in colour, and is sold at a higher price because it has been artificially bleached.

PEARL BARLEY.—During the years 1910–1928, 707 samples were examined, and of these 25 bought in the years 1913 and 1914 were adulterated by facing either with talc or rice flour. Fourteen samples had been faced with talc, 0·2–0·7 per cent. being present. Nine samples had been faced with 0·5–2 per cent. of rice flour, and 2 samples were faced with both rice and talc. Cautions were given to retailers, wholesale dealers and a London wholesale house, and all the samples examined in subsequent years have been unfaced.

This is apparently another example of adulteration to give increased whiteness. In later years bleaching by sulphur dioxide may have taken the place of facing previously used. Last year 6 samples contained from 10–120 parts of sulphur dioxide per million. Its presence is prohibited by the Preservatives Regulations of 1925.

In the years 1912 to 1920, 32 per cent. of the samples contained over 1·1 per cent. of ash, but in the next eight years only 12 per cent. exceeded that figure, indicating a great improvement.

There has also been improvement in the cleanliness of the samples of pearl barley. In the six years, 1918–23, 55 per cent. of the samples contained mites, either alive or dead, but in the following five years the percentage fell to 30. It is obviously advisable that pearl barley should be washed before use.

Of the samples of barley examined in England and Wales during the years 1920–7, 2·8 per cent. were reported adulterated.

VINEGAR.—In 1893 four samples of vinegar were submitted to Dr. Hill for analysis in one day, and were found to be artificial vinegar. Prosecutions ensued, and the vendors were fined. One of the vendors appealed to Quarter Sessions, and, after a lengthy hearing, the appeal was dismissed with costs. Since that

date the sale of artificial vinegar in Birmingham has been unusual, only 2·4 per cent. of the samples being thus adulterated, and in some cases duplicate samples were obtained from one vendor.

One vendor, last year, who sold artificial vinegar both as vinegar and malt vinegar, was defended by the makers of the vinegar. The vendor, who was a foreigner, stated that she did not hear the word "malt," but her evidence was unconvincing. I gave evidence that either vinegar or malt vinegar should be prepared by fermentation and acetification, and that artificial vinegar was simply prepared by diluting and colouring acetic acid. I was shown a label which was said to have been on the cask from which the article was taken, but not visible to the purchaser. As this label stated "Pure Vinegar," I said that it was a false one.

The defence also suggested that a case at Leeds Assizes had settled, for the whole of the country, that artificial vinegar could be sold as vinegar. This was a case in which the makers of the artificial vinegar claimed damages for libel from a vinegar brewery company. All this case settled was that the artificial vinegar makers were libelled; the jury assessed the damages as one farthing without costs. The magistrates imposed a fine of £1.

BAKING POWDER.—In 1894 an appeal case decided that baking powder was not legally a food, although a large proportion of it was of a starchy nature. In 1899 the definition of "food" was enlarged to include "any article which ordinarily enters into or is used in the composition or preparation of human food." The necessity of samples being taken under the Sale of Food and Drugs Act was shown by the fact that in the next year 6 of the 19 samples contained alum, the proportion present varying from 13–30 per cent., and the vendors were prosecuted for selling an article injurious to health. One of these samples was marked "Prize Medal Baking Powder," and the label on another actually claimed that the article would make bread more digestible, in spite of the presence of 25 per cent. of alum.

Since that year 57 samples have been analysed. In 1916 one sample contained 12·4 per cent. of calcium sulphate and 25 parts of arsenic per million, and a second sample of the same make had a similar composition. The presence of these impurities was due to the acid calcium phosphate used being of inferior composition.

In 1922 one sample contained an excess of calcium sulphate. In 1927–8 four samples were of inferior quality, yielding 3·4 to 4·6 per cent. of carbonic acid gas, while samples of good quality yielded from 6·0 to 9·4 per cent. The practical value of the powder is proportional to the amount of carbonic acid gas yielded by it. Last year a manufacturer who was cautioned undertook to increase the strength of his powder.

J. F. LIVERSEEGE.

Legal Notes.

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

AMMONIATED TINCTURE OF QUININE.

AT Thames Police Court, a pharmacist was summoned for selling on September 28th, 1928, ammoniated tincture of quinine which was deficient in ammonia to the extent of 15·8 per cent. The pharmacist stated that he had had one pint of the tincture in stock for three days only, and that it must have been received by him at practically the same strength as he had sold it.

The analyst for the defence agreed with the figures of analysis, but regarded the loss of ammonia as negligible and due to loss in filtration during manufacture and in opening the bottle while dispensing. Similar evidence was given by the

analyst to the wholesale firm which supplied the tincture. The solicitor for the defence stated that the British Pharmacopoeia gave no tests for the strength of ammoniated tincture of quinine, and that the theoretical composition did not constitute a standard. He quoted the figure of "about 0·9 per cent. w/v of absolute ammonia" given in Squire's Companion to the British Pharmacopoeia, and produced a pamphlet described as "Local Government Dept., Revised Standards for Pharmacopoeial Preparations," which, he stated, contained a standard for ammonia in the tincture.*

Rebutting evidence was given for the prosecution by Mr. D. Henville, Public Analyst for Stepney, who explained that tests regulating the composition of each constituent of ammoniated tincture of quinine were definitely detailed in the British Pharmacopoeia, that the manufacture of the tincture consisted in the simple admixture of these standard constituents, followed by filtration, and that it was only reasonable to assume that the resulting product would closely approximate in composition to the theoretical standard.

Results of a series of experiments made to ascertain the amount of loss of ammonia caused by dispensing a pint of the tincture in small quantities over a period of three days were given. This loss amounted to less than 1 per cent. The witness did not accept the minimum standard in the pamphlet produced.

Further evidence for the prosecution was given by Mr. R. A. Cripps, F.I.C., who gave figures showing the small loss of ammonia during dispensing and during manufacture under various conditions of filtration. The experiments on which this evidence was based, were as follows:—

	Loss of original Ammonia Per Cent.
After filtration in uncovered filter	2
,, ,, ,, lightly covered filter	Nil
(Loss due to experimental error would not exceed 0·008 per cent.)	
After exposure in open bottle for 19½ hours	13·5
,, ,, ,, beaker ,, 2½ ,, at 15° C.	12·5
,, ,, ,, ,, ,, ,, ,, 18° C.	18·0
(In draught from open window)	
On serving out ½ to 1 oz. from 12 oz. bottle containing 11 ozs. of tincture, last portion poured out	0·5
2nd test .. .. loss	0·7
Total loss, sample "A" bad conditions of filtration, serving out 17 times	2·5
Total loss, sample "B" usual conditions, and serving out 17 times	0·7

The case was adjourned a number of times, and at the last hearing, on March 25th, the magistrate (Mr. Cairns) fined the defendant £2, with £15 15s. costs.

SULPHUR DIOXIDE IN GROUND GINGER.

On May 29 an adjourned summons against a trading company was heard at West Ham, London, for the sale of ground ginger containing 1564 parts of sulphur dioxide per million.

The Medical Officer for West Ham (Dr. Collins) said that he was not pressing the case, but the presence of this amount of sulphur dioxide in the ginger was contrary to the Regulations.

The solicitor for the defence said that the company took every precaution to assure themselves that all their goods complied with the regulations. After the

* No intimation as to the origin of this pamphlet was given, and the standard was a minimum figure only. It was later found that this pamphlet was a publication of the Irish Free State.

matter had been discussed at several meetings of the Spice Association, it had been decided that guarantees should be given, but it appeared that there was still controversy with the Ministry of Health. In India and Cochin, owing to the humidity of the moisture, a preservative had to be used during the drying of the roots, and they were then bleached with lime. The lime retained the sulphur dioxide, but in cooking it would disappear to a large extent. When the regulations first came into force the importation of this ginger from Cochin and India was stopped. The whole ginger roots could be sold as bleached ginger, but when powdered they could not be sold. The Company was perfectly innocent of any cognisance of an offence in this case.

Mr. St. John Morrow said that he understood this, and that the case would be met by the defendant company paying £1 as costs. There would be no conviction.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

Food and Drugs Analysis.

Copper Content of Plant and Animal Foods. C. W. Lindow, C. A. Elvehjem and W. H. Peterson. (*J. Biol. Chem.*, 1929, **82**, 465–471.)—The copper content of about 160 samples of common food materials has been determined by the modified Biazzo method, as outlined by Elvehjem and Lindow (*J. Biol. Chem.*, 1929, **81**, 435 ; ANALYST, 1929, **54**, 245). The copper content is calculated on the dry basis and fresh basis, and a table gives the results. The figures range from 0·1 mgrm. of copper per kilo of fresh celery to 44·1 mgrms. per kilo. of fresh calf liver. The classes of foods in descending order of their average copper content per kilo. of fresh material are :—10 nuts, 11·6 mgrms.; 4 dried legumes, 9·0 mgrms.; 19 cereals, 4·7 mgrms.; 8 dried fruits, 4·2 mgrms.; 4 kinds of poultry, 3·0 mgrms.; 17 kinds of fish, 2·5 mgrms.; 13 animal tissues, 1·7 mgrms.; 2 green legumes, 1·7 mgrms.; 11 roots, tubers, stalks and bulbs, 1·4 mgrms.; 14 leafy vegetables, 1·2 mgrms.; 27 fresh fruits, 1·0 mgrm.; and 10 non-leafy vegetables, 0·7 mgrm. The copper content of leafy vegetables does not place them in the pre-eminent position that they hold with reference to their iron content. A wide variation was found in the copper content of livers from different animals ; calf liver was highest (44·1 mgrms.) and hog liver was lowest (6·5 mgrms.). The copper content of oysters was strikingly high (30·7 mgrms. per kilo.), and surpassed all the sea foods in this element. Unlike the data obtained for the iron content of salt water and fresh water fish (*J. Biol. Chem.*, 1928, **78**, 215 ; ANALYST, 1928, **53**, 444), the average figures for the copper content of the two groups are practically the same. A table shows that the degree of variation in the copper content of foods falling in the same class was less than that of either manganese or iron. There is a wide distribution of copper in food materials ; no food examined was without this element. Certain milled cereals, such as polished rice and patent wheat flour, are very low in copper, as compared with the whole grain from which they were made. P. H. P.

Copper Content of Feedingstuffs. C. A. Elvehjem and E. B. Hart. (*J. Biol. Chem.*, 1929, **82**, 473–477.)—In a previous paper by Skinner and Peterson (*J. Biol. Chem.*, 1928, **79**, 679) the manganese and iron content of 51 feeds was given. The same series has now been analysed for copper in order to compare the relative abundance and distribution of the three elements in the same feeds. Experiments were also carried out to determine whether or not fertilisation with copper salts would influence the copper content of the crop grown. The copper content of 47 common feeds is given. The average copper content of 42 of the feeds is 13·5 mgrms. per kilo of dry matter. The average iron and manganese contents of the same 42 feeds are 199·0 and 65·8 mgrms. per kilo. of dry matter, respectively. Therefore the average copper content of these feeds is one-fifth of the manganese and one-fifteenth of the iron content. Certain manufactured feeds are unusually high in copper, probably owing to contamination, *e.g.* gluten feed 89·5, and distillers' grain 38·4 mgrms. per kilo. of dry matter. The feedingstuffs arranged in ascending order of copper content are as follows :—Straws and stovers, hays and grasses, and seeds and seed products. Crops grown on a plot treated with copper sulphate at the rate of 50 pounds of the salt per acre, added in solution to insure uniform distribution, showed a small but definite increase in their copper content. In a single experiment, in which only lettuce was involved, the copper fertilisation was increased tenfold, *i.e.* 500 pounds of copper salt per acre were added, and an increased copper content of 148 per cent. was shown by the lettuce over that produced on untreated soil. Therefore the copper content of the crop can be increased within certain limits by fertilisation of the soil with a copper salt. P. H. P.

Determination of Reducing Sugars, particularly of Glucose, by Alkaline Copper Solutions in the presence of Hydrocyanic Acid. H. Herissey and A. Chalmeta. (*Ann. Falsif.*, 1929, **22**, 214–223).—The presence of hydrocyanic acid will cause a loss or even a total apparent disappearance of reducing sugars when determined in alkaline copper solutions, whether the determination is finished colorimetrically or gravimetrically, partly owing to combination of the reducing sugar in the alkaline medium with the hydrocyanic acid, and partly by formation of a double cyanide of sodium and copper from the cuprous oxide in alkaline solution. Hydrocyanic acid may be eliminated before the determination in simple cases either by heating (evaporating the solution to half the volume or to dryness and redissolving) ; or, in others, by passing through the solution a current of air for a prolonged period, or chemically by the addition of a very slight excess of silver nitrate, followed by filtration and addition of sodium chloride to remove any traces of silver salt. In the case of liquors obtained by hydrolysis of a glucoside by emulsin precautions are necessary, such as the effecting of certain preliminary separations before evaporation. D. G. H.

Divinylglycol as the Cause of the Bitter Flavour of Wines suffering from Bitterness. E. Voisenet. (*Comptes rend.*, 1929, **188**, 1271–1273.)—A

compound isolated from a Burgundy wine exhibiting the disease known as "bitterness," was found to consist of divinylglycol, which is doubtless formed from acrolein by the action either of the reductases normally present in wines or of a hydrogenase secreted by the " bitter " organism. T. H. P.

Detection of Fruit Wine in Grape Wine by Means of Dibenzal-sorbito' C. von der Heide and K. Hennig. (*Z. Unters. Lebensm.*, 1929, **57**, 240–241.)–According to Werder (*Mitteilg. a. d. Gebiete d. Lebensmitteluntersuchung u. Hygiene*, 1928, **19**, 294) 100 c.c. of wine are boiled for 3 minutes with 10 grms. of active charcoal, filtered hot, and the filtrate and washings evaporated under reduced pressure (70° C.) to a colourless syrup. This is well shaken with 4 drops of benzaldehyde and 0·8 c.c. of 50 per cent. (by volume) sulphuric acid, and on the following day, diluted with 100 c.c. of water, when a white precipitate (dibenzal-sorbitol) indicates fruit wine. The authors consider it necessary to identify this precipitate, on account of the separation of other substances (*e.g.* gypsum) from grape wine. The precipitate is filtered on a sintered glass crucible, washed free of acid, dried with alcohol and ether, and extracted with benzene for 3 hours. This dissolves only the dibenzal-sorbitol, leaving the organic impurities (benzaldehyde, benzoic acid, glycerol, etc.). The solution is evaporated, and the residue recrystallised from benzene. It has m.pt. 162° C., whilst tribenzal-mannitol has m.pt. 213 to 217° C. (both uncorr.). J. G.

Analysis of the Bitter Substances of Hops. W. Windisch, P. Kolbach, and M. Winter. (*J. Inst. Brew.*, 1929, **35**, 269–270.)—In the usual method of determining the α-bitter acid or humulone of hops by precipitating it from methyl alcoholic solution as lead salt, a slight excess of the dilute lead acetate solution (1 per cent.) must be used, but the precipitate is appreciably soluble in excess of the reagent, and more so in solutions containing other hop resins than in solutions of pure humulone. It is recommended that a series of test-tube trials be made with 2 c.c. of the resin solution, 2 c.c. of methyl alcohol, and varying amounts of lead acetate solution, these being heated for 5 minutes in a water-bath at 65–70° C. and filtered, and the filtrates tested with sulphide, so that the proper amount of the lead salt to use may be ascertained. For the actual analysis, 10 c.c. of the resin solution, 10 c.c. of methyl alcohol, and the requisite volume of lead acetate solution are heated together for 5 minutes in a gently boiling water-bath, and allowed to stand for 10 minutes before filtering, the precipitate being then washed 6 times with 5 c.c. portions of methyl alcohol, dried, and weighed. No oxidation of humulone to resinous substances occurs during this procedure, but even with extracts from fairly fresh hops the precipitate is apt to be slightly contaminated with resins. This may be ascertained from the weight of lead in 1 part by weight of the precipitate ; the correct lead factor is 0·3653, but values varying from 0·360 to 0·364 were found for different samples of lupulin. Moreover, the humulone obtained by acidifying the precipitates gave iodine values of 147–149, the correct value being 151·3–151·5.

As hops age, the small inaccuracies indicated by the above figures become more serious, owing to the appearance of an excessive proportion of hard resins, which contaminate the lead humulate and, later, of other resinous decomposition products. Hard resins may be eliminated by extracting the hops with hexane, which dissolves only the soft resins and bitter acids. After protracted ageing, the hops contain a disturbing substance soluble in hexane, but this may be removed by shaking the extract with an aqueous phosphate buffer solution of P_H 6·4, the humulone being afterwards precipitated as usual. The humulone content furnishes the most trustworthy indication of the state of preservation of hops, as it undergoes far greater changes than the soft resin content found by extraction with petroleum spirit or hexane. T. H. P.

Composition of Spinach Fat. J. H. Speer, E. C. Wise and M. C. Hart. (*J. Biol. Chem.*, 1929, **82**, 105–110.)—Sixty-eight kilos. of dried spinach were extracted with cold acetone, the extract obtained by concentration under nitrogen at reduced pressure dissolved in boiling alcohol, the insoluble material filtered off, the alcohol evaporated, the residue dissolved in ether and washed with 1 per cent. sulphuric acid, the free fatty acids removed, followed by removal of chlorophyll and its degradation products. The yield was 550 grms. of fatty acids, 47 per cent. of which were present as glycerides and 53 per cent. free. The solid acids weighed 26·5 grms. (separation not quantitative) consisting chiefly of palmitic and stearic acids, with 3 per cent. of cerotic acid. Of the 145 grms. of liquid acids recovered from the fractionation processes, at least 12·7 per cent. were linolenic, 24·7 linolic, and 26·3 per cent. oleic acids. Volatile acids, if present at all, were only so in traces. D. G. H.

Glycerides of Chaulmoogra Oil. A. Bömer and H. Engel. (*Z. Unters. Lebensm.*, 1929, **57**, 113–147.)—To eliminate changes due to rapid atmospheric oxidation the oil was hardened in the presence of 0·1 per cent. of a 1 per cent. mixture of palladium and kieselguhr at 170 to 200° C. The hardened oil (m.pt. 27 to 28° C.), in which a ring double-bond had been satisfied with two hydrogen atoms, was optically inactive. As a result of a large number of fractional crystallisations from acetone and ether at about 0° C. curves were plotted showing the m.pt. and the weight of the fractions, and the presence of 79 per cent. of dihydro-chaulmoogro-dihydro-hydnocarpin (m.pt. 30·7° C., corr.), 13 per cent. of dihydro-hydnocarpo-di-dihydro-chaulmoogrin (m.pt. 42·2° C., corr.), and traces of a slightly soluble glyceride (possibly tripalmitin or a stearo-dipalmitin) was established. The results were checked by determinations of the saponification, iodine and acid values of the fractions, and by fractional precipitation of the fatty acids with magnesium acetate. The natural oil therefore contains the corresponding unsaturated glycerides chaulmoogro-di-hydnocarpin and hydnocarpo-di-chaulmoogrin in corresponding proportions, whilst the fatty acids of the hardened oil have the composition : 40 per cent. dihydro-chaulmoogric acid and 59 per cent. dihydro-hydnocarpic acid (*cf.* Dean and Wrenshall, ANALYST, 1921, **46**, 52).

Tridihydro-chaulmoogrin (m.pt. 51·0° C., corr.) was synthesised by the action of the lead salt of dihydro-chaulmoogric acid on tribromhydrin in the presence of xylene at 170 to 180° C. for 10 hours, and tri-dihydro hydnocarpin (m.pt. 39·2° C., corr.), di-dihydro chaulmoogrin (m.pt. 60·7° C., corr.), trilaurin and trimyristin were obtained in an analogous manner. Dihydrochaulmoogric and dihydro-hydnocarpic acids are three and four times more soluble in alcohol than stearic and palmitic acids, respectively. J. G.

Nitrobenzaldehyde as Reagent for Organic Medicines. H. W. Van Urk. (*Pharm. Weekblad*, 1929, **66**, 429–435.)—The reagent consists of a 1 per cent. alcoholic solution of *o*-, *m*- or *p*-nitrobenzaldehyde to which dilute sulphuric acid is added until the acid strength is about 2 per cent. A little of the sample is heated or evaporated on the water bath with 5 to 10 drops of reagent, and a coloured residue obtained. The results are tabulated for numerous substances. Amino-compounds do not react, and the author's *p*-dimethyl-amino-benzaldehyde (*id.*, 1929, **66**, 101; ANALYST, *infra.*) reaction should be used. Simple phenols and polyphenols react, whilst mandelic acid and homatropine do not, though tropic acid and atropine give a positive reaction. Salicylic acid gives no marked reaction with the *m*- or *p*-compounds, but salol reacts with all three. Other exceptions are the purines, pyridine derivatives, nicotine, papaverine, and cotarnine. The phenetidines, pyrroles, and alkaloids containing hydroxyl groups react. Narcotine reacts only with the *o*- compound, and hydrastine gives a negative reaction unless previously heated with hydrochloric acid. Morphine differs from codeine in that it gives a green colour with the *o*-reagent, if previously heated, instead of the yellow normally given by both substances, while the aqueous solution of the residue is brown instead of orange-yellow. In general, the three isomers behave similarly. J. G.

Microchemical Reactions of Piperine. M. Wagenaar. (*Pharm. Weekblad*, 1929, **66**, 405–406.)—Piperine, $C_{17}H_{19}NO_3$, is a very weak base crystallising in badly-defined prisms, m.pt. 128° to 129° C., refractive index, 1·70 and 1·55 (Kley). It is insoluble in water, and soluble in ether, chloroform, and cold (1 in 30) or hot (1 in 1) alcohol. The acetate may be prepared in prisms about 1 mm. long by cooling a solution of a little solid piperine in the minimum amount of warm 30 per cent. acetic acid, and, if necessary, by salting out with sodium acetate. The alkaloid (50 m.grms.) is precipitated from a solution in a drop of acetone as fine needles, 100μ long, by the addition of a drop of water. J. G.

Microchemical Reactions of Physostigmine. M. Wagenaar. (*Pharm. Weekblad*, 1929, **66**, 381–382.)—Physostigmine, $C_{15}H_{21}N_3O_2$, has strongly basic properties and crystallises in shining leaflets, m.pt. 102° to 103° C., refractive index 1·66 and 1·54 (Kley), slightly soluble in water, and readily soluble in alcohol, ether or chloroform. Sodium salicylate solution gives a precipitate with the sulphate of doubly refracting cubic or hexagonal crystals, 200μ in size, which may be obtained from super-saturated solutions by addition of ammonium sulphate. If the sulphate

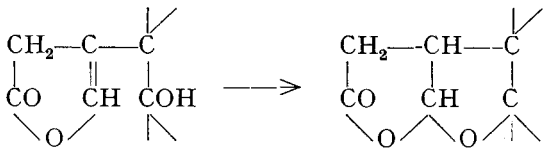
is acidified with dilute hydrochloric acid and a crystal of sodium bromide added, followed by a drop of gold trichloride solution, there is a brown-red precipitate. The limiting concentrations for these reactions are 1:200 and 1:500, and the smallest amounts detectable are 10 and 5 m.grms., respectively. J. G.

New Reactions of Cantharidin. H. W. Van Urk. (*Pharm. Weekblad*, 1929, **66**, 313–317.)—(1) Cantharidin (0·1 mgrm.) is nitrated by evaporation with 5 c.c. of 50 per cent. nitric acid, and the residue reduced by the addition of two drops of a fresh solution of stannous chloride in 25 per cent. hydrochloric acid. After three minutes on the water-bath 1 drop of a 1 per cent. solution of sodium nitrite is added in the cold, the excess of reagent removed by urea, and a violet-red azo dye produced by the addition of a fresh 1 per cent. solution of α-naphthol in 10 per cent. ammonia. (2) The nitrated cantharidin is mixed with 10 drops of a 1 per cent. alcoholic solution of *p*-dimethyl-amino-benzaldehyde, and dilute sulphuric acid added till an acid strength of 2 per cent. is obtained. In the presence of 0·5 mgrm. of cantharidine a yellow-red residue is obtained on evaporation, and a yellow solution or precipitate on the addition of water. (3) David's test (*Pharm. Ztg.*, 1927, p. 56) may be used as a ring-reaction sensitive to 5 mgrms. of cantharidin by adding a layer of an alcoholic solution of vanillin to a solution of nitrated cantharidin in strong sulphuric acid, when an orange-red ring is obtained. Blank tests on the reagents should be made in the above cases, as these may yield yellow colorations (*cf. Pharm. Weekblad*, 1929, **66**, 101). J. G.

An Impurity in Commercial Narceine which gives a Colour Reaction with Sodium Nitroprusside. J. J. L. Zwikker. (*Pharm. Weekblad*, 1929, **66**, 445–449.)—The methylation of narcotine involved in the synthesis of narceine may result in the production of methyl narceine which gives a red colour in the Bitto sodium nitroprusside reaction (cf. *id.*, 1929, **66**, 50). The test is carried out by the addition of 2 drops each of a 10 per cent. solution of reagent and 4 *N* sodium hydroxide solution to a solution of 50 mgrms. of narceine hydrochloride in 2 c.c. of water, and it is capable of detecting 2 per cent. of methyl narceine. These conclusions were confirmed by the methylation of pure narceine and by the extraction of the methyl compound from commercial narceine, followed by determinations of the m.pt. (234° C., corr.) and equivalent weight of the hydrochloride. J. G.

The Digitalis Glucosides. III. Gitoxigenin and Isogitoxigenin. W. A. Jacobs and E. L. Gustus. (*J. Biol. Chem.*, 1929, **82**, 403–409.)—In a previous communication by Jacobs and Gustus (*J. Biol. Chem.*, 1928, **79**, 553) the conclusion was reached that gitoxigenin is a $\Delta^{\beta,\gamma}$-lactone like digitoxigenin and the related cardiac aglucones, but certain dissimilarities were observed in the derivatives of isogitoxigenin. It is now definitely shown that isogitoxigenin, like the other *iso*-compounds, is a lactone or the lactol form of a hydroxyaldehyde. Contrary to the former belief, isogitoxigenin when saponified displays great stability towards alkali. Renewed study has resulted in a much improved yield of the *iso*-compound from gitoxigenin. Re-investigation of the preparation and composition of the

so-called isogitoxigenonic methyl ester has confirmed the formula, $C_{24}H_{34}O_6$, previously reported. This ester only consumes 1 equivalent of 0·1 *N* alkali when saponified by the method which decomposed both ester and lactone group in the case of the analogous isodigitoxigenin, isostrophanthidin, etc., derivatives, but by the use of stronger alkali and higher temperature, a relatively resistant lactone group can be detected in the isogitoxigenin derivative. Therefore, this substance is a ketolactone ester, and, in conformity with the analogous substances obtained from the other iso-compounds, should be called *isogitoxigenic methyl ester*. The acid obtained on oxidation with hypobromite of isogitoxigeninic acid (prepared by saponification of isogitoxigenin) was given the incorrect formula $C_{21}H_{30}O_6$. It has now been obtained as a pure, anhydrous substance of the formula $C_{23}H_{34}O_6$, and this also consumed an extra equivalent of alkali with stronger alkali and higher temperature. Therefore this substance, *isogitoxigenic acid*, is a lactone acid isomeric with isoperiplogenic acid and isosarmentogenic acid, but with a more stable lactone group. The retention of the secondary hydroxyl in isogitoxigenin was shown by its oxidation to the ketone *isogitoxigenon*. Strong hydrochloric acid converted isogitoxigenic acid into *anhydroisogitoxigenic acid*, by removal of the additional tertiary hydroxyl as water. The lactone group of the anhydro acid was readily decomposed by dilute alkali. Probably the proximity of the extra tertiary hydroxyl group plays a rôle in the stability of the lactone group of isogitoxigenic acid, and for similar reasons isogitoxigeninic acid exists only as the stable lactol and not as a hydroxyaldehyde. Hydroxylamine with isogitoxigeninic methyl ester gave, instead of an oxime, a substance which owed its origin to the intermediate formation of a hydroxamic acid which then lost water with the lactol hydroxyl. It is definitely concluded that gitoxigenin, like digitoxigenin, is a tetracyclic $\Delta^{\beta,\gamma}$-lactone in which a carbon atom, presumably γ to the lactone γ carbon atom, carries a tertiary hydroxyl group. In the formation of isogitoxigenin this hydroxyl functions in an oxidic union between the two carbon atoms with a disappearance of the double bond as follows :—



Attempts at further structural correlation of gitoxigenin with digitoxigenin by means of the iso-compounds will shortly be presented. P. H. P.

Standardisation of Tincture of Digitalis. F. Wokes. (*Quart. J. Pharm.*, 1929, **2**, 48.)—The average potency of eight samples of English digitalis leaves was similar to that of the international standard, but the samples varied in themselves from 64 to 140 per cent. of the average. This accounts for the great variation found in the potency of the tincture. The only safeguard against this variation is a determination of their strength by biological assay. When tested by the cat

method and by the frog method, samples that are fresh give the same results, but if kept more than a month or so the frog method gives somewhat lower results, until, after a few months, the potency (as found by the frog method) has decreased to one-half to two-thirds of its original value, at which point it remains fairly constant for some years. With the cat method, however, the potency decreases much more slowly. R. F. I.

Biochemical.

Acetone as a Control Substance for Respiration and Gas Analysis Apparatus. T. M. Carpenter, E. L. Fox and A. F. Sereque. (*J. Biol. Chem.*, 1929, **82**, 335–343.)—The use of acetone for control tests of the gasometer method, and of the Haldane portable and the Haldane-Carpenter gas analysis apparatus is described, and also the use of combinations of alcohol and acetone with the Benedict universal apparatus. Acetone has several advantages over ethyl alcohol, which was for many years the standard substance for control tests of respiration apparatus and gas analysis apparatus, but it very readily penetrates rubber. Therefore the apparatus for the supply of acetone to the burner must either have mercury-sealed joints, or else be made entirely of glass. A diagram shows the apparatus used for control tests, with acetone, of the gasometer method. It consists of a burette made on the Mariotte principle, a lamp, and a small spirometer which is raised and lowered by a wind-screen wiper. The average of sixteen periods with the gasometer method was 0·746 for the ratio of CO_2 to O_2, and 99·9 per cent. and 100·5 per cent. for the recovery of the theoretical carbon dioxide and oxygen values. The average value of CO_2 to O_2 with the Haldane portable gas analysis apparatus was 0·751 when the changes in the composition of the air current were 2 per cent. or over. The average ratio with the Haldane-Carpenter gas analysis apparatus was 0·746. The ideal control test of a respiration apparatus would be the one that most closely imitates the biological processes, in which there is practically no constancy, *i.e.* a test in which there are quantitative and qualitative variations. The use of alcohol and acetone in various combinations was used to meet these requirements. The average ratio of CO_2 to O_2 found for the various mixtures of ethyl alcohol and acetone was 0·704 with the Benedict universal apparatus, compared with a theoretical average of 0·709. The apparatus for these experiments lacked the perfect prevention of leakage which is now known to be necessary, and with which the percentage results would probably have been comparable with those obtained with the gasometer method. P. H. P.

Effect of Boron Deficiency on the Growth of Tobacco Plants in Aerated and Unaerated Solutions. J. E. McMurtrey. (*J. Agric. Res.*, 1929, **38**, 371–380.)—Normal tobacco plants cannot be grown to maturity in solutions containing only the usually accepted essential elements in distilled water, but if tap water is substituted growth is possible, or if boron (0·5 p.p.m.) is supplied in the distilled water solution. In boron-deficient media growth is much reduced, but still more striking is the injury to the terminal bud, which is the more pronounced in the

more vigorously growing plants, such as those growing in aerated rather than unaerated solutions. The bases of the young leaves are affected, whereas calcium deficiency in the plant affects the tips and margins. D. G. H.

Purification of Picric Acid for Creatinine Determination. S. R. Benedict. (*J. Biol. Chem.*, 1929, **82**, 1–3.)—Purification of picric acid may be satisfactorily carried out by two methods. (1) *From glacial acetic acid.*—The picric acid is dried and 100 grms. dissolved by heating in 150 c.c. of glacial acetic acid. After reaching boiling-point it is filtered hot, allowed to stand for some hours, and if the picric acid has not crystallised out, stirred vigorously or seeded with a minute crystal of pure acid. After 2 hours the liquid is filtered, and the precipitate washed by means of suction with about 35 c.c. of cold glacial acetic acid, as free of acid as possible, and dried at 80–90° C. The yield is about 60 grms. of pure acid which should read 12·5–13·5 mm. by the Folin–Doisy test. (2) *As sodium picrate.*—Six litres of water are heated to boiling in a large porcelain enamelled pail, 250 grms. of anhydrous sodium carbonate added, and when this has dissolved, 500 grms. of the moist technical picric acid are added gradually. Filtration is not usually necessary, but the clear solution is decanted from any dirt settling on the bottom. After standing, the crystallised sodium picrate is filtered off, washed with 2 litres of 10 per cent. sodium chloride solution, dried by suction as completely as possible, the suction stopped, and 500 c.c. of dilute (1:4) hydrochloric acid poured over the mixture, which is then stirred, and attached to the suction pump, 3 more portions of acid being added in succession. Finally the picric acid is washed with 2 litres of cold water, dried at 90° C., and powdered. It should read about 13·5 to 14 mm. by the Folin–Doisy test. D. G. H.

Use of Molybdic Acid as a precipitant for Blood Proteins. S. R. Benedict and E. B. Newton. (*J. Biol. Chem.*, 1929, **82**, 5–10.)—Molybdic acid may be satisfactorily used instead of tungstic acid as a blood protein precipitant, and thioneine and all non-protein blood constituents may be determined in the filtrate as with tungstic acid filtrates. Direct uric acid determinations will be too high, due to the larger amount of thioneine in such filtrates, and the modified indirect method should then be used (*J. Biol. Chem.*, 1922, **51**, 204). The molybdic acid must be of the highest purity, with practically no ammonia. Twenty-five grms. of the acid and 125 c.c. of *N* sodium hydroxide are heated to boiling, and after solution of the acid, filtered, and the residue washed with 100 c.c. of boiling water. The filtrate is cooled and diluted to 500 c.c., and the precipitating reagent is prepared by diluting 1 volume of this solution with an equal volume of 0·4 *N* sulphuric acid. The mixed solution will keep for 6 weeks. For precipitation of blood proteins 1 volume of blood is diluted with 7 volumes of water, 2 volumes of reagent added, and the mixture shaken and filtered. D. G. H.

Colorimetric Determination of the Serum Proteins. D. M. Greenberg. (*J. Biol. Chem.*, 1929, **82**, 545–550.)—Wu (*J. Biol. Chem.*, 1922, **51**, 33 ; ANALYST, 1922, **47**, 265) and Wu and Ling (*Chinese J. Physiol.*, 1927, **1**, 161) published a

method for the determination of plasma proteins based on the colour developed with Folin's phenol reagent. Folin and Ciocalteau (*J. Biol. Chem.*, 1927, **73**, 627) improved the phenol reagent by the addition of lithium sulphate. A colorimetric method is now described by the author for the determination of serum proteins based on the colour developed with Folin's phenol reagent, and on the method of Howe (*J. Biol. Chem.*, 1921, **49**, 93 ; ANALYST, 1922, **47**, 128) of salting out the globulin with sodium sulphate solution. The sodium sulphate was found to have almost no effect on the colour developed ; hence the albumin can be determined directly on an aliquot part of the filtrate. The total serum protein and the albumin fraction can be determined, and the globulin found by subtraction, but it is better to determine both albumin and globulin separately. The method is rapid and relatively simple, only 0·5 c.c. of serum is needed for a determination, and results were obtained accurate to about 5 per cent. The tyrosine equivalents of the serum proteins were determined for human blood, with composite samples of serum, by parallel determinations by the colorimetric method, and by Kjeldahl's nitrogen method. The average value of the factors obtained, in terms of mgrms., of protein that give a colour equivalent to that given by 1 mgrm. of tyrosine are:—Total protein, 16·0 ; albumin, 16·6 ; globulin, 14·4. P. H. P.

Plant Haemagglutinins with Special Reference to a Preparation from the Navy Bean. V. R. Goddard and L. B. Mendel. (*J. Biol. Chem.*, 1929, **82**, 447–463.)—A non-toxic, highly potent, soluble, haemagglutinating protein having the characteristics of an albumin has been prepared in dry form from navy beans (*Phaseolus communis*). The method of Osborne, Mendel and Harris (*Amer. J. Physiol.*, 1905, **14**, 259) for the preparation of ricin was closely followed. A quantitative, macroscopic method for the measurement of haemagglutination has been devised, and used for a study of the variables which affect the reaction. The procedure used is as follows :—Rabbit blood freshly removed from the ear vein and defibrinated was centrifuged until the serum could be removed. The corpuscles washed twice with 0·89 per cent. sodium chloride solution were suspended therein, 2·5 c.c. of packed corpuscles being suspended in a total volume of 100 c.c. of the isotonic sodium chloride solution. Aliquot parts of this suspension were used for the individual tests. As a rule, 0·5 c.c. of the corpuscle suspension was added to 0·5 c.c. (making a total volume of 1 c.c.) of 0·89 per cent. sodium chloride solution containing varying amounts of the agglutinin preparation to be tested. The reaction was carried out in chemically clean glass tubes, 75 × 8 mm., placed in a rack which would keep them at an angle of 40°, and incubated at 40–44° C., for 12 hours. Under these conditions, when agglutinin was present, the cells became firmly attached to the glass, and remained unmoved when the tube was returned to the vertical position ; this adherence of cells never occurred in the absence of haemagglutinin, nor when the latter was too dilute to bring about clumping detectable upon microscopic examination. Results show that agglutinin is still active, for rabbit erythrocytes, at a dilution of 1 in 6,000,000; by analogy with immunological terminology this figure could be called its titre,

since clumping is effected in a total volume of 1 c.c. of solution containing only 0·0006 mgrm. of agglutinin by dry weight. The agglutinin gave very similar results with the erythrocytes of man, rabbit, dog and duck. The erythrocytes of the hen required 10 times as much agglutinin as rabbit erythrocytes. The indispensability of electrolytes and the inhibiting influence of certain proteins, notably those of egg albumin and the serum proteins, are demonstrated. Chemical changes in the protein which lead to denaturation or hydrolytic cleavage are shown to be accompanied by a lessened haemagglutinative potency. The mode of action, the chemical nature, and some practical aspects relating to the application of the procedures to the production of therapeutic serums are discussed. A sample of agglutinin from the bean, which had been stored for over 2 years, showed no appreciable loss in activity. The substance was obtained in the form of a nearly white powder. P. H. P.

Determination of Sugar in Blood. I. Observations upon Benedict's Alkaline Copper Solution. M. R. Everett. (*J. Biol. Chem.*, 1929, **82**, 369–376.)—The work of Benedict (*J. Biol. Chem.*, 1925, **64**, 207 ; ANALYST, 1925, **50**, 414 ; *J. Biol. Chem.*, 1926, **68**, 759 ; ANALYST, 1926, **51**, 467) and Folin (*J. Biol. Chem.*, 1926, **67**, 357 ; ANALYST, 1926, **51**, 309 ; Folin and Svedberg, *J. Biol. Chem.*, 1926, **70**, 405) reveals an effect of sulphite in alkaline copper reagents which has been only partly appreciated heretofore. A careful study of the effect of sulphite upon alkaline copper reagents has now been made, and the effect is a general one. It is not entirely inactive with any copper mixture, and it always causes intense reduction by itself, if sufficient is added to a copper mixture. Without exception there is a lowering of the apparent blood sugar values, regardless of the nature of the other components of the alkaline copper solutions. Blood sugar values 10 to 20 per cent. lower than similar Folin–Wu values were always obtained with sulphite and copper reagents which contained malate, glycine salicylate, or pyridine in place of tartrate, or with reagents in which tartrate was combined with these substances, etc., and the Folin–Wu copper reagent was found to give similar low values when proper amounts of sulphite were added to it. The sensitiveness of alkaline copper mixtures to reduction by sulphite is quite variable, *e.g.* decreasing alkali concentration increases the sensitiveness. The author was studying the preparation of an ideal copper mixture, for securing low blood sugar values, containing glycine, sodium sulphite, and an inactive salt, when the new alanine, tartrate and sulphite reagent of Benedict (*J. Biol. Chem.*, 1928, **76**, 457 ; ANALYST, 1928, **53**, 230) was reported, and further work on the glycine reagent was unnecessary. However, it is demonstrated that Benedict's new blood sugar method does not give true blood sugar values. The apparently low values for blood sugar, given by the sulphite-containing reagents, are due partly, if not entirely, to an unequal and deceptive fading of the colours in the standard and the unknown, and not to an increased specificity for glucose. Benedict's new method has given negative values for hydrolysable blood sugar when other methods have proved the presence of appreciable amounts of such sugar. The rate of fading appears

to be different for different samples of blood filtrate. The use of 4 c.c. of Folin's acid molybdate solution seems to eliminate the unequal fading, and is suggested as a possible modification. Figures given show that the original blood sugar values are close to the Folin values, and that added glucose is apparently recovered from fermented filtrates. P. H. P.

Dextro-rotatory Sterol of Yeast. Zymosterol. H. Penau and G. Tanret. (*Comptes rend.*, 1929, **188**, 1317–1319.)—Analysis of zymosterol isolated from yeast-fat by Smedley and Maclean (*Biochem. J.*, 1928, **22**, 22) indicates the formula, $C_{27}H_{42}O_2,H_2O$, corresponding with that of an oxyergosterol. This compound is markedly more soluble than ergosterol, 1 part dissolving at 18° in 18 parts of absolute alcohol, 26 of 95 per cent. alcohol, 11 of ether, or 15 of acetone ; 1 grm. dissolves in 80 vols. of olive or sesame oil, whereas 325–350 vols. are required with ergosterol. The iodine value (190–201) confirms the presence of three ethylenic linkings in the molecule, which contains also two alcoholic groupings. One kilo. of fresh yeast contains from 1 to 1·5 grm. of ergosterol and 1 grm. of zymosterol. T. H. P.

Cytological Study of Water-Soluble and Fat-Soluble Constituents of Citrus. J. Dufrenoy. (*J. Agric. Res.*, 1929, **38**, 411–429.)—This work was undertaken in order to get a clearer notion of the biochemical changes which take place in the normal citrus tree and result in the production of highly attractive fruit, and to learn what undesirable biochemical phenomena are associated with various pathological conditions or blemishes. A study has therefore been made of the cells of both normal and pathological tissues ; first, in the living condition, with the use of vital dyes ; secondly, in the post-vital condition ; and thirdly, after killing with suitable killing fluids. Twenty-one figures of histological sections are given showing stained cells of leaves and fruit. The results show that cells of green parts of citrus leaves or fruits normally contain one large vacuole, which can be stained in the living cell by the use of neutral red in 10 per cent. cane-sugar solution as a dye. Gentle excitations tend to cause the large vacuole to break into a number of smaller vacuoles. Greater shock may result in the browning of the vacuolar content and ultimately in the collapse of the cell, when the vacuolar material is thrown out of colloidal states and mixed with the cytoplasmic constituents, on which it exercises a coagulating effect. Cells of green parts of citrus contain in their cytoplasm short rod-like mitrochondria and starch-forming chloroplasts. A number of conditions may result in the breaking down of the lipoprotein complex of which the normal mitochondria and plastids are made, resulting in such cytological phenomena as are naturally observed in the leaf tissues affected by puncturing of the cells, or such as may be experimentally induced in the peel of the fruit by the ethylene-gas treatment for artificial colouring. The natural colouring process of the fruit is concomitant with starch translocation from the chloroplasts in the cells of the three upper layers in the peel. As starch disappears, fat bodies develop in the chloroplasts, and the orange pigment that gives the fruit its colour goes into solution in the fat bodies inside the chloroplasts.

Artificial gas treatment gives the same result. The action of selenium salts may induce mitochondria which seem to be inactive normally, to develop red pigment.
P. H. P.

Antineuritic and Water-Soluble *B* Vitamins in Beef and Pork. R. Hoagland. (*J. Agric. Res.*, 1929, **38**, 431–446.)—As a result of feeding experiments with pigeons, the author reported (*U.S. Dept. Agric. Bull.*, 1923, **1138**, 48 pp. *Amer. J. Physiol.*, 1924, **67**, 300) that lean pork was an excellent source of vitamin *B* (antineuritic vitamin), but that beef contained much less of this vitamin. In view of the present knowledge concerning the multiple nature of water-soluble vitamin *B* it is evident that the antineuritic value of lean pork or beef is not necessarily an indication of the amount of the vitamin *B* complex present. Therefore the relative amounts of the antineuritic and the composite water-soluble *B* vitamins in lean pork and beef have now been studied by feeding tests with pigeons and with rats, respectively. It is shown that lean pork, and fresh and smoked ham are excellent sources of the antineuritic vitamin, and compare favourably in this respect with brewers' yeast. Five per cent. of dried lean pork in a ration protected pigeons against both polyneuritis and loss in weight for 8 weeks and longer. This is equivalent to a daily intake of 1 grm. of dried pork for a pigeon weighing 400 grms. Beef contains much less of the antineuritic vitamin ; from 35 to 40 per cent. of dried lean beef were required for protection, corresponding to a daily intake of from 7 to 8 grms. of dried beef for a 400 grm. pigeon. Lean pork is a good source of water-soluble *B* vitamins, but not so good as either brewers' or bakers' yeast. From 15 to 25 per cent. of dried lean pork in the diet furnished sufficient water-soluble *B* vitamins for excellent growth in rats, as compared with 5 per cent. of dried brewers' yeast. During a period of 60 days, approximately 1·6 grms. of dried lean pork or 0·5 grm. of dried brewers' yeast daily proved adequate for growth in male rats. No material difference was observed between fresh and smoked hams as sources of the water-soluble *B* vitamins. The fact that dried pork was approximately as rich in the antineuritic vitamin as dried brewers' yeast, whilst the latter was from 3 to 4 times richer in water-soluble *B* vitamins than the former, indicates that the dried brewers' yeast was correspondingly richer in the heat-stabile vitamin than dried lean pork. Lean beef contained much less water-soluble *B* vitamins than lean pork ; from 40 to 70 per cent. of dried, fresh beef was required for excellent growth in rats, as compared with 15 to 25 per cent. of dried lean pork or 5 per cent. of dried brewers' yeast. P. H. P.

Chemical Detection of Vitamin *C*. B. Glassmann and A. Posdeew. (*Z. Unters. Lebensm.*, 1929, **17**, 191–200.)—Exhaustive experiments on the qualitative and quantitative uses of Bezssonow's phosphomolybdotungstic acid reagent for vitamin *C* (ANALYST, 1921, **46**, 411, 462 ; 1924, **49**, 594) have shown that the reagent is not reliable, since it reacts with tannins present in plant substances at ordinary temperatures, and with carbohydrates and other plant substances at 100° C. A close correspondence was found between the colour produced and the carbohydrate contents of a number of vitamin-containing juices, while 0·5 to 1·5

mgrm. of tannic acid in 10 c.c. gives a colour which can be matched accurately against that produced with the standard solution of hydroquinone used in the experiments. It is concluded that animal experiments are at present the only reliable means of estimating vitamin *C*. J. G.

Organic Analysis.

Cold Test of Fatty Oils. R. R. Matthews. (*J. Amer. Leather Chem. Assoc.*, 1929, **24**, 242.)—A report has been issued on the " Cloud " and " Pour " points of neatsfoot oil by a joint committee of the American Society for Testing Materials and the American Leather Chemists' Association. The A.S.T.M. standard method D97–28 for Cloud and Pour Point of petroleum products was used by 13 analysts on two neatsfoot oils, one of a low and one of a high cold-test. The report states that the Pour Points agreed closely. The table given shows that for the low cold test oil eleven members returned it as 20° F., one as 15° F., and one as 10° F. For the high cold test oil ten members reported the Pour Point as 25° F., and the other three as 15° F., 20° F. and 30° F. Two further samples of oil were sent out for the purpose of comparing the test at 2° intervals with that at 5° intervals. Complete concordance was not obtained in either case, and no advantage is gained by taking readings at 2° intervals.

The test for Cloud Point was not reliable, and it was decided to do nothing further on it so far as fatty oils are concerned. R. F. I.

Action of Bromine on Insect Oils. J. Timon-David. (*Comptes. rend.*, 1929, **17**, 1122–1124.)—The iodine values of certain insect oils, particularly of the Lepidoptera, are high. The iodine values (Wijs) of the following caterpillar oils were :—*Vanessa urticae* L., 159·7 ; *Pieris brassicae* L., 149·9 ; *Saturnia Pernyi* Guer., 140·4 ; *Arctia caja* L., 133·3 ; *Malacosoma franconica* Esp., 138·0; and, of the Chrysomelids of the Coleoptera, the iodine value of the oil from the larvae of *Colaspidema atra* Ol. was 113·4; that of the imago of *Leptinotarsa decemlineata*, Say., 108·6, and of *Galerucella luteola*, Mull. 118·2. The action of bromine showed that the oils may be classified as : (1) Those giving high hexabromide values (*Saturnia pernyi*, Guer., *Pieris brassicae* L.) ; lower hexabromide values (*Colaspidema atra* Oliv., *Leptinotarsa decemlineata* Say., *Thaumetopoea pityocampa*, Sch.) ; and no hexabromide values (*Ergastes faber* I., *Pyrausta nubilitatis*, Hubn.). The differences are probably largely due to methods of feeding. D. G. H.

Determination of Hygroscopic Moisture in Coals. H. Löffler. (*Chem. Ztg.*, 1929, **42**, 411.)—The method recommended, especially suitable for brown coals, is a modification of that of Abderhalden and Blacher in which the coal is dried in a vacuum at 60° to 70°. The modification consists in a more regular application of the heat. One or two grms. of the coal are placed in a weighing-tube inserted horizontally in the neck of a wider tube, through which the vapour of a liquid of the required boiling point freely passes. The liquid is contained in an electrically heated conical flask, loss being avoided by a globe condenser. The

end of the weighing-tube is connected with one end of a retort of special shape, the narrow end of which leads to the vacuum pump. In the body of the retort is placed granulated calcium chloride. During the test the pressure is reduced to 10 mm., and the heating continued for one hour at most. The weighing-tube containing the dried coal is then removed, the stopper inserted, the whole cooled in a desiccator and the loss of weight determined. Agreement in duplicates is within 0·1 per cent. The apparatus is obtainable from Messrs. Woytacek, Starhemberggasse, Vienna IV. R. F. I.

Determination of Nitrogen by the Kjeldahl Method, applied to the Analysis of Colouring Matters and Intermediates. P. Sisley and M. David. (*Bull. Soc. Chim.*, 1929, **45**, 312–324.)—The various modifications suggested to render the Kjeldahl method applicable to compounds in which the nitrogen present is united either to another nitrogen or to an oxygen atom, are discussed. The low results obtained with nitrobenzene, *p*-nitrotoluene, etc., are due to loss of the substance by volatilisation ; preliminary sulphonation helps in these cases, but is not of universal application.

The following procedure yields accurate results for the nitrogen content of a large number of nitro-, nitroso-, azoxy-, and azo- compounds. From 0·5 to 1 grm. of the substance, according to its nitrogen content, is heated in a 250 c.c. pyrex flask with 10 c.c. of pure alcohol and 5 c.c. of water. From 2 to 4 grms. of sodium hydrosulphite are then added in 1 grm. quantities, the liquid being heated to boiling under a reflux condenser after each addition. With substances which are readily soluble and reducible, it is not necessary to heat to boiling, decolorisation being rapid ; substances sparingly soluble in water must be very finely powdered. After completion of the reduction, which requires at most 10–15 minutes, the flask is allowed to cool, 10 c.c. of sulphuric acid (66° Bé.) being then added. The liquid is heated gently, with the neck of the flask inclined, so that the bulk of the alcohol is expelled. When the liquid begins to froth, 0·5 grm. of copper sulphate, 6–8 grms. (10 grms. less the weight of hydrosulphite used), and 12 c.c. of sulphuric acid are added. The heating is continued, at first gently and afterwards more strongly, for 20 to 30 minutes, when the liquid should have a pure blue colour. The solution is diluted to 300 c.c. in a 700 c.c. Erlenmeyer flask, and is then treated with excess (100 c.c.) of sodium hydroxide solution (36° Bé.), 5 c.c. of freshly prepared 20 per cent. sodium sulphide solution, and a little granulated zinc. The distillation is carried out in the Wagner apparatus, the boiling being continued for 45 minutes. The best indicator is methyl red. T. H. P.

Inorganic Analysis.

Separation of Beryllium from Aluminium, Iron, and Copper by o-Hydroxyquinoline. M. Niessner. (*Z. anal. Chem.*, 1929, **76**, 135–145.)—As beryllium does not form an insoluble hydroxyquinolate, the reagent permits of the following rapid and accurate separations :—*From aluminium.*—The neutral or feebly acid solution (200 to 300 c.c.) is heated to 70° C., stirred vigorously, and an

excess of precipitant (a mixture of strong ammonium acetate and 2 per cent. alcoholic hydroxyquinoline solutions) is added. The precipitate is left to settle on a water-bath at 70° C., collected on a porous glass crucible, and washed with hot water until the washings are colourless. It is weighed after drying to constant weight at 110° C. The beryllium in the filtrate is precipitated by boiling with ammonia. *From iron.*—The operation is like the preceding, 2 grms. of tartaric acid having been added to the solution. *From copper.*—The copper is precipitated according to Berg's directions (ANALYST, 1927, **52**, 302) from acetate solution, and the beryllium in the filtrate is precipitated with ammonia. (*Cf.* Kolthoff and Sandell, ANALYST, 1928, **53**, 508.) W. R. S.

Uranyl Zinc Acetate as Reagent for the Quantitative Determination of Sodium. I. M. Kolthoff. (*Chem. Weekblad*, 1929, **26**, 294–298.)—The investigations of the author and others on this reagent (Blanchetière, ANALYST, 1923, **48**, 456; Kolthoff, *id.*, 1927, **52**, 304; 1928, **53**, 456) are summarised. Sodium (0·002 to 0·04 per cent.) may be detected in salts of zinc, iron, potassium, copper, lead, bismuth, mercury, or cadmium. Arsenates, ferrocyanides and oxalates interfere, phosphates should be removed by magnesia mixture, and sulphates as barium sulphate. Ammonium, zinc and magnesium salts do not interfere. For quantitative work an accuracy of +0·5 to −0·2 per cent. is normally obtainable if the alcohol used for washing purposes is previously saturated with the triple salt, which is soluble in water, insoluble in the reagent or in 95 per cent. alcohol, and slightly soluble in absolute alcohol. Sodium in potassium chloride is determined by the addition to 1 grm. of sample in 5 c.c. of water of a warm solution of 2 grms. of ammonium perchlorate in 3 c.c. of water and 25 c.c. of 95 per cent. alcohol. The cooled mixture is then filtered, washed 5 times with 2 c.c. of 95 per cent. alcohol, the filtrate evaporated, the residue dissolved in 1 c.c. of water, and the sodium precipitated with 10 c.c. of reagent. An accuracy of 1 per cent. is obtainable for potassium salts containing 0·1 per cent. of sodium. Sodium in lithium salts is determined, after removal of the lithium, by Palkins' method (*id.*, 1917, **42**, 54), or as carbonate or fluoride. A solution of 0·2 grm. of salt in 10 c.c. of water is allowed to stand for 20 hours with 5 c.c. of a 10 per cent. ammoniacal solution of ammonium fluoride and 10 c.c. of 96 per cent. alcohol, and the lithium fluoride filtered off and washed with 50 per cent. alcohol containing a little ammonium fluoride. The filtrate is evaporated with 8 c.c. of 6 *N* hydrochloric acid, and the sodium determined in the residue. The method, which may be combined with the determination of lithium, may be used for 0·01 per cent. of sodium in 0·5 grm. of lithium chloride. J. G.

Simultaneous Determination of Orthophosphate and Pyrophosphate. (*a*) **R. Dworzak and W. Reich-Rohrwig.** (*Z. anal. Chem.*, 1929, **77**, 14–37.) (*b*) **W. Stollenwerk and A. Bäurle.** (*Id.*, 81–111.)—(*a*) The gravimetric method of Berthelot and André was modified as follows: The solution of the phosphates (total phosphorus about 0·2 grm.) is added to a mixture of 100 c.c. of acid magnesia mixture ($MgCl_2.6H_2O$, 55 grms.; NH_4Cl, 105 grms. per litre, faintly

acidified with hydrochloric acid against methyl red), and 20 c.c. each of ammonium chloride and acetate solutions, both saturated in the cold; the precipitate is dissolved by addition of 40 c.c. of 2 *N* acetic acid. The liquid (200 to 300 c.c.) is heated on the water-bath for 4 to 5 hours in the covered beaker, after which the magnesium pyrophosphate is collected and washed with hot water containing ammonium chloride and acetate, and acetic acid. Concentration of the filtrate on the water-bath yields another 0·5 to 3 per cent. of the pyrophosphate present, which is treated like the bulk. The filtrate will now remain clear, even on more protracted heating; the orthophosphate contained therein may be determined as usual. The pyrophosphate precipitates are dissolved off the filters in warm dilute nitric acid, and the solution boiled for an hour; its orthophosphate content is then determined. The method is satisfactory except when the relative amount of pyrophosphate is small; in this case the addition of the 2 *N* acetic acid is reduced to 1 to 5 c.c., and the solution evaporated on the water-bath to small bulk.—A volumetric method was elaborated: it answers well except for mixtures containing very much pyrophosphate. It is based on the property of metallic (*e.g.* stannic) pyrophosphate precipitates of re-dissolving, with the formation of soluble complexes, so long as the soluble pyrophosphate is in excess; when an excess of metallic salt is added to such a solution, a cloudiness appears as soon as a definite ratio of base to acid is exceeded. A solution of uranium acetate (20 grms. per litre) is used, and its uranium content determined gravimetrically. Twenty-five grms. of the salt to be tested are dissolved in 500 c.c. of water, and 25 c.c. portions measured out. A preliminary test is made, in which the solution diluted to 150 c.c. is titrated, with constant stirring and dropwise addition of the uranium solution, to permanent cloudiness. For the final determination, the aliquot portions are treated with a quantity of *N* ammonia depending on the ascertained volume of uranium solution: 0·1 for 10, 0·5 for 20, 1·5 for 30, 2·5 for 40, and 4 c.c. of ammonia for 50 of uranium solution. The ten portions are then titrated with the uranium solution, the first portion being given 5 to 7 c.c. less than was ascertained for the preliminary test, and each succeeding portion one c.c. more than its predecessor. After an interval of at least 6 hours, the assays are inspected, some being clear, the others cloudy. The volume of uranium solution required lies between those of the last clear and the first cloudy test; the intermediate reading is estimated according to the depth of the cloudiness. The calculation is based on the ratio $U=2P_2O_5$, the formula of the complex probably being $Na_6[UO_2(P_2O_7)_2]$, whence $Na_4P_2O_7=2{\cdot}234U$.

(*b*) The analytical application of the pyrophosphates of silver, copper, the alkaline earths, beryllium, aluminium, and lead was investigated; they proved unsuitable for quantitative purposes, with the exception of the alkaline-earth compounds. The process worked out is an indirect one, the phosphates being precipitated by baryta of known strength, with subsequent gravimetric or volumetric determination of the excess of precipitant. The precipitates are always the fully saturated salts $Ba_3(PO_4)_2$ and $Ba_2P_2O_7$. For the gravimetric determination, 1 grm. of the salt is dissolved in 200 c.c. of distilled water; an aliquot volume is diluted to 50 c.c. in a 200 c.c. flask, boiled, treated with an excess of

0·025 *N* barium hydroxide, cooled to room temperature after insertion of a soda-lime tube, and made up to bulk with carbon dioxide-free water. After settling, 100 c.c. are filtered through a dry Gooch crucible, and the excess of baryta in the filtrate determined as sulphate. In another portion the total P_2O_5 is determined as $MgNH_4PO_4$ after 15 minutes' boiling with dilute nitric acid. Let B=BaO combined with P_2O_5; P=total P_2O_5; X=ortho-P_2O_5; $(P-X)$=pyro-P_2O_5; then $\frac{460{\cdot}11X}{142}+\frac{306{\cdot}74}{142}(P-X)=B$, whence $X=\frac{142B-306{\cdot}74P}{153{\cdot}37}$. For the volumetric determination, the salts must be converted into NaH_2PO_4 and $Na_2H_2P_2O_7$ by neutralisation with acid against methyl orange; a measured excess of 0·1 *N* barium hydroxide is then added:

(1) $2NaH_2PO_4+3Ba(OH)_2=Ba_3(PO_4)_2+2NaOH+4H_2O$.

(2) $Na_2H_2P_2O_7+2Ba(OH)_2=Ba_2P_2O_7+2NaOH+2H_2O$.

The excess of baryta is ascertained in one-half of the filtrate, which is run into an excess of standard acid to prevent precipitation of carbonate; the excess acid is titrated with standard alkali (1 c.c. 0·1 *N* alkali=0·00355 grm. ortho-P_2O_5 and 0·0071 grm. pyro-P_2O_5). Let x=c.c. 0·1 *N* alkali combined to orthophosphate, a=c.c. total alkali, and P=total P_2O_5, then $P=0{\cdot}00355x+(a-x)\ 0{\cdot}0071$.

W. R. S.

Colour Indicators for Permanganate Titrations. (*a*) **Determination of Ferrocyanide. J. Knop.** (*Z. anal. Chem.*, 1929, **77**, 111–125.) (*b*) **Determination of Iron. J. Knop and O. Kubelkova.** (*Id.*, 125–130.)—(*a*) The two following triphenylmethane dyes are suitable as indicators in permanganate (not dichromate) titrations: (1) "*Erioglaucin A*" and (2) "*Eriogrün B*," made by *Anilinfarben- und Extraktfabriken vorm. J. R. Geigy*, Basle. They are used in 0·1 per cent. aqueous solution. One c.c. of (1) (blue), added to 200 to 400 c.c. of an acidified solution, produces a green colour, giving a grey transition tint with 0·1 c.c. of 0·01 *N* permanganate; a further 0·2 c.c. changes the colour to red. Two c.c. of (2) (blue-green) under the same conditions produces a yellow liquid giving a full orange-yellow tint with 0·1 c.c. of the permanganate solution. The colour changes are reversible, and the coloration is more intense than that of permanganate. For the titration of ferrocyanide, less than 1 grm. of salt is dissolved in 400 c.c. of water and 20 c.c. of 8 *N*-sulphuric acid. The above quantities of either indicator are added, and the solution titrated with 0·05 *N* permanganate. The colour changes in presence of ferricyanide are: Yellowish-green to orange-brown for (1), full yellow to orange-yellow for (2). Artificial light does not vitiate the result, and the end-point is more easily observed. (*b*) In the permanganate titration for iron, the transition in the acid sulphate solution is from grass-green, over grey to red for (1), from yellow to orange for (2). The indicators proved specially suitable in micro-work.

W. R. S.

Determination of the Purity of Potassium and Sodium Ferrocyanides by Titration with Zinc Sulphate Solution. Farbsalz-Gesellschaft, Berlin. (*Chem. Ztg.*, 1929, **53**, 399.)—Discrepant results having been obtained owing to

the use of different methods of analysis, most of the firms interested in ferrocyanides now precipitate with zinc sulphate, the product formed containing two $Fe(CN)_6$ groups per three atoms of zinc. The titre of the zinc sulphate solution, containing 28·755 grms. of the pure salt per litre, is determined by means of a solution containing 10 grms. of potassium ferrocyanide in 500 c.c. Fifty c.c. of the latter, diluted with 100 c.c. of water, and mixed with 10 c.c. of 0·1 *N* iron-free sulphuric acid, are titrated with the zinc sulphate solution at 15–20° C. To determine the end of the titration, 2 or 3 drops of the liquid are transferred by a thin glass rod to the same place on a strip of ash- and iron-free filter-paper and, after 20 or 30 seconds, when the liquid has spread, a similar amount of aqueous 15 per cent. pure ferric ammonium alum is placed close by on the paper : no blue colour should appear at the contact point of the liquids within 2 or 3 minutes.

In carrying out the drop test, small depressions should be made in the filter-paper with a glass rod and the drops should be placed in depressions 1–1½ cm. apart. The drops should flow together slowly, so that the precipitated zinc ferrocyanide does not come into contact with the ferric ammonium sulphate. The preliminary test should be followed by a more exact one.

The procedure is similar in determining the purity of a commercial ferrocyanide. The titre of the zinc sulphate solution must be determined with either sodium or potassium ferrocyanide, according to whether the sodium or potassium salt is to be analysed, since different results are obtained with the two salts. Moisture in ferrocyanides is determined by drying to constant weight at 125° C., the excess (over the water of crystallisation) corresponding with the proportion of pure salt present being extraneous moisture. (*Cf.* ANALYST, 1929, 38.)

T. H. P.

Reviews.

ESSENTIALS OF QUALITATIVE CHEMICAL ANALYSIS. By J. C. WARE, Sc.M., Ph.D. Pp. xii + 351, with 27 illustrations and 6 coloured plates. London: Chapman & Hall, Ltd. 1928. Price 17s. 6d. net.

This volume is intended for the use of students who propose to adopt the profession of analytical chemistry, and provides a thorough grounding in the theoretical principles of analysis and their application in practical work. For those merely taking chemistry as a subsidiary subject in a degree course the book is too detailed in the earlier portions, since before taking up practical work the beginner is expected to possess a reliable knowledge of ionisation, equilibria, solubility products, partition coefficients, etc., for which, unfortunately, the time of this class of student is too limited.

The book is divided into five parts, the first of which deals with the various fundamental factors involved in solution and precipitation. Parts II and III give the reactions and separations of some two dozen common metallic radicals and of a similar number of acidic radicals in simple solution. Part IV provides complete schemes and separation tables for the analysis of complex mixtures, both solid and in solution, based upon the earlier work, and the text is brought to a close by suggestions for the arrangement of courses, the preparation of reagents and other solutions, the management and equipment of a general academic laboratory, and various tables of service in analytical work.

The illustrations comprise, in addition to 11 figures of apparatus, some 15 half-tone reproductions of photographs taken in various American industrial laboratories, together with one of historic interest, depicting Liebig's famous laboratory as it appeared in 1846. The idea of these is, no doubt, to stimulate the interest of the student, but whether this is achieved or not, such pictures add to the value of the work. The plates depict the colours obtained in various reactions, flame tests and borax beads, and, with one or two exceptions, give an accurate rendering of the actual tints observed in practice. It is unfortunate that the references to these plates in the text are not indicated by page numbers, although these occur in the index.

The text is legible and lucid, although occasional traces of American grammar and expression are evident, but these do not introduce any ambiguity. The preface provides sound and useful advice for the student, and contains a few somewhat facetious sentences on the names given to the colours of precipitates, etc. Notwithstanding this, in the text such descriptions as "seal brown," "shell pink," and "flesh colour" occur, the last being given as the hue of manganese sulphide on p. 109, whereas the same precipitate is stated to be "pink" on p. 165.

This volume is remarkably free from typographical and other errors, the few encountered being of minor importance, but it is probable that English demonstrators will disagree with the advice given on p. 116: "it is not necessary to obtain perfect tests (for a radical) by all methods. Obtain one good test and be satisfied with that."

The analytical schemes, whilst in general following the usual groupings, are excellent, and include some novel modifications which facilitate precipitation and separation and avoid the usual students' wastage of hydrogen sulphide.

An admirable feature of this volume is the series of questions inserted at the end of each chapter and also introduced throughout the text. These are so framed as to test the student's knowledge of the reactions he is performing and to ensure a complete understanding of the basic principles of analysis.

The text-book is, as a whole, an admirable production, is provided with an adequate and accurate index, and is by far the best and most educative work on elementary qualitative analysis that the reviewer has yet seen. At the price charged the volume is exceedingly good value, and well deserves the attention of all interested in the teaching of analytical chemistry. T. J. WARD.

THE PROBLEM OF FERMENTATION: THE FACTS AND HYPOTHESES. By M. SCHOEN, with an introduction by Professor A. FERNBACH. Translated from the French by H. LLOYD HIND, B.Sc., F.I.C., and revised and enlarged by the author. Pp. xii + 211. London: Chapman & Hall. 1928. Price 21s. net.

The appearance of a work on this important subject is a welcome addition to scientific literature, and the author—so long associated with Professor A. Fernbach at the Pasteur Institute in Paris—is well qualified for the task of writing it. It may, indeed, be said that he has produced a book that will be appreciated by all who are interested in the fundamental branch of biochemistry with which it deals.

From the earliest times the study of fermentation phenomena has attracted the attention of those on whose work modern biochemical science is founded. The chief interest has, for obvious reasons, been centred in the change brought about by the action of yeast on sugar. But dating from the classical work of Pasteur to the present time, it has been recognised that alcoholic fermentation has a significance far beyond the production of beer and wine.

Alcoholic fermentation has, as Professor Fernbach remarks in the introduction to the book before us, "become the prototype of chemical actions brought about by micro-organisms," and we may add that together with its congeners it has been shown to occur in anaerobic respiratory processes in the higher organisms. A book on so comprehensive a subject should therefore command a wide circulation.

Pasteur's theory that fermentation is life without air has been frequently attacked, because that eminent *savant* could not show that in presence of air fermentation of sugar by yeast is completely arrested. As Dr. Schoen points out, Pasteur proved that the quantity of alcohol formed by unit weight of yeast in unit time is less in presence than in absence of air. But he recognised also that multiplication of cells can only occur continuously in presence of oxygen, observations that have been made use of in the manufacture of fermented beverages, as well as in that of yeast itself. This matter is discussed in the opening chapter of Dr. Schoen's book, in which the recent observations of Meyerhof are cited as completely proving Pasteur's theory.

The book is divided into fourteen chapters in which a connected account is given of the chemistry of fermentation, which may now be regarded as one of the forms of intramolecular or anaërobic respiration. The latest views on the nature of the intermediate products of alcoholic fermentation are discussed, and the conclusion arrived at by the author is that pyruvic acid is an indispensable link in the chain of reactions leading to the conversion of sugar into alcohol and carbon dioxide. He does not, however, deny that this acid may be formed through the intermediary of methyl-glyoxal, although this latter has never been isolated from fermentation products. A clear and concise account is given of the significance of the hexose phosphates (discovered independently by Harden and Young and by Iwanoff in 1906), in alcoholic fermentation, muscle contraction (Embden) and ossification (Robison). We presume that the work of Robison on the existence of a

monophosphoric ester of trehalose, and that of Morgan and Robison on the constitution of hexosediphosphoric acid, appeared too late for an account to be included in the text.

In dealing with the hexoses, the author speaks of the epimeric α- and β- forms as tautomeric forms. As regards the structure of the hexoses, the normal forms are stated, quite correctly, on the evidence of Charlton, Haworth and Peat (1926), to possess an amylene oxide ring, but no mention is made of the observation of the last-mentioned authors that the γ-sugars possess a butylene oxide ring. Indeed, Irvine's work is cited, without qualification, that γ-glucose possesses a propylene oxide ring, and that γ-fructose possesses an amylene oxide ring. Structural considerations are of the utmost importance to the subject matter of the book, for recent work has indicated that the γ-sugars and the normal sugars each play their own specific rôle in metabolism.

The chapter on hydrogen and the phenomena of fermentation, which appears only in the English edition, is dealt with historically and constitutes one of the most valuable portions of the book. In an earlier chapter the author had rightly claimed that recent facts—more especially those put forward by Meyerhof—apparently far removed from fermentation phenomena, had accorded to Pasteur's conception of fermentation the demonstration he desired. The idea that active hydrogen, and not active oxygen, is the initiator in oxidation-reduction changes was first shown to be possible by Pasteur's discovery of anaërobic organisms. This idea of active hydrogen as the initiator of respiratory changes has been developed by Wieland and by Thunberg, who believe it to apply generally. Warburg, on the other hand, favours the view that active oxygen in presence of iron and other metals is the initiator in changes of the kind under discussion. The arguments *pro* and *con* are set forth clearly in the book. May it not be, however, that both views are correct according to circumstances? The autoxidisable substance, glutathione, discovered by Hopkins, is present in tissues, for the most part in the reduced form; but both the oxidised and the reduced forms are catalysed by iron and other metals, as shown by Harrison.

The author does well in this chapter to point to the observations of Quastel and of Kostytschew, who throw doubt on the existence of so many specific enzymes which it has become the fashion of workers—especially in Germany—to postulate. For every supposed intermediate product between sugar and alcohol—even although these may be present in mere traces—the existence of a specific enzyme has been assumed by some investigators. Pasteur likened fermentation to an explosion, and if the change of sugar into alcohol and carbon dioxide consists of a series of explosions—and this is coming very near to Liebig's original hypothesis—it appears to the reviewer that it is unnecessary to assume the existence of so many and various intermediate products. The fact that there are products besides alcohol and carbon dioxide and that the quantity of these may be varied according to conditions, simply shows that the explosive wave can proceed in more than one direction.

Taking a general survey of this work, we can unhesitatingly recommend it to chemists who desire to obtain in a concise form the latest facts and theories concerning fermentation phenomena. The sequence is such that the book may be read from beginning to end and the arguments followed, so well are they connected. To the translator, Mr. H. Lloyd Hind, must be accorded praise for the excellent manner in which he has rendered the original French text into English. A useful bibliography and a subject-matter index are appended to the book.

ARTHUR R. LING.

DAIRY BACTERIOLOGY. By BERNARD W. HAMMER. Pp. 473 + xii. New York: John Wiley & Sons, Inc.; London: Chapman & Hall. Price 25s.

This book, which is based on the author's course in Dairy Bacteriology at Iowa State College, has for its main theme the application of bacteriological investigation to the problems of dairy practice. Such matters as the elements of laboratory technique or the detailed construction of dairy plant are not dealt with, but the numerous references to the research literature of the subject and the wealth of experienced advice contained therein make this work one which the student will take with him into practice; to the British or European worker it will, among other things, afford a key to the vast volume of investigation which appears in the bulletins of the splendidly equipped agricultural research institutions of the United States.

In Chapter I the relative advantages and disadvantages of the various methods of making routine bacterial counts in milk are discussed at length; in judging the American municipal standards for milk counts, legalised or proposed, which are given here, it may be well to remember that the standard meat extract, peptone and salt agar at 37° C., as laid down by the American Public Health Association, is by no means an ideal medium for the growth of the organisms which develop in milk under usual conditions; under the heading "Research Counts" evidence is given as to the increased counts obtained on the addition of sugars to such a medium, while in Chapter VIII, under "Pin-point colonies from Pasteurised Milk," one notes with interest that "the small amount of lactose carried over in a 1/100 c.c. dilution of milk was a factor enabling certain organisms to grow on the plates." In Chapter XVI, on the tests for the quality of milk, it is seen that the methylene blue reductase test has been gradually gaining favour in America. The classification of milk according to reduction times, as originally worked out by Barthel and Orla Jensen, is given here, but the directions as to the concentration of the dye to be used are rather vague; although some subsidiary investigations on the subject are noted, no reference is made to the pioneer work of the investigators just mentioned.

Chapter II, on milk fermentations, contains some detailed descriptions of the biological and cultural features of common milk organisms, but lacks a general survey of the relationships of the various groups of lactic acid organisms to one another. Although the necessity of repeating work of this nature under local

conditions is fully recognised, yet it must be remarked that this chapter is singularly lacking in references to modern European work. There is some very useful general advice on the investigation of milk defects. In Chapters III and IV we find a valuable survey of numerous practical trials by American workers, in which every conceivable source of milk infection has been critically investigated. The conclusions reached as to the most effective means of reducing milk contamination (not always the most obvious at first sight) will be of interest to all concerned with the improvement of milk supplies. Chapter V, dealing with bacterial growth in milk at various temperatures, treats almost exclusively of the work of American investigators; some European references would have rendered the account more complete, as, for example, in the case of milk held at temperatures above 37° C. Chapter VI treats of body cells in milk, and Chapter VII, which extends over 84 pages, gives a very full account of the most important American, British and European investigations of the spread of diseases through milk. Chapter VIII, on the preservation of milk, deals efficiently with the bacteriology of pasteurisation, in which field American workers have done such valuable pioneer work.

The sections and chapters dealing with the bacteriology of milk powder, condensed milk, ice cream, butter and cheese, contain much information that will be of use to those interested in these products. Chapter XII deals in a very practical way with the preparation of butter cultures, and is particularly valuable, as it contains an account of Professor Hammer's important researches on the associated aroma bacteria. In Chapter XIV the manufacture of butter is naturally considered with regard to American practice, but there is much that will be of interest to those concerned with butter-making in other countries, especially the information dealing with the flavour and keeping power of butter, as they are affected by the use of ripened or unripened cream, and the addition of culture to unripened cream immediately before churning.

This work will undoubtedly take its place as a standard text-book of Dairy Bacteriology, and the author is to be warmly congratulated on its production.

PAUL ARUP.

ORGANIC SYNTHESES: AN ANNUAL PUBLICATION OF SATISFACTORY METHODS FOR THE PREPARATION OF ORGANIC CHEMICALS. Vol. VIII, pp. 141+vii, and Vol. IX, pp. 108+v. Editors in chief: R. Adams and J. B. Conant, New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. Price, Vol. VIII, 10s. net; Vol. IX, 8s. 6d. net.

Workers in the field of organic chemistry are often delayed by the lack of some particular substance. On looking up the literature it is found that the methods given are in some way unsatisfactory, and, in consequence, much time is wasted in the efforts to evolve a suitable procedure. It was in order to minimise the recurrence of this state of affairs that a band of American chemists determined to publish annually a list of organic preparations which "will work." Thus "Organic Syntheses" sprang into being, and nine useful volumes have, so far, been published.

The essence of the whole scheme is that the editors have taken steps to check each preparation submitted to them, and where they have failed to repeat one, they carefully refrain from publishing it.

Each volume contains details of a number of preparations arranged as follows: First comes an equation or scheme representing the reactions involved; second, a detailed procedure; third, a set of notes giving additional information concerning various points in the method, condition of reagents, etc.; fourth, a summary of methods that have been used to prepare the substance in question; and finally, a list of references.

The volumes under review are up to the standard of clearness and conciseness set by the previous members of the series. Every effort has been made to make them as useful as possible, and, in some cases, where the question of the availability of raw material may cause difficulty to workers in various countries, alternative methods are given. Thus in Vol. VIII alternative preparations are given for β-chloropropionic acid and for trimethyl acetic acid.

The preparations given are, for the most part, those of substances which might be needed as the starting point for some line of research. Thus in Vol. IX details are given for cyanoacetamide, iodobenzene and *ac*-tetrahydro-β-naphthylamine, and in Vol. VIII details are given for benzoylformic and cyanoacetic esters. It is therefore obvious that any one requiring a substance not otherwise obtainable will do well to consult these volumes. The reviewer has rather painful memories of trying to obtain a satisfactory yield of *o*-bromotoluene and now finds in Vol. IX, p. 22, not only a simple method of preparation for this substance, but also the cause of the comparative failures which hampered the progress of research. Teachers, too, will find many a preparation which can be given to keen students, and the latter will find many practical details worthy of attention.

Excellent and characteristically American Indexes are provided. Vol. VIII contains an author index for all volumes from I to VIII, and also a subject index of the same range. Volume IX contains only a subject-index for volumes I to IX.

The volumes are of sterling value. All engaged in manufacture or research will, when in need of a substance, do well to consult these volumes before starting on their work. On the whole, the books are very useful additions to the literature of practical organic chemistry and deserve a place on the book shelves of all chemists.

HAROLD TOMS.