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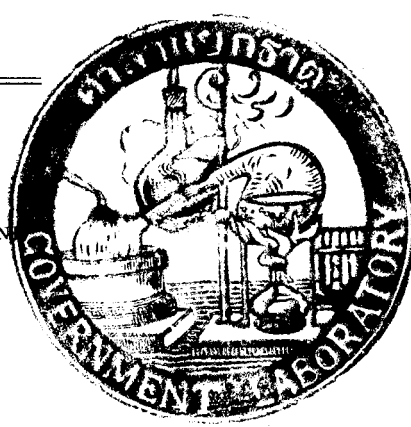


THE ANALYST

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

AN Ordinary Meeting of the Society was held on Wednesday, December 6th, at the Chemical Society's Rooms, Burlington House. The President, Mr. P. A. Ellis Richards, F.I.C., was in the chair.

Certificates were read for the first time in favour of Messrs. George Henry Appleyard, F.I.C., John Walter Black, B.Sc., John Matthew Wilkie, B.Sc., F.I.C., and Arthur William Starey, A.R.C.S., B.Sc., A.I.C.

Certificates were read for the second time in favour of Messrs. Henry Aldous Bromley, Robert Faraday Innes, F.I.C., Osman Jones, F.I.C., Alan West Stewart, D.Sc. (Brux.), A.I.C., William Heaton Thorns, Walter Horace Clulow, and William Plenderleith Lewellen Hope.

The following were elected Members of the Society:—Messrs. Frederick John Martin, M.A. (Cantab) A.I.C.; George Scott Robertson, D.Sc., F.I.C.; and Frederick Stanley Shadbolt, A.I.C.

The following papers were read:—"A Note on the Estimation of Formaldehyde and Acetaldehyde," by E. W. Blair, B.Sc., D.I.C., A.I.C., and T. Shirlock Wheeler, B.Sc., A.R.C.Sc.I., A.I.C.; "A Sliding Scale for the Convenient Titration of Strong Liquids by Dilution and Use with Aliquot Parts," by C. H. Douglas Clark, B.Sc., D.I.C., A.I.C.; "Note on the Presence of Sulphur Dioxide in Cattle Foodstuffs after Fumigation," by H. A. Peacock, B.Sc.; "Some Observations with regard to the Unsaponifiable Matter and Sterols of Edible Fats," by D. W. Steuart, B.Sc.; and "Note on the Sulphuric Acid Test for Fish Liver Oils," by Norman Evers, B.Sc., F.I.C., and H. J. Foster.

Deaths.

WE regret to record the deaths of two of our Members:

Horace Fabian Cheshire, on Nov. 8th, 1922.
Robert George Grimwood, on Dec. 24th, 1922.

Obituary notices will be published later.

The Colorimetric Estimation of Pyrogallol, Gallotannin and Gallic Acid.

By C. AINSWORTH MITCHELL, M.A., F.I.C.

(*Read at the Meeting, November* 1, 1922.)

Most of the published analyses of galls and similar materials, in which the proportion of tannin has been estimated by the hide-power or similar methods, are only relatively useful as an indication of the value of those substances for the preparation of ink, since they do not take into consideration the amounts of gallic acid and other gallotannin derivatives.

For this reason I have, for some years, used a colorimetric method in preference to any of the adsorption, precipitation or oxidation methods of estimating tannin, but it is only recently that I have had the opportunity of determining the significance of the reaction, of making it less empirical, and of adapting it to the estimation of gallotannin and gallic acid in the presence of each other.

The method is based upon the fact that the presence of a tartrate causes ferrous sulphate to react immediately with gallotannin to form a soluble compound which, unlike the ink produced by ferrous sulphate alone, is fairly stable. The coloration ranges from reddish-violet in a very dilute solution to bluish-violet in a relatively more concentrated solution, and its intensity is proportional to the amount of tannin substance present.

The Ferrous Tartrate Reagent.—After experiments with mixtures of various ferrous salts and tartrates, I found a solution of 0·1 grm. of ferrous sulphate and 0·5 grm. of Rochelle salt in 100 c.c. of water to be the most generally suitable for the purpose.

This reagent should be freshly prepared and added to the tannin solution, which may afterwards be diluted to match the standard. The violet coloration is still perceptible in a solution containing 0·0001 grm. of gallic acid in 100 c.c.

The reagent does not give any coloration with phenol or other monohydroxylated compounds, nor with salicylic acid or other compounds containing two hydroxyl groups. So far as my observations go, the violet colour is specific for the pyrogallic grouping, and affords a measure of that group in different compounds.

Quantitative Colorimetric Comparisons.—Either pure pyrogallol or pure gallic acid may be used as the standard for the comparison, but gallic acid is preferable, since it is more stable in solution. I use a solution of 0·1 grm. of either substance in 100 c.c. of water and make the comparison in Nessler tubes provided with Hehner's side tubulures and taps. In the case of gallotannin or gallic acid, 0·1 grm. of the substance is also dissolved in 100 c.c. of water, and 1 c.c. of each solution is added to about 95 c.c. of water in the respective cylinders. Two c.c. of the reagent are then added to each tube, the solution made up to 100 c.c., and the colours compared both vertically and horizontally. If the

colours are both dark, the contents of the tubes are run down to 50 c.c., the colours again compared, and the darker liquid then run out until the colours match. The contents of both cylinders are then diluted to 100 c.c. and again compared at different levels. If the solution of the unknown substance is so dilute that it gives the reddish-violet colour, the estimation should be repeated with only about a third of the quantity of the gallic acid solution in the standard tube prior to the addition of the reagent.

The coloration is so stable that the process of dilution and comparison of the liquids at different levels can be repeated several times without recharging the tubes.

COMPARISON OF PYROGALLOL WITH GALLIC ACID.—The same rich violet coloration was given by each substance on the addition of the reagent, but the 65 c.c. of the dilute pyrogallol solution had to be diluted to 100 c.c. to match the 100 c.c. of gallic acid solution. That is to say, the ratio between the two compounds was 1:1·53. The gallic acid lost 9·3 per cent. of water of crystallisation at 100° C. (theoretical amount 9·5 per cent.), and the solution of anhydrous acid (100 c.c.) was then matched by 74 c.c. of the pyrogallol solution (Ratio = 1:1·35).

Now if we compare the molecular weights of the three substances, we get the following ratios:

		Molecular Weight	Ratio
Pyrogallol	$C_6H_2(OH)_3.H$	125	1
Crystalline Gallic Acid	$C_6H_2(OH)_3.COOH+H_2O$	188	1·50
Anhydrous Gallic Acid	$C_6H_2(OH)_3.COOH$	170	1·36

In each instance, therefore, the pyrogallic group, $C_6H_2(OH)_3$, is the tinctogenic agent, and the carboxyl group and the water of crystallisation act merely as diluents of that group.

THE PROPOSED FORMULÆ FOR GALLOTANNIN.—In order that the method should be capable of general application, it was necessary also to establish a definite relationship between gallotannin and gallic acid, and here I was at once faced with the uncertainty of our knowledge of the constitution of gallotannin.

The long-accepted formula of Schiff (*Ann. Chem. Pharm.*, 1873, **162**, 43), which represents gallotannin as an anhydride of digallic acid, is not in keeping with the fact that gallotannin has been shown by the boiling-point method to have a molecular weight of over 600. In this old formula, $C_6H_2(OH)_3CO=O(OH)_3H_2C_6$, the proportion of pyrogallic groups is 78·1 per cent. and the ratio of pyrogallol to tannin would be as 1:1·28.

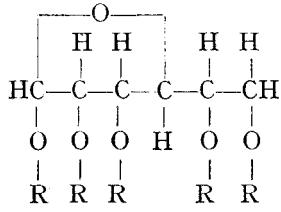
In 1908 Nierenstein (*Ber.*, **41**, 78, 3015) proposed a formula more in keeping with the observed optical properties of gallotannin, although he was firmly of opinion that dextrose or other glucose was not an integral part of the tannin molecule.

Evidence of the want of uniformity of gallotannin was furnished by Iljin (*Ber.*, 1909, **42**, 1731), who fractionally precipitated, from a solution of gallotannin, a series of preparations differing in elementary composition and varying in optical rotation, up to $\alpha_D = +76·5$.

Further evidence of the same kind was brought by Feist (*Arch. Pharm.*, **1912**, **250**, 668) and by Feist and Haun (*ibid.*, **1913**, **251**, 468), who separated from Aleppo galls a crystalline compound yielding 35·5 per cent. of glucose, and also fractionated methyl-gallotannin into compounds corresponding with substituted glucoses containing 2, 3 and 4 galloyl groups.

In the meantime Emil Fischer (*Ber.*, 1912, **45**, 922; 1914, **47**, 922; 1918, **51**, 1760; 1919, **52**, 829) had prepared his pentadigalloyl glucose synthetically, and had shown that it closely resembled gallotannin in composition and in many of its characteristics.

The formula for this synthetic tannin is represented structurally as



where R represents the digalloyl group—$C_6H_2(OH)_3.CO.O.C_6H_2(OH)_2CO$. This compound has a molecular weight of 1700·4, and, containing five pyrogallic groups, its colorimetric ratio to pyrogallol would be as 1:2·72 (gallic acid = 1:1·81).

The specimens of gallotannin with which Fischer compared it were prepared by purifying commercially "pure" gallotannin by treatment with ether and ethyl acetate. They were, therefore, fractionated compounds, and had an optical rotation similar to that of one of Iljin's fractions (*loc. cit.*). There is no evidence to show that they were typical of an average gallotannin not fractionated to such an extent. They yielded about 7 to 8 per cent. of glucose in the case of Chinese gall tannin, and about 12 to 14 per cent. in the case of Aleppo gall tannin.

Nierenstein (*J. Chem. Soc.*, 1921, **119**, 275) has criticised Fischer's formula for gallotannin on several grounds, and in particular, because, when methylated and hydrolysed, gallotannin yielded tetramethyl-glucose, whereas Fischer's synthetic tannin, under the same conditions, yielded glucose.

This proved that in the case of the natural gallotannin four of the hydroxyl groups in the glucose molecule could not have been replaced by digalloyl groups.

The "long-chain" formula which Nierenstein proposes (*J. Soc. Chem. Ind.*, 1922, **41**, 29T) as being more in keeping with the observed facts is that of a glucoside of a polyleucodigalloyl digallic anhydride—

$$(OH)_3C_6H_2CO.[C_6H_2(OH)_3CO]x$$
$$O.C_6H_2(OH).CO.O.C_6H_2(OH)_2.CH(OH).O.C_6H_2(OH)_2$$
$$|\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad|$$
$$O\text{———————————————————}O$$

Omitting the glucose and taking x to represent unity, this compound would have a molecular weight of 779, and contain 2 pyrogallic groups, so that its colorimetric ratio (250:779) would be as 1:3·11. If a molecule of glucose were added, the molecular weight would become 959, and the ratio as 1:3·8.

It may be pointed out, in passing, that a glucoside of this composition must contain at least 18·7 per cent. of glucose.

"PURE" GALLOTANNIN USED FOR COLORIMETRIC COMPARISON.—I am indebted to Mr. G. Booker for the sample of specially selected "pure" gallotannin which I have used for my colorimetric comparisons. It was a very light bulky powder of a pale cream tint, and contained 1·2 per cent. of moisture and 10·5 per cent. of gallic acid.

Its optical rotation in aqueous solution, which was kindly determined by Mr. T. J. Ward, was $\alpha_D = +51·8$. When exposed to the air for three weeks it absorbed 5 per cent. of moisture, and its optical rotation fell to $\alpha_D = +20$. This high optical rotation in aqueous solution indicated that its origin was Chinese galls, since the tannin from Aleppo galls has a low rotation in aqueous solution ($\alpha_D = +2·5$ to $+5$, Fischer and Freudenberg, *Ber.*, 1914, **47**, 2495; 6·5, Feist and Haun, *Arch. Pharm.*, 1913, **25**, 524), whilst that of Chinese galls has a rotation varying up to about $\alpha_D = +73$, according to the extent of purification.

This gallotannin was very difficult to hydrolyse with acid—a characteristic of gallotannin noted by Fischer and by Feist. Mr. Ward boiled it for 45 minutes with 0·1 per cent. hydrochloric acid without changing its optical rotation, and I heated it on the water bath with 5 per cent. sulphuric acid for 18 hours, and then allowed the flask to stand for another 54 hours. The liquid was then quite dark, but a very large proportion of the tannin was still undecomposed, and no glucose was found in the filtrate from the precipitated tannin and gallic acid.

I then inoculated a 2 per cent. solution with *Penicillium*, and left it for 8 days. A deposit of gallic acid had formed, but tannin was still present. It was removed by treatment with lead acetate, and the excess of lead precipitated with hydrogen sulphide. The solution then showed an optical rotation of only $\alpha_D = +0·2$, so that no appreciable amount of glucose was present. In the light of the experiments to be described presently it is conceivable that glucose had been liberated and had then recombined with the gallic acid.

In comparing this tannin with pyrogallol and gallic acid, I made allowance for the 10·5 per cent. of gallic acid in each case.

COMPARISON WITH PYROGALLOL.—One c.c. of a 0·1 per cent. solution of this gallotannin was diluted to 100 c.c., and then required 33·5 c.c. of the standard pyrogallol solution to match the colour produced by the reagent. The 10·5 per cent. of gallic acid was colorimetrically equivalent to 7 per cent. of pyrogallol. Hence:

Gallotannin Grm.	Gallotannin (after deducting 10·5 % gallic acid) Grm.	Pyrogallol Grm. Grm.
0·1	0·0895	=0·0335 − 0·0070
		=0·0265

Ratio = 1 : 3·37, or, allowing for 1·2 per cent. of moisture in the tannin, 1 : 3·32.

COMPARISON WITH GALLIC ACID.—A series of estimations made on different occasions and with freshly prepared solutions (with deduction as before for the

10·5 per cent. of gallic acid in the tannin) gave results of which the following are typical:

1. Gallotannin. Gallic Acid.

0·1 grm., less 0·0105 grm. = 0·0895 grm. = 0·05 grm. (less 0·0105 grm.) = 0·0395 grm.

Ratio = 1 : 2·26.

2. Gallotannin. Gallic Acid.

0·096 grm., less (10·5 per cent.) = 0·0856 grm. = 0·05 grm. (less 0·0104 grm.) = 0·396 grm.

Ratio = 1 : 2·16.

These results are consistent with those previously obtained by comparison with pyrogallol ($2{\cdot}22 \times 1{\cdot}5 = 3{\cdot}33$).

Comparison with Fractionated Gallotannin.—Fifty grms. of the "pure" gallotannin were stirred up with 100 c.c. of cold ether, the yellow solution filtered, the residue of about 3 grms. allowed to dry spontaneously and then dissolved in water, and the solution evaporated to dryness at 100° C.

This residue was free from gallic acid. When compared colorimetrically in 0·1 per cent. solution with the standard gallic acid solution, it required 100 c.c. to match 60 c.c. of the standard solution (ratio = 1 : 1·66). This fraction, therefore, was obviously of a different composition from that of the original tannin. Its colorimetric value was not far from that of Fischer's pentadigalloyl glucose (1 : 1·8).

The Colorimetric Factors for Gallotannin.—The results thus experimentally obtained with the original material agree fairly well with the theoretical value required by Nierenstein's formula (without glucose), *viz.* 3·11, but do not agree with the value required by Schiff's formula or by that of Fischer's synthetic pentagalloyl glucose.

There is still, however, the difficulty of accounting for a low percentage of glucose in association with a relatively low molecular weight (600 to 800 for Aleppo galls tannin, 1100 to 1200 for Chinese gallotannin). The molecular weight of Fischer's synthetic gallotannin (1700) is much higher than has been found in the case of any fraction of natural gallotannin. On the other hand, a low molecular weight involves a higher percentage of glucose, which has only been definitely found in the case of Feist's fraction (*vide supra*), and Fischer and Freudenberg (*loc. sit.*) were unable to isolate a similar compound from the Aleppo galls which they examined.

If we assume, in accordance with Nierenstein's observations, that four hydroxyl groups in a glucose are not replaced by digalloyl groups, and make the further assumption that two are so replaced, we should have a di-digalloyl glucose with the formula: $C_6H_{10}O_6[C_6H_2(OH)_3.CO.O.C_6H_2(OH)_2CO]_2$. This would have a molecular weight of 788 and contain two pyrogallic groups, so that its colorimetric ratio would be as 1 : 3·15. Its elementary composition (C = 51·78; H = 3·55) is in accordance with the results obtained by different chemists with fractions from

gallotannin, and one of Feist's methylated fractions (*loc. cit.*) contained 44·5 per cent. of methoxyl, corresponding with a digalloyl glucose.

On the other hand, such a compound should yield 22·5 per cent. of glucose, whereas the amounts found (with the exception of Feist's fraction) vary, as already stated, from about 7 to 14 per cent.

The general conclusion which I would put forward tentatively is that the average "pure" tannin is a mixture of different glucosides, but mainly of didigalloyl glucose, with a digallic anhydride of the type described by Nierenstein.

A mixture of about one-third of such a substituted glucose with about two-thirds of Nierenstein's anhydride would yield the required proportion of glucose and contain the necessary pyrogallic groups, although, in the case of Chinese tannin, it would require the presence of a small amount of one of the higher substituted glucoses to raise the molecular weight to the observed value. This would not materially affect the colorimetric factor of 2·1 for Chinese galls, which is in accordance with the results I have obtained with samples of those galls.

In the case of Aleppo galls there is a different mixture, having a lower molecular weight and giving a higher yield of glucose. Here there is apparently a considerably higher proportion of one or more of the lower substituted glucoses, and it is therefore reasonable to expect a somewhat lower colorimetric factor. This I have found to be in accordance with the facts. A factor of 2·1 gives in some cases results too high for the proportion of soluble extract, and a factor of 1·85 is required, as shown below. It is obvious, therefore, that each tannin has its own average factor. Where, as in the case of tea, there is no exact knowledge of the constitution of the tannin, the factor must provisionally be based on the average results for tannin obtained by other methods.

In the subsequent test experiments with my gallotannin, I used the factor 2·2, which I had obtained empirically.

Enzymic Hydrolysis of Gallotannin.—Ink manufacturers have long been acquainted with the fact that by allowing an infusion of galls to become mouldy and then exposing it to the air, they eventually obtained a product requiring only about half an much iron as the original solution to make an ink.

In the case of gallotannic acid it was assumed that the change taking place in this "fermentation," as it was termed, was simply the hydration of one molecule of gallotannic acid or digallic anhydride to form two molecules of gallic acid. Since, however, pure gallic acid was known to require only about half the proportion of iron required by gallotannin, the two gallic groups in gallotannin could only have had one half of the tinctogenic power of free gallic acid, if the old formula were correct.

If, however, my theory of diluted pyrogallic groupings is correct, the two original groups in the gallotannin should appear again in the fermented product in a much more concentrated form, and in the correct proportion to agree with the formulæ for both substances.

In making tests to determine this question, I inoculated two 0·1 per cent. solutions of the gallotannin (containing 10·5 per cent. of gallic acid), *i.e.* 0·895 grm.

of gallotannin, with moulds which had formed on the surface of two stronger solutions, and exposed the liquids in loosely covered flasks at the ordinary temperature (60°–65° F.). The flasks were shaken, occasionally, and each day 1 c.c. of each solution was taken, and its colorimetric equivalent estimated in terms of pure gallic acid.

In each instance the hydrolysis proceeded to the anticipated extent, the gallotannin solution doubling its tinctogenic power in four to five days.

From this stage onwards, however, the solutions behaved differently, apparently owing to the action of the different mould fungi present. In each case there was first a gradual decrease in the tinctogenic power, while one solution turned progressively yellow, and the other remained nearly colourless.

In the case of the yellow solution this decrease continued until the liquid hardly gave any coloration with the reagent, whereas in the case of the other solution the colorimetric value, having reached the minimum corresponding with gallotannin, began to rise again, and continued in a state of unstable equilibrium.

The results thus obtained are shown in the following series, in which the values are expressed in comparison with a 1 per cent. solution.

ENZYMIC HYDROLYSIS OF 0·1 PER CENT. SOLUTION OF GALLOTANNIN. RESULTS IN COMPARISON WITH GALLIC ACID AS 100.

Days ..	1	2	3	4	5	6	7	8	9	10
A. Gallic Acid Equivalent	50	—	—	—	—	95	85	62	53	—
B. Gallic Acid Equivalent	50	50	62	83	—	—	79	60	43	64

Days ..	11	12	13	14	15	16	17	18	19
A. Gallic Acid Equivalent	—	49	—	42	37	30	—	—	15
B. Gallic Acid Equivalent	—	69	62	72	—	—	—	—	—

In the first case, therefore, the hydrolysis proceeded practically to the complete destruction of the gallic acid formed, and in the second case the enzymic reaction appeared to be reversible.

Mr. Ward has made cultivations, on agar containing tannin, of the moulds from these two flasks. In the case of A gelatinous masses formed below the surface and nothing above, whilst B yielded typical mould growths on the surface.

THE ENZYMIC DESTRUCTION OF GALLIC ACID.—In experiments to ascertain the nature of the change occurring in the destruction of the gallic acid, I exposed 0·1 per cent. solutions of pure gallotannic acid to the air. After a day they turned slightly yellow and, compared with freshly prepared solutions of gallic acid, showed a slight decrease in tinctogenic power. For example, one solution, after standing for a week, was only equivalent to 0·082 per cent., whilst, after another two days, the equivalent was 0·0876 per cent., and, after another week, it had risen to 0·09 per cent.

A 0·1 per cent. solution of pure gallic acid was inoculated with some of the mould from the experiment A, and a trace of ammonium nitrate and potassium phosphate added. After standing for a month, the liquid no longer gave any coloration with the reagent, so that there seems to be little doubt that there can be enzymic destruction of gallic acid.

A 0·1 per cent. solution of pyrogallol, similarly exposed for a week, but not inoculated with mould, fell 31 per cent. in colorimetric value, and became bright orange in colour, and this oxidation change was progressive.

It seems not improbable, therefore, that in the case of the deep yellow solution of gallotannin (A) the enzymic hydrolysis had ultimately resulted in the formation of pyrogallol which became oxidised on exposure to the air, and so on progressively, until practically the whole of the gallic acid had been destroyed.

I am studying this oxidation reaction of pyrogallol with a view to its possible use in the estimation of pyrogallol in the presence of gallic acid.

ESTIMATION OF COMMERCIAL GALLIC ACIDS.—Two samples of technical gallic acid were tested in comparison with pure gallic acid. The first was a dark buff powder containing 11·36 per cent. of water, and the other a dirty grey powder with 11·49 per cent. of water. The first corresponded with 76 per cent. of the pure acid, and the second with 97 per cent. A third sample matched the standard exactly and was therefore of 100 per cent. purity.

ESTIMATION OF GALLOTANNIN IN THE PRESENCE OF GALLIC ACID.—The two substances are first estimated together in terms of gallic acid, the gallotannin then precipitated, and the gallic acid in the filtrate again estimated. The difference between the two results multiplied by the appropriate factor gives the amount of gallotannin.

I have found quinine hydrochloride to be the most suitable precipitant, its use being suggested to me by the fact that the manufacturers of detannated orange wine have long been in the habit of testing the freedom of their products from tannin by adding a pinch of that quinine salt to a small quantity of the wine.

I afterwards noticed that precipitation with quinine sulphate was made the basis of a gravimetric method of estimating tannin in tea by Tatlock and Thompson (ANALYST, 1910, **35**, 104), but the objection to that method is that colouring matters are also precipitated simultaneously with the tannin, and that the composition of the precipitate will therefore vary with the nature of the original material. In the case of my colorimetric method the removal of colouring matters is an advantage, and the presence of quinine in the filtrate does not interfere with the colour reaction.

GALLIC ACID IN GALLOTANNIN.—In the test experiments with my "pure" specimen of gallotannin, 0·1 grm. was equivalent colorimetrically to 0·05 grm. of gallic acid. Ten c.c. of the 0·1 per cent. solution were then treated with a slight excess of a solution of quinine hydrochloride, the precipitate filtered off after about five minutes, and washed several times with water (about 25 c.c. in all), and the filtrate and washings made up to 100 c.c. Ten c.c. of this solution (corresponding with 1 c.c. of the original solution) were then compared, as before, with the standard, and the following results were obtained:

Gallotannin, 0·1 grm.	matched	Gallic Acid, 0·05 grm.
Filtrate from 0·1 grm.	matched	Gallic Acid, 0·0105 grm.

Amount of Gallic Acid = 10·5 per cent.

Two other estimations gave the proportion as 10·5 and 10·7 per cent. respectively, and in the subsequent experiments the lower value of 10·5 per cent. was deducted from the amount of this gallotannin taken and added to the gallic acid standard.

MIXTURES OF GALLOTANNIN AND GALLIC ACID.—The following results were obtained with test mixtures of this gallotannin and pure gallic acid, the gallotannin in each case being multiplied by the empirical factor 2·2:

MIXTURES OF GALLOTANNIN AND GALLIC ACID.

Gallotannin Grm.		Gallotannin (less 10.5% of gallic acid) Grm.	Gallic acid Grm.		Gallic acid +10.5% Grm.	Gallic acid Equiv. of Mixture Grm.	Gallic Acid Equiv. of Filtrate Grm.	Difference Grm.	Tannin (Difference × 2.2) Grm.
0·05	=	0·0447	0·05	=	0·0553	0·078	0·058	0·020	0·0440
0·05	=	0·0447	0·05	=	0·0553	0·075	0·056	0·019	0·0418
0·025	=	0·0221	0·075	=	0·0779	0·087	0·076	0·011	0·024
0·010	=	0·0085	0·090	=	0·0910	0·095	—	0·004	0·0088

Making allowance for the fact that the gallotannin was not a pure substance, I think that these results prove that the method may be regarded as trustworthy.

COMMERCIAL TANNIC ACIDS.—A sample of technical tannic acid was of a pale brown colour and contained 10·8 per cent. of moisture. The total gallic acid equivalent of 0·1 grm. was 0·0350 grm., and the gallic acid equivalent of the filtrate from the quinine precipitation was 0·0081 grm.

Hence the gallotannin $= (0{\cdot}0350 - 0{\cdot}0081) \times 2{\cdot}1 = 56{\cdot}49$ per cent.; and the gallic acid $= 8{\cdot}1$ per cent.

In the case of another commercial sample the following results were obtained: Total gallic acid equivalent, 35 per cent.; gallic acid in filtrate, 8·1 per cent.; tannin $(26{\cdot}9 \times 2{\cdot}1) = 56{\cdot}4$ per cent.

A third sample, which was about 15 years old, contained 10 per cent. of moisture and 16·2 per cent. of an insoluble reddish compound. The soluble matter consisted of 4·5 per cent. of gallic acid and 79·6 per cent. of tannin. Total constituents estimated = 100·3 per cent.

EFFECT OF COLOURING MATTERS.—Certain products, such as roasted galls or myrobalans, yield a deep yellow extract which, even in very dilute solution, gives a slight yellow tint, and so modifies the colour produced by the reagent.

In such cases the simplest plan is to tint the standard solution with caramel until it matches the solution under examination, prior to the addition of the reagent.

Another method is to attach a Lovibond tintometer glass of the right tint in the 52 series, by means of rubber bands, to the bottom of the Nessler cylinder.

NUT GALLS.—These are the most important raw material of the ink maker and, as their value depends entirely upon the proportions of gallotannin and gallic acid present, a rapid method of estimating both of these constituents has long been needed.

The method I use is to take about twelve galls from a sample and to crush them to a coarse powder, which is then thoroughly mixed. Five grms. of the mixture are then boiled for an hour each time with successive portions of about 150 c.c. of water, the extracts filtered, and the whole made up to 500 c.c. Ten c.c.

of this 1 per cent. extract are diluted to 100 c.c., and 1 c.c. of this 0·1 per cent. extract compared in the usual way with the standard gallic acid solution. The following results are typical of those obtained:

COMPOSITION OF ALEPPO GALLS.
(*Factor* = 1·85.)

	Moisture Per Cent.	Total extract Per Cent.	Tannin Per Cent.	Gallic acid Per Cent.	Total pyrogallic equivalent Per Cent.
1. Blue Galls, old	10·25	80·4	53·5	11·2	26·5
2. ,, ,, ,,	11·01	74·0	61·9	1·5	23·2
3. ,, ,, new	10·85	86·4	79·5	7·0	33·3
4. ,, ,, ,,	13·05	86·4	77·5	3·5	28·8
5. Green Galls	11·00	73·4	61·8	2·5	23·9
6. White Galls (same lot)	10·72	72·4	52·9	7·4	24·0
7. Roasted Galls	10·51	59·6	37·6	20·0 (=13·3 Pyro.)	26·6

If the factor 2·1 were used for converting the gallic acid equivalent of the precipitated tannin into tannin, the amount of the combined tannin and gallic acid would in some cases exceed that of the total extract.

If, however, we deduct from the total solids in the extracts from new galls the amount of anhydrous gallic acid found and the average amount of gum in nut galls (2 per cent.), and divide the difference by the gallic acid equivalent of the precipitated tannin, we get in the case of the new galls (3 and 4) factors of 1·82 and 1·96 respectively. The average of various estimations gave 1·85.

For comparing the tinctogenic values, however, it is advisable to express the total results in terms of pyrogallol. After reporting on Nos. 3 and 4, I was informed that the price asked for No. 3 was 13 per cent. higher than for No. 4. Tinctogenically it is worth nearly 16 per cent. more.

THE WHITE GALLS (No. 6), *i.e.* those from which the larvæ had escaped, leaving an open channel, were picked out of the consignment of green galls No. 5. White galls, as is well known, are regarded in commerce as of lower value than blue or green galls.

From the tanning point of view this opinion is justified, but from the tinctogenic point of view much will probably depend upon the extent to which the oxidation has proceeded within the galls. In the case of this particular sample it will be seen that, while the tannin has been reduced, the gallic acid has increased, so that the total tinctogenic value is the same in each case.

Some light may be thrown on this question by a comparison of the results given by English oak-apple galls at different stages of their growth.

ROASTED GALLS.—Galls are sold not only in their natural state or in a crushed condition, but also after being roasted. This process of roasting, which is done by a rule of thumb method, causes the galls to become friable and dark brown in colour; after the treatment they yield a darker ink than in the raw condition. This fact is well known, but so far as I am aware no explanation has been given of its cause.

When galls are heated to 220° C. the gallotannin is decomposed, first with the formation of gallic acid and then of pyrogallol, some of which separates as a sublimate.

The decomposition appears to be far from quantitative, for in two experiments in which I heated 0·1 grm. of gallotannin for 30 minutes (one at 220° C. and the other up to 300° C.), the amounts of sublimate collected were only equivalent colorimetrically to 0·85 and 0·65 per cent. of pyrogallol respectively.

Some years ago Schluttig and Neumann made a series of experiments upon the stability to sunlight and air of colour washes on paper of the iron compounds of various trihydroxylated substances. They found that the gallotannin compound faded to pale yellow, the gallic acid to grey, and the pyrogallol compound to dark brown, and expressed their surprise that gallotannin, with its accepted composition of a digallic anhydride, should not have exceeded the other two in depth of colour and permanence.

The facts I have already brought out will explain why pyrogallol comes first and gallotannin last in the series; and if we assume that the production of pyrogallol is the cause of the increase in the blackness of ink from roasted galls, we ought to be able to obtain some evidence of this by the colorimetric method.

A reference to the results given in the table (No. 7) shows that this is the case. Roasting the galls reduces the amount of soluble extract and gallotannin, but greatly increases the non-tannin colorimetric equivalent.

Oak-Apple Galls.—Although our British galls are of no commercial importance, I have included a few analyses of them, as they show the general applicability of the method to bio-chemical work, and the information obtained as to the variation in their composition at different stages of growth may eventually be found to apply also to the commercial product.

I have to thank Mr. R. M. Prideaux for these different specimens, all of which are of this year's (1922) growth.

I have also, for comparison, added an analysis of a specimen of a rose gall, which contained a large number of living larvæ.

Composition of British Galls.

(*Factor*=2·1.)

	Moisture Per cent.	Soluble extract Per Cent.	Tannin Per Cent.	Gallic acid Per Cent.
1. Black gall.	35·66	6·00	3·25	—
2. Yellow, empty	14·70	19·00	12·93	1·25
3. Pale brown	18·93	62·0	36·74	1·50
4. Blind gall, brown	17·78	31·25	28·25	0·93
5. Rose gall	—	—	19·74	0·0

No. 1 had a white, spongy interior; it contained no larva in the centre, but had eight or ten small larvæ round the periphery.

No. 2 was a pierced gall, and contained a good deal of dust.

No. 3 had a large larva in the central cavity.

No. 4 was a blind gall without a central chamber, though it had one small larva on the exterior just below the epidermis.

It is interesting to note that the gall containing the living larva in the centre chamber was the richest in tannin, and that this fact supports the general practice of collecting Aleppo galls at a period shortly before the larva reaches maturity.

A comparison of these results strongly suggests that the true larvæ (of *Cynips terminalis*) produce tannin as an excretory product, whereas the parasitic larvæ (as typified by No. 1) are unable to effect this decomposition.

CHINESE GALLS.—Two commercial samples obtained from different sources gave the results shown in the following table:

CHINESE GALLS.

(*Factor* = 2·1.)

	Moisture Per Cent.	Total extract Per Cent.	Tannin Per Cent.	Gallic acid Per Cent.	Total pyrogallol equivalent Per Cent.
Chinese Galls I.	9·71	63·2	60·9	4·0	22·0
,, ,, II.	11·01	63·8	52·7	6·4	21·0

It is probable that the soluble extract of Chinese galls consists almost entirely of tannins and gallic acid. Gums do not appear to be present. It is interesting to note that the total tinctogenic value of Chinese galls is lower than that of good nut galls. This is contrary to the commonly accepted belief.

MYROBALANS.—Sometimes a small proportion of myrobalans is mixed with galls for the preparation of writing ink, but they are mainly used as a raw material for copying inks, for which purpose their high proportion of gums renders them particularly suitable. It is well known that roasting improves myrobalans for ink-making, but the changes brought about by the process have not hitherto been ascertained.

In calculating the amount of tannin from the pyrogallol equivalent I availed myself of the recent researches of Freudenberg and Fick (*Ber.*, 1918, **52**, 1238; 1920, **53**, 1728), who have shown that chebulinic acid, the tannin of myrobalans, is a crystalline compound of digalloyl glucose, with a dibasic phenolic acid, $C_{14}H_{14}O_{11}$, which loses two molecules of water in the process of combining. This phenolic acid yields pyrogallol when distilled under reduced pressure, and we may therefore assume that it contains a pyrogallic group. A second pyrogallic group is present in the digalloyl group as shown in the formula:

$$[C_6H_2(OH)_3.CO.O.C_6H_2(OH)_2.CO].C_6H_{11}O_6+C_{14}H_{14}O_{11}-2H_2O.$$

The molecular weight of this compound, $C_{34}H_{30}O_{23}$, is 806, which agrees fairly well with the results experimentally obtained, and the colorimetric ratio between two pyrogallic groups and the tannin is therefore as 1:3·22, or, in terms of gallic acid, as 1:2·14, which is practically the same as in the case of gallotannin from Chinese galls. This factor was accordingly used in the following analyses of myrobalans:

COMPOSITION OF MYROBALANS.

(*Factor* = 2·16.)

	Moisture Per Cent.	Extract Per Cent.	Tannin Per Cent.	Gallic acid Per Cent.	Total pyrogallol equivalent Per Cent.
Raw	7·25	32·8 (6 days) 33·0 (14 ,,)	21·6	2·8	8·53
Roasted (*a*)	9·70	49·6	34·5	7·2	15·40
,, (*b*)	—	50·8	33·4	7·5	15·33

As a rule, roasted myrobalans do not yield a much higher extract than the raw product, but, in the case of this sample, the roasting process had caused not only the pericarp, but also the kernel, to become dark brown and friable.

MISCELLANEOUS TANNIN MATERIALS.—A few commercial samples of materials sometimes used in the manufacture of ink, though more frequently on the Continent than in England, have been examined, with the following results. In the absence of more exact information, the factor 2·0 has been used to multiply the gallic acid equivalent of the tannin. Two samples of tea are also included.

MISCELLANEOUS TANNIN MATERIALS.

(*Factor* = 2·0.)

	Moisture Per Cent.	Total extract Per Cent.	Tannin Per Cent.	Gallic acid Per Cent.	Total pyrogallol equivalent Per Cent.
Valonia	13·15	49·0	46·0	3·0	17·3
Divi-divi.	11·49	74·0	32·8	2·6	12·66
Commercial gall liquor	97·32	2·68	1·6	0·2	0·66
Commercial myrobalans liquor	97·08	2·92	1·62	0·5	0·87
Sumach	—		No coloration		nil
Chinese tea	—	—	3·32	0·84	1·66
Tea (blend)	—		7·90	0·8	2·8

The samples of gall liquor and myrobalans liquor were typical of those sometimes sold for the manufacture of ink. Obviously it is a dear method of buying tannin.

The result given by the sample of sumach was surprising, but, on referring to the literature on the subject, I found that Löwe [*Zeitsch. anal. Chem.*, 1873, **12**, 128) had found that only Sicilian sumach gives the reactions of gallotannin, and that other species of sumach do not yield gallic acid on hydrolysis with sulphuric acid. This sample of mine gave a greenish black coloration with ferric chloride, but apparently its tannin did not contain a pyrogallic group or a pyrocatechol group.

TEA.—In using the method for the estimation of tannin in tea a 1 per cent. decoction should be made, and the colorimetric comparisons made with the undiluted extract. The constitution of the tannin has not yet been determined, but I have used the factor 2 provisionally. If the pyrogallic equivalent is stated, results can always be subsequently adjusted should another factor be found to be more correct. The method is rapid and presents no difficulties.

COFFEE.—Freudenberg has shown that the tannin of coffee is a simple glucoside with a low molecular weight. It gives an olive-green coloration with my reagent, and therefore does not contain a pyrogallic group.

HOPS.—A decoction of hops also gives an olive-green coloration with the reagent. It seems probable that the method could be extended to the iron-greening types of tannins by basing the factor on pyrocatechol instead of on pyrogallol, and I am making experiments on these lines.

TANNIN LOZENGES.—The B.P. requires tannin lozenges to contain 0·03 grm. of tannin. I have examined two samples, bought from different pharmacists;

both of these were stamped "B.P." The lozenges in the first of these averaged 1·1488 grm. in weight and in the second 1·1062 grm. In each case a 1 per cent. solution was made and tested colorimetrically, with the following results:

	Total gallic acid equivalent Per Cent.	Gallic acid Per Cent.	Tannin (*Factor* 2·1) Per Cent.
(1)	1·2	0·4	1·68
(2)	1·31	0·4	2·0

Even if we multiply the total gallic acid equivalent by the tannin factor, the results (viz. 0·0252 grm. and 0·0275 grm.) are still somewhat too low.

TANNINS IN INK.—In order to apply the method to ink, it will be necessary first to liberate the tannin and gallic acid from their combination with the iron, and then to extract them with a suitable solvent. I have obtained promising results in this way, but have not yet worked out the method in sufficient detail to bring them before the Society.

In conclusion, I wish to thank Mr. T. J. Ward for his help in some of the experimental work, and Mr. W. Baker, of Messrs. Mabie, Todd & Co., for the specimens of roasted galls and myrobalans.

DISCUSSION.

Dr. T. A. HENRY said that he considered the author's suggestion, that the tannin in galls was an excretory product, was sound, and referred to Nierenstein's view that there might be a kind of symbiotic relationship between the grub and the plant. He suggested that the author's method might throw light on the vexed question of the tannin content of China tea, as compared with that of Indian and Ceylon teas. The usual methods for estimating tannin in tea were not very satisfactory, and indicated, possibly quite erroneously, that China tea generally contained less tannin than Indian and Ceylon teas.

Mr. A. E. PARKES asked whether the author's method gave reliable results for the estimation of tannin in tea and coffee. It seemed to him that 3 per cent. for China tea and 8 per cent. for blended tea were low as compared with the figures he had been accustomed to get, namely, 5 to 8 per cent. for China teas, and 9 to 12 per cent. for other teas, when estimated by the quinine or cinchonine methods. He enquired if the author had checked the methods of estimating tannin by precipitation with quinine or cinchonine by comparison with his colorimetric method.

Mr. MITCHELL, replying to Dr. Henry, said that he thought it quite possible that tannin might be present in some form of combination in woody fibre, and that the grubs might be able to digest the lignin and leave the tannin as an excretory product. Referring to Mr. Parkes' question, he said that so far the tannin of tea had not been isolated, and that it was therefore conceivable that a much higher colorimetric factor than that provisionally adopted might be required, but that if the pyrogallol equivalent were recorded in each case, it would always be possible to adjust the calculations subsequently. He had not yet made comparative estimations of the tannin in tea by the colorimetric and precipitation methods.

The Estimation of Codeine.

By HAROLD EDWARD ANNETT, D.Sc., F.I.C., and RAM RICHHPAL SANGHI.

(*Read at the Meeting, November* 1, 1922.)

The present paper is to be regarded as a continuation of one already published (Annett and Sen, Analyst, 1920, **45**, 321). We have improved and simplified the method there described for the estimation of codeine. The basis of our method is the same, but we have omitted the basic lead acetate treatment.

Modified Method.—The following is the procedure now recommended:—Eight grms. of opium are triturated with 2 grms. of slaked lime and 80 c.c. of water during half an hour, as in the B.P. (1914) process. Fifty c.c. of the filtrate (=5 grms. of opium) are extracted with 3 successive portions, each of 50 c.c., of toluene. The toluene from each successive extract is passed through a dry filter into a distillation flask. It is then concentrated under diminished pressure to a small bulk (about 25 c.c.), and dry hydrogen chloride gas bubbled through it for half a minute. Codeine hydrochloride rapidly separates in a flocculent form, together with colouring matter, etc. The toluene is filtered through a dry filter, the codeine hydrochloride dissolved in water and the solution filtered through the same filter into a small round-bottomed glass dish, a deep magenta colour developing. The liquid is then evaporated on the water bath. When almost dry, the dish is transferred to a water oven and dried to constant weight. This treatment renders the colouring matter insoluble, as required in the next process. The substance is now dissolved in hot water and transferred to a 50 c.c. flask, the volume made up to 50 c.c., 0·2 grm. of fresh slaked lime added, and the flask shaken during half an hour. The liquid is then filtered, and 40 c.c. (=4 grms. of opium) are taken for extraction with 3 successive portions, each of 40 c.c., of toluene. The toluene is filtered through a dry filter into a distillation flask, as before, each portion of toluene being put through the filter immediately after extraction. In this way the final filtration is of the toluene least concentrated in codeine. Finally the filter paper is washed with a small amount of toluene. The toluene is concentrated to a small bulk (about 25 c.c.), as before, and dry hydrogen chloride gas passed through it for half a minute. The toluene is filtered off, the codeine hydrochloride dissolved in water, and the solution filtered into a small weighed round-bottomed glass dish. The liquid is evaporated almost to dryness in a glass dish and now develops practically no colour. When only a small amount of water is left, a little weak alcohol is added to encourage crystallisation, and rosettes of almost colourless crystals of codeine hydrochloride are obtained. The dish is dried to constant weight in the water oven and weighed, and the residue taken as $C_{18}H_{21}NO_3HCl+1\frac{1}{2}H_2O$.

The method has been tested with pure codeine and on opium, with and without added amounts of codeine.

We would refer here to a surprising criticism of our method which has been made by Rakshit (ANALYST, 1921, **46**, 485). He states that the shaking of the opium extract with toluene gives rise to an emulsion which cannot be broken. We need only reply that we always recover 144 to 145 c.c. of the 150 c.c. of toluene actually used for the extraction, and have had no trouble at all with emulsion formation in the many hundreds of codeine estimations we have made. Five assistants have worked the method with success. If the opium extract be vigorously shaken up with toluene, then of course one gets great difficulty owing to emulsion formation, but one naturally assumes that ordinary manipulative skill will be exercised.

EXPERIMENTS WITH PURE CODEINE.—Three portions of 0·2992, 0·1995 and 0·0997 grm. respectively of pure anhydrous codeine were analysed as in the above method, and the following results obtained:

Anhydrous codeine taken Grm.	Codeine hydrochloride corresponding with half the codeine taken*		Codeine recovered Per Cent.
	Actual yield Grm.	Theoretical yield Grm.	
0·2992	0·1738	0·1809	95·96
0·1995	0·1148	0·1206	95·10
0·0997	0·0580	0·0603	96·16

* NOTE.—Eighty c.c. of water and 2 grms. of lime were triturated with the codeine, and 50 c.c., or $\frac{5}{8}$ of this, taken for analysis. Four-fifths of this were used in the final operation. Hence the codeine hydrochloride finally obtained corresponds with $\frac{4}{5} \times \frac{5}{8}$ or half of the codeine taken.

Hence the method accounted for 95 to 96 per cent. of the codeine taken. Many other experiments have been carried out on similar lines, and a recovery of 94 to 96 per cent. is always obtained.

EXPERIMENTS WITH OPIUM, WITH AND WITHOUT KNOWN ADDED AMOUNTS OF CODEINE.—A sample of opium well powdered in a mortar, and 5 portions, numbered 1, 2, 3, 4 and 5, each of 8 grms. were then taken. To portions numbered 3, 4 and 5, quantities of 0·0994, 0·0497 and 0·0248 grm. of pure anhydrous codeine were added respectively. Portions 1 and 2 received no additions. All 5 portions were then analysed by our method, with the following results:

Sample No.	Opium taken Grm.	Anhydrous codeine added Grm.	Codeine hydrochloride		Codeine in opium	
			Actual yield Grm.	Theoretical yield on basis of average analyses of samples 1 and 2 Grm.	Found Per Cent.	Theory based on average result for samples 1 and 2 Per Cent.
1.	8·00	nil	0·1284	0·1305	2·65	2·69
2.	8·00	nil	0·1326		2·74	
3.	8·00	0·0994	0·1856	0·1910	3·83	3·94
4.	8·00	0·0497	0·1544	0·1607	3·19	3·32
5.	8·00	0·0248	0·1398	0·1456	2·89	3·01

Several other similar series were carried out and the results were just as satisfactory.

PURITY OF THE PRODUCT.—The codeine hydrochloride recovered by us is practically colourless and crystallises in beautiful rosettes of needles. These are indications of its purity. We have, however, examined the product polarimetrically, as follows: We took the following samples of codeine hydrochloride for examination:

(1) 0·2524 grm. recovered from analyses of opiums; (2) 0·2942 grm. recovered from analyses of opiums containing added amounts of codeine; (3) 0·4000 grm. recovered from the analysis of pure codeine.

The substance in each case was dissolved in 50 c.c. of water, and the solution filtered and read in a 200 mm. tube in a Hilger saccharimeter with the use of white light. The readings obtained were −3·31, −3·70 and −5·04 respectively, each figure being an average of 10 closely agreeing readings. Assuming the codeine hydrochloride in 3 to be pure, the readings for 1 and 2 should theoretically have been −3·18 and −3·71 respectively. Our product would therefore appear to be of a satisfactory degree of purity.

CONCLUSIONS.—The method we have devised for codeine estimation gives satisfactory results and recovers approximately 95 per cent. of the codeine present in opium. The modification here described has been in continual use in this laboratory during the past year, and has proved itself trustworthy in practice.

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.

QUANTITATIVE MICROSCOPY UNIT.

I HAVE read with much interest the note by J. F. Liverseege and Una Liverseege upon the need for a unit for comparison in quantitative microscopy. I am in agreement with the authors as to the desirability of establishing some such unit of reference, and it was with this end in view that I made determinations of the number of spores per mgrm. of lycopodium (ANALYST, 1919, **44**, 321). By using 94,000 lycopodium spores as the unit of reference and stating the number of counted particles in a weight of the substance equal to the weight of 94,000 spores, one has immediately the number of countable particles per mgrm. of the substance. It would seem preferable to use 94,000 spores, as I suggested in July, 1919, instead of 100 spores as the unit, because American and Continental workers commonly express their results in particles per mgrm., and the results obtained by those who use the lycopodium method would then be directly comparable with figures obtained by other methods. The chief advantage of using a smaller unit, such as 100 spores, would seem to be that smaller numbers result as representing the various materials; this advantage, however, would not outweigh the disadvantage of yielding numbers which are not directly comparable with results expressed in particles per mgrm. This is especially evident when one realises that in various instances the numbers per mgrm. are comparatively small, *e.g.* 500 to 2000 pollen grains per mgrm. of insect powder (Lehmann and Trottner, *Revist. Pharm.*, 1917) and 400 to 600 starch grains of 40 microns and over per mgrm. of wheat starch (Wallis, ANALYST, 1922, **47**, 516); these numbers would be represented by fractions, if 100 lycopodium spores were used as the unit.

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THE TEMPERATURE COEFFICIENT OF THE REFRACTIVE INDEX OF AMERICAN TURPENTINE.

WITH reference to the note in the ANALYST (1922, **47**, 469), the value of this coefficient was given by me in the Journal of the Society of Chemical Industry (1905, p. 717) as 0·00048 for genuine American turpentine for temperatures between 10° and 25° C., as determined with an Abbe refractometer. This is in practical agreement with the value found by the writer of the note referred to, *viz.* 0·000468, the Abbe instrument not indicating the fifth decimal.

It was stated by Pulfrich (*Zeitsch. fur Instrumentenkunde*, 1898) that the temperature effect due to the glass of the prisms was negligible over a range of 30° C.

It may also be pointed out that there are a few essential oils with rather low temperature coefficients. For example, sandalwood oil has a temperature coefficient of 0·00039 and cedarwood oil one of 0·00042.

CHINGFORD. T. F. HARVEY.

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.

THE SALE OF MILK.

Dewey v. *Faulkner.*

THIS was an appeal against the decision of a Metropolitan Magistrate heard in the King's Bench Division, on December 13, 1922, before the Lord Chief Justice, Mr. Justice Darling and Mr. Justice Salter.

The following facts were admitted or proved at the hearing:—The appellant had sold to the respondent one half-pint of milk, a sample of which was proved by the analyst's certificate to have had 10 per cent. of its original fat abstracted. Before the hearing the respondent had given notice under section 20 (1) of the Sale of Food and Drugs Act, 1899, that he intended to rely upon a written warranty alleged to be contained in (1) a memorandum of agreement for the sale of "new milk" between a dairy company of Sherborne, Dorset, and the respondent; (2) a written label attached to the churns sent by the dairy company to the respondent, bearing the words "guaranteed pure unskimmed milk with all its cream." On May 22 the respondent collected from Vauxhall railway station eight churns of milk despatched by the dairy company and took them to his shop in Camberwell, where they were kept until he started on his round.

The magistrate held (*a*) that the memorandum and label constituted a warranty, (*b*) that Vauxhall railway station was the respondent's station for delivery free, in accordance with the agreement, and that the respondent was therefore protected by the warranty until the milk was delivered to him at that station; (*c*) that the respondent had proved that the milk was part of that received from the dairy company, and that he had sold it in the same condition as that in which he had received it.

Mr. Disturnal, for the appellant, contended that the label did not form part of the contract of sale, and that therefore there was no warranty (*Jeynes* v. *Hindle*, *Times*, L.R. 454; [1921] 2 K.B. 581). *Rook* v. *Hopley* (38 L.T. 649). *Iorns* v. *Van Tromp* (72 L.T. 499).

Mr. Whiteley, for the respondent, pointed out that *Hunt* v. *Richardson* (32, *The Times*, L.R. 560; [1916] 2 K.B., 446) showed that a buyer of milk was entitled to receive new milk as it came from the cow. As the dairy company had contracted to supply respondent with "new milk," he was entitled to accept that provision in the contract as a warranty that the milk would be in accordance with the regulations. The label identified the churn as part of the deliveries under the contract. Referring to *Irving* v. *Callow Park Dairy Co.* (18, *The Times*, L.R. 3), he suggested that the case might be referred to the magistrate to find whether there was a verbal agreement that every consignment should bear the guarantee shown on the label. The respondent was not responsible until the milk was delivered to him at the station (*Parker* v. *Alder* (15, *The Times*, L.R. 3; [1899] 1 Q.B. 20), and what happened subsequently was a question of fact for the magistrate.

The Lord Chief Justice, in giving judgment, said that for the respondent to succeed he must show that he purchased the milk with a written warranty. The cases to which reference had been made had gone to the length that if it was a term of a contract of purchase that a written warranty should accompany the article, that warranty satisfied the Statute. In the present case the magistrate had expressed the opinion that the agreement with the label on the churns, taken together, constituted a warranty. In his opinion that decision was wrong. There was nothing to connect the label with the stipulation in the agreement, which purported to contain all the terms agreed upon between the parties. The agreement and the label could not be read together to obtain a warranty which was not in one of them. The agreement by itself referred to "new milk." What was required was a guarantee that the milk satisfied the requirements of the Statute. The words "new milk" did not constitute such a guarantee, and the guarantee, therefore, did not satisfy the Statute.

The other Justices agreed, and the appeal was therefore allowed, but without costs.

DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH.

REPORT OF THE FOOD INVESTIGATION BOARD FOR THE YEAR 1921.*

INTRODUCTION.—During the year the Low Temperature Station for Research in Biochemistry and Biophysics has been built and equipped. It has been planned to give temperatures ranging from freezing point to −10° F., whilst in some of the chambers electric thermostats have been installed, so as to give a range of 25° to 80° F.

The report upon the theory of the freezing of tissues has been completed and published (ANALYST, 1922, **47**, 392), and a report upon the bacteriology of canned meat and fish has also been published (ANALYST, 1922, **47**, 350, 513).

FISH PRESERVATION COMMITTEE.—The investigation into the bacteriology of "pink" in dried salted fish has been continued throughout the year. One type of the pink growth is due to a coccus (*Rhodococcus*), whilst a different variety of the growth appears to be due to another cause.

MEAT COMMITTEE.—Experiments on the effect of low temperatures upon the production of lactic acid in muscle have been continued. It has been found that during the exposure to 0° C. there is a great increase in the soluble sugar present in the muscle; also that muscle possesses the power of synthesising hexose-phosphate from glucose and sodium phosphate. Experiments on the effect of the rate of freezing on the autolysis of beef have proved that the more rapidly the meat is frozen, the more closely does the subsequent hydrolysis approach to that of normal unfrozen beef.

* H.M. Stationery Office, pp. 47. Price 2s. net.

Work has also been continued upon anaerobic bacilli and upon the identification of meat moulds, special attention being paid to the temperature relations of these fungi.

ENGINEERING COMMITTEE.—Investigations on the same lines as before have been continued (*cf.* ANALYST, 1921, **46,** 453), and an official report has been issued giving the results of researches on the thermal conductivity of heat insulators in relation to cold storage (ANALYST, 1922, **47,** 119).

FRUIT AND VEGETABLES COMMITTEE.—The scope of the enquiry has followed the three main lines:—(*a*) The preservation of fruit by cold or by "gas"; (*b*) the chemistry of the process of ripening; (*c*) the diseases of fruit when stored.

Tyrosinase and reductase have been detected in stored apples, and it has been found that the diseases of apples in store are fungal or physiological. Apple scald belongs to the latter class. Whilst carbon dioxide and diminished oxygen do not of themselves cause scald, the relatively stagnant conditions of the atmosphere in "gas" storage chambers, as at present operated, are favourable to its growth.

The following fungi have been found on apples:

(1) Common rot-producing fungi: *Sclerotinia fructigena, Penicillium glaucum, Botrytis, Rhizopus nigrans, Cephalothecium roseum, Gleosporium* and *Sphæropses malorum.*

(2) Common scab-producing fungi: *Cladosporium, Venturia.*

(3) "Spot"-producing fungi: *Pleospora pomorum, Polyoperus purpureus, P. pomi, Alternaria grossulariæ, A. pomicola, Dematium pullulans.*

(4) Fungi usually associated with other fruits: *Fusarium sporotrichoides, Rhizopus circinans, Cytosporina ludibunda.*

(5) Several other fungi not fully identified.

An attempt is being made to obtain data on the question of the relative immunity of the different varieties of apples to fungal attack. Results so far obtained show a marked relation between the hydrogen ion concentration of the apple sap and the resistance to infection by *Pleospora.*

Pectin in Fruits.—Investigations into the quantitative estimation of pectin in fruits has been continued (*cf.* ANALYST, 1921, **46,** 453). In an ordinary store there is a progressive increase in pectin, which is then followed by a decrease, full ripeness being reached shortly before the stage of maximum pectin content.

In cold store there is also a progressive increase in pectin content, but apples in cold store contain less pectin at any given date than those in an ordinary store.

A method of estimating pectin has been based by C. P. Dutt upon the formation of acetone during the conversion of the pectin into pectic acid. The acetone is distilled, other organic compounds destroyed by a second distillation with dilute sulphuric acid and potassium permanganate, and the acetone then estimated by treatment with excess of iodine in the presence of alkali, and titration of the excess of iodine.

OILS AND FATS COMMITTEE.—The study of oleic acid has shown that a thorough revision of the subject is necessary, since even the properties of the pure substance have not been ascertained with certainty. In order to obtain a practically pure acid it is necessary to reject a large proportion of the mixed lead salts which are ordinarily retained by ether at ordinary temperatures; thus separation can be effected by freezing out the ether or benzene solutions. Subsequent recrystallisation of the barium salts from mixtures of moist benzene or toluene and amyl or ethyl alcohol then yields barium oleate which is contaminated with only about 1 to 2 per cent. of palmitic acid.

The setting point of oleic acid is but slightly depressed by small additions of palmitic acid, and so, by extrapolation, it is now possible to say that the usual setting point of pure oleic acid is 13° C. or slightly higher. As oleic acid is dimorphous, the best methods of estimating the amount of palmitic acid in such samples were found to be by oxidising the oleic acid and isolating the palmitic acid, or by the use of a bromine absorption method.

Further work on "mannitol fat" (ANALYST, 1921, **46**, 454) has been carried out, and it has been found to be essentially a mannitan dioleate.

The study of the formation of fat in yeast has been continued, and it has been shown that a large part of the fat is held in combination in the cell, and built up into some complex which is broken down by acid hydrolysis. In the normal yeast cell grown on a medium containing nitrogen only, from about a third to a half of the fat present appears to exist in the free state.

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY.

THIRD CONFERENCE.*

THE third Conference of the International Union of Pure and Applied Chemistry was held at Lyons from June 27 to July 24, 1922, under the Presidency of Professor Charles Moureu. It was attended by 89 delegates, representing 24 nations. The British delegates were: Messrs. J. T. Hewitt, T. M. Lowry, S. Miall, E. Mond, and Sir William Pope, who also represented Australia. Canada was represented by Dr. Charron.

The reports of the different Committees were discussed and adopted.

I. NOMENCLATURE OF MINERAL CHEMISTRY.—The Committee was of opinion that the *Formula Index* used in the tables of *Chemical Abstracts* should be generally adopted for the classification of mineral substances. It recommended that the members of National Committees should discuss the changes suggested since the establishment of the International Committee, and send a report of their discussions to the Central Bureau of the Union before January 1, 1923.

II. NOMENCLATURE OF ORGANIC CHEMISTRY.—Acting upon the suggestion of Sir William Pope, the Committee recommended that a small committee of editors should be appointed. A Committee composed of the editors of the three most important journals of organic chemistry—Messrs. Greenaway, Marquis and Patterson—was accordingly appointed to prepare an outline of a system of nomenclature, and to submit it to the six Presidents of the National Committees elected in 1921 for discussion and report to the General Secretary of the Union.

III. NOMENCLATURE OF BIOLOGICAL CHEMISTRY.—It was decided that the preliminary report of M. G. Bertrand should be printed, and that a copy should be sent to each member of the Committee, with a view to a report being submitted at the next Conference.

IV. SIGNS OF THE POTENTIALS OF ELECTRODES.—It was decided that: (1) When the potential of a metal in solution is mentioned, the sign of the potential of the metal should be used. (2) When a numerical value of a potential is given, the basis of reference should be indicated, together with all details necessary for reproducing the value.

* Report by C. Moureu, *Bull. Soc. Chim.*, 1922, 31, 944–960.

V. ASSOCIATION OF CHEMICAL PERIODICALS.—A Committee composed of representatives of chemical periodicals proposed that the abbreviations of the titles of chemical periodicals should be those used in *Chemical Abstracts*. In accordance with the wish of the National Research Council of Japan, the abbreviation for the word "Japanese" should be "Jp.," not "Jap." The Committee also expressed the following views:

(1) That Central Bibliographic Bureaus should be formed in each country, and that arrangements should be made for the co-ordination of their work.

(2) That a list of record offices undertaking this work should be published by the Union.

(3) That the decimal system of the International Institute of Bibliography should be adopted for the classification of publications on pure and applied chemistry, and that the decimal index should be given at the head of any review of these publications.

(4) That a central registry of chemical publications and patents should be established, the publications being grouped under the names of the authors, eventually in collaboration with the editorial Committee of the *International Catalogue of Scientific Literature.*

(5) That all chemical publications should give the address of the author or that of the laboratory in which the work was done.

(6) That periodical journals should give résumés of their articles in such a form that they could be published in a journal of abstracts.

A provisional Committee was appointed to continue the consideration of all the problems connected with chemical publications.

VI. INTERNATIONAL INSTITUTE OF CHEMICAL STANDARDS.—A. *The Committee on physico-chemical standards* recommended that countries which have not yet chosen a correspondent should be asked to communicate with the Secretary of the Bureau. Also, that the annual subsidy of 10,000 francs should be made an integral part of the budget of the International Union.

B. *The Committee on pure products for research* decided that, in order to obtain immediate practical results, its work should be limited to defining the characteristics which the analytical reagents most commonly used should have, and to embodying these characteristics in an international codex, which should specify the impurities from which each reagent should be free and the maximum permissible quantity of other impurities.

This Committee recommended: (1) That each delegate of the Committee should hold office for at least three years. (2) That each delegate should collect publications bearing on the work of the Committee, and consult specialists in their respective countries. (3) That each delegate should receive from the General Secretary of the International Union all the publications relating to reagents for analysis. (4) That the use of pure sodium chloride, preferably of the sea salt type, should be studied as a standard for the comparative volumetric estimation of halogens in sea water.

C. *The Committee on technological and industrial products* recommended that a classified list of the different manufacturers of chemical products should be prepared and made available for reference at the Central Bureau, and that each country should have a delegate in touch with the central service at Paris.

VII. ESTABLISHMENT OF A THERMOCHEMICAL STANDARD.—Benzoic acid was adopted as the substance for standardising calorimetric apparatus. It is prepared by Bureau of Standards, Washington, and distributed by the Bureau of the International Institute of Physico-Chemical Standards, Brussels.

It was recommended that authors publishing details of the heat of combustion of organic substances should always give the value found by them for the heat of combustion of benzoic acid in their calorimeter.

VIII. ANNUAL INTERNATIONAL TABLES OF CONSTANTS.—The Union approved of the expenditure of the Committee for 1921, and decided that the report embodying the tables should be sent to the International Council of Researches. It was decided to establish an international fund for future working, and to ask the French Government to obtain by diplomatic means the assistance of the Governments of other countries within the Union.

IX. PRESERVATION OF FOODS BY MEANS OF CHEMICAL SUBSTANCES.—It was decided to ask delegates to prepare a summary of the work done in each country on the methods of preserving solid and liquid foods, with a view to raising the question again at the next Conference.

X. NATIONAL AND INTERNATIONAL LABORATORIES FOR COMBUSTIBLES AND CERAMICS.—It was decided to ask each country within the Union to appoint a delegate or committee to study: (1) The nomenclature of various combustibles, their legal and industrial definitions, and their physical and chemical properties. (2) Methods and apparatus for their analyses and investigation. (3) The Sub-Committee recommended that the connotation of "ceramic" should be widened, and that the American work on the subject should be made the basis of investigations to be discussed at the next Conference.

XI. SCIENTIFIC AND INDUSTRIAL PATENTS.—The Committee reported that the establishment of an international patent presented serious difficulties, but that it was more feasible to classify the nations into two or three groups, with a single bureau for each group, and so to effect a material economy in time and money. It was decided that the Committee should continue its endeavour to discover a solution acceptable to the Governments and to the patentees of the respective nations.

XII. INDUSTRIAL HYGIENE.—The Committee reported that it had been unable to make any material progress. It was decided that the Conference should, each year, concentrate its attention upon special questions. For example, it was proposed that at the next meeting the problem of industrial smoke and that of the absorption and recovery of gases and toxic vapours should be discussed.

XIII. FINANCE OF THE UNION.—It was decided that the various Committees should continue their work, that the budget of the Union should be based entirely on the normal receipts, and that these should be assigned in certain specified proportions.

XIV. CHEMICAL ELEMENTS.—The Committee nominated two new members: Messrs. Baxter (U.S.A.) and Leduc (France). It is intended to publish, in January, 1923, tables of isotopes, radio-active elements and atomic masses.

XV. ELECTION OF THE BUREAU.—The following were elected for a period of three years:—*President*, Sir W. Pope (Great Britain); *Vice-Presidents*, W. D. Bancroft (U.S.A.), E. Bülmann (Copenhagen), E. Paterno (Rome), and E. Votoček (Prague); *General Secretary*, J. Gérard (Paris).

The next Conference will be held at Cambridge from June 17 to 23, 1923.

Ministry of Health.

MILK AND DAIRIES (AMENDMENT) ACT, 1922.

SPECIAL DESIGNATIONS. Circular 356.*

THIS Circular (dated December 12, 1922) has been issued to the County Councils and Sanitary Authorities of England and Wales, to call attention to the Order No. 1332 (*vide infra*).

The Order provides for the issue of licences as follows:—

(1) By County and County Borough Councils (and in certain circumstances by Urban and Rural District Councils) to Producers of "Grade A" Milk. (2) By Sanitary Authorities to: (*a*) Distributors of "Certified" Milk, (*b*) Distributors of "Grade A" Milk, including "Grade A (Tuberculin Tested)" and "Grade A (Pasteurised)." (*c*) Distributors of "Pasteurised" Milk. (NOTE.—A licensed producer who distributes his milk to consumers direct from the farm will not require a licence from the Sanitary Authority.)

The Minister will for the present issue licences to producers of "Certified" and "Grade A (Tuberculin Tested)" Milk, who will be entitled to sell the milk produced under such licences as "Grade A" Milk without further licence from a Local Authority.

The Minister is advised that the conditions now prescribed for "Grade A" Milk are such as should provide milk that is superior to the ordinary milk of the country and reasonably safe under all ordinary circumstances, and at the same time bring the production and distribution of the milk within the competence of all careful dairymen.

PROCEDURE OF COUNTY COUNCILS.

On receipt of an application from a farmer for a licence to sell "Grade A" milk, it will be necessary for the County Council to nominate a Veterinary Surgeon to examine the herd, and until further notice the Minister concurs in the nomination of any person for the time being holding the appointment of Local Veterinary Inspector of the Ministry of Agriculture and Fisheries or the appointment of Veterinary Inspector to a Local Authority under the Diseases of Animals Acts, without reference to the Minister. If in any case it is considered desirable to nominate a Veterinary Surgeon who does not hold either of these appointments a special request should be made for the concurrence of the Minister.

It is also desirable that the County Council, on receiving such an application, should require the applicant to have one or more samples of his milk submitted to bacteriological examination at his own expense, and should satisfy themselves that the results of the examinations are such as to make it reasonably probable that the milk will comply with the prescribed tests at the time of distribution.

Where the producer delivers milk to the consumer direct from the farm it will be necessary for the County Council also to issue the distributor's licence and to exercise or arrange for the exercise of the functions of a Sanitary Authority with regard to such milk.

PROCEDURE OF SANITARY AUTHORITIES.

The action to be taken by a Sanitary Authority on receipt of an application from a distributor for a licence will depend on the designation proposed to be used. In every case except that of "Pasteurised" Milk it will be necessary for them to ascertain the source of supply and then to satisfy themselves that the corresponding licence has been issued to the producer.

In the case of "Certified" Milk a licence may ordinarily be issued without further enquiry.

In the case of "Grade A (Tuberculin Tested)" or "Grade A" Milk the Authority must verify that the distributor has an efficient arrangement for bottling, and that, until after bottling, the milk is being dealt with in a part of the premises separate from other milk. The Authority should also arrange for an examination of the caps and seals proposed to be used, and should require the applicant to have one or more samples of the milk submitted to bacteriological examination at his own expense.

* H.M. Stationery Office. Price 1d.

Apart from procedure in connection with new licences, the principal function of a Sanitary Authority will be to take samples of milk from time to time and arrange for them to be submitted to bacteriological examination. The Minister should be informed of the laboratories to which it is proposed to send the samples, and of the estimated cost. In taking samples of "Certified" Milk the Authority will be acting as agents for the Minister, who will require to be satisfied as to the arrangements proposed and will repay the approved cost of the examinations. The Minister thinks it desirable that samples of "Certified" Milk should be taken on the average at the rate of about one sample a month for each producer, the samples generally being taken from time to time from all the distributors concerned. A set of instructions as to the taking and testing of samples will be issued shortly.

When an unfavourable report is received with regard to a sample of "Grade A (Tuberculin Tested)" or "Grade A" Milk the special attention of the distributor should be drawn to the matter, and he should be advised to take steps to discover the cause of the defect and to remedy it if it is due to any default of his own, or to arrange with the producer to do so if the latter is responsible. If a further unfavourable report is received, it will be necessary for the Authority to consider whether they should take steps under Article 9 of the Order to suspend or revoke the licence, and in this connection it may be observed that a distributor who cannot deliver milk that regularly satisfies the prescribed tests is not entitled to hold a licence, even though the failure may be due to the conditions of his source of supply, and not to any fault of his own.

Notwithstanding the precautions taken in the examination of the herd it may occasionally happen that tubercle bacillus is found in milk of any grade. If this should at any time be reported, full information should at once be given to the Minister or the County Council, as the case may be, who will inform the producer and require him to take steps to ascertain which animals are affected and to remove them from the herd. The steps taken would generally include bacteriological examinations of the milk of individual cows or groups of cows, and the licensing authority will no doubt facilitate the enquiry by arranging, where necessary, for the bacteriological examinations at an appropriate charge.

"Pasteurised" Milk.

The Order lays down the general conditions governing the sale of milk as "Pasteurised," the essential condition in the matter of treatment being that the milk must be held at a temperature between 145° and 150° Fahrenheit for a period of at least half an hour. It is desirable for purposes of control that every pasteurising plant should be provided with an automatic recording apparatus.

The prescribed conditions definitely exclude the "flash pasteurisation" process by which milk is heated to a high temperature (*e.g.* 170°) for a short period. But it is also to be observed that the apparatus must be such that all the milk is held for the prescribed period, and in examining other types of pasteurisers, it will be for the Licensing Authority to determine whether the prescribed conditions are complied with. The Minister is advised that no process except that which is commonly known as the "positive holder" process has yet been shown to satisfy these conditions.

In laying down the nature of the process which is for the present to be recognised as pasteurisation the Minister wishes to make it clear that he will at any time be prepared to consider the question of making a further Order extending the definition of the term so as to embrace any other pasteurising process which can be shown to produce similar results in regard both to the destruction of pathogenic and other bacteria and to the preservation of the original properties of the milk.

General.

The Order prescribes the amounts of the fees payable for the various licences. These fees will be paid to the Authorities by which the relative licences are issued, and they have been fixed at such amounts as may be expected on the average to cover the expenditure of Licensing Authorities under the Order. But it should be understood that the amounts of the fees will be reconsidered and, if necessary, revised when sufficient experience has been gained of the working of the scheme.

It will be seen that Section 3 of the Act makes it an offence in certain circumstances to describe or refer to milk by a prescribed designation *except under and in accordance with a licence*. It would appear therefore that legal proceedings might be taken against a licensee who sold milk under such a designation contrary to the conditions governing his licence, but it seems to the Minister that as a general rule such a contravention would best be dealt with by the revocation of the licence, though a prosecution might be desirable in an especially flagrant case. The section would, however, be appropriate for enabling proceedings to be instituted against a person who uses a prescribed designation without a licence. Under Section 10 the power of taking proceedings for such an offence is conferred both on the County Council and on the Sanitary Authority. It is thought, however, that such proceedings would most conveniently be taken by the Authority having power to issue or refuse the licence or, where such power is retained by the Minister, by the Authority exercising functions under the Sale of Food and Drugs Acts.

I am to add that the Minister will be pleased to assist Local Authorities in making their arrangements in connection with licences, and he would be glad to receive, during the next year, statements at intervals of three months, showing the numbers of licences of each kind which have been issued. Lists of the persons at present licensed by the Minister to sell graded milk are being prepared for every area, and the appropriate list will be sent to every Licensing Authority in whose area any person is so licensed, as it may be expected that applications will be received from such persons. As the Order will come into operation on the 1st January, 1923, licences in these cases may at once be issued, having effect as from that date. It is not necessary that an application for a licence should be in any special form, but it will probably be found convenient for all parties that suitable forms should be issued and used. Copies of suggested forms suitable for ordinary cases are contained in the appendix to this Circular, together with a copy of the approved form of Veterinary Surgeon's Certificate.

STATUTORY RULES AND ORDERS, 1922, No. 1332.

DAIRY, ENGLAND.

The Milk (Special Designations) Order, 1922, dated December 9, 1922, made by the Minister of Health under Section 3 of the Milk and Dairies (Amendment) Act, 1922 (12 & 13 Geo. 5, c. 54.

68,101

The Minister of Health, in exercise of the powers conferred on him by Section 3 of the Milk and Dairies (Amendment) Act, 1922, and of any other powers enabling him in that behalf, hereby orders as follows:—

1. This Order may be cited as the Milk (Special Designations) Order, 1922.

2. (1) In this Order, unless the context otherwise requires—"The Minister" means the Minister of Health; "Licensing authority" means, in relation to any application for the grant of a licence, the authority which has power under Section 3 (1) of the Milk and Dairies (Amendment) Act, 1922, or this Order to grant the licence, and in relation to any licence which has been granted means the authority by which the licence was granted; "District Council" means an urban or rural district council and in relation to London means a metropolitan borough council and the Common Council of the City of London; "Herd" means the milch cows kept on the farm in respect of which any licence is applied for or granted, as the case may be, and includes any other bovine animal kept in contact with such cows; "Sell" includes offer or expose for sale, and "sale" shall be construed accordingly; "Dealer" means any person who sells milk either by wholesale or retail under any of the special designations referred to in this Order; "Producer" means a dealer owning or having the control of a herd from which milk sold under any of the special designations is obtained; "Examination" of an animal means a clinical examination carried out in such manner as the Minister may direct, by a veterinary surgeon nominated by the licensing authority with the concurrence of the Minister, and "veterinary surgeon's certificate" means a certificate, in a form approved by the Minister, signed by the person who has made such examination and certifying the result thereof; "Tuberculin test" of an animal means a test made with tuberculin in such manner as the Minister may direct, by a veterinary surgeon nominated by the Minister; and "Certificate of a tuberculin test" means a certificate, in a form approved by the Minister, signed by the person making the test and certifying the result thereof; "Sample" means a sample taken by a person duly authorised in that behalf by the licensing authority. (2) The Interpretation Act, 1889 (*) applies to the interpretation of this Order as it applies to the interpretation of an Act of Parliament.

3. The special designations under which milk may be sold or offered or exposed for sale in pursuance of this Order are the following, namely:—"Certified," "Grade A (Tuberculin tested)," "Grade A," and "Pasteurised."

4. Subject to the provisions of this Order, and without prejudice to the power to grant licences conferred on the Minister by Section 3 (1) of the Milk and Dairies (Amendment) Act, 1922, every County Council and County Borough Council is hereby authorised to grant licences to producers to sell milk as "Grade A," and every District Council is hereby authorised to grant licences to any person other than a producer to sell milk as "Certified," "Grade A (Tuberculin tested)," "Grade A," and "Pasteurised": Provided that in any case in which the Minister is satisfied that a County Council are unwilling or do not propose to exercise in any District the power of granting licences hereby conferred on them, the Minister may authorise the District Council to grant such licences in place of the County Council.

(*) 52–3 V. c. 63.

5.—(1) Every licence granted under this Order shall be in the form contained in the First Schedule hereto with such modifications, if any, as the circumstances may require. (2) Subject as hereinafter provided, every licence shall be valid for a period ending on the 31st day of December in the year in respect of which it is granted. (3) A licence granted by a local authority shall not entitle the holder to sell milk under the terms of the licence outside the area of the authority except by wholesale. (4) Where the holder of a licence desires to sell milk under the designation specified in the licence by retail outside the area of the licensing authority from a shop or other premises within that area, the licensing authority of the area in which he proposes to sell the milk, may grant him a supplementary licence entitling him to sell milk under that designation in their area from such shop or other premises, but in that event nothing in this Order shall authorise the licensing authority, either before the grant or during the currency of the supplementary licence, to exercise any powers under this Order outside their area.

6.—(1) Application for a licence shall be made in writing to the licensing authority. (2) Before granting a licence to a producer the licensing authority shall require him to produce a veterinary surgeon's certificate showing the results of an examination of the herd carried out not more than one month before the date of the application, and, in any case in which the conditions of the licence, if granted, would include the tuberculin test, a certificate of a tuberculin test of the herd carried out within a period of three months. (3) Subject to the provisions of this Order, the licensing authority shall require every applicant to satisfy them that his arrangements for the production, storage, treatment and distribution of the milk, as the case may be, are such as to comply with the conditions upon which the licence may be granted.

7.—(1) Every licence granted under this Order shall be subject to the general conditions set out in the Second Schedule to this Order. (2) In addition to the general conditions every licence for the sale of milk as "Certified," "Grade A (Tuberculin tested)," "Grade A," and "Pasteurised" shall be subject to the conditions set out in Parts I, II, III and IV of the Third Schedule to this Order respectively.

8. The fees payable for licences shall be those set out in the Fourth Schedule to this Order, but where a licence is granted for a period of less than a year, the licensing authority may grant the licence upon payment of such sum being less than the full fee as they think fit, having regard to the period for which the licence will operate.

9.—(1) A licensing authority may, if they are satisfied that any of the conditions upon which the licence is granted are not being complied with, suspend or revoke the licence, but the licence shall not be suspended or revoked before the licensing authority have served on the holder a notice stating the grounds on which it is proposed to suspend or revoke the licence, and the holder shall be afforded a reasonable opportunity of making such representations to the authority in regard thereto as he thinks fit; provided that a producer's licence shall not be suspended or revoked by reason only of his milk being found not to comply with the conditions of the licence after it has left his custody and control if he proves that such non-compliance was not due to any act or default of himself or of his servants or agents. (2) Where application for a licence has been made to or a licence has been granted by any licensing authority other than the Minister, a person aggrieved by a decision of the licensing authority to refuse or to suspend or revoke a licence may within seven days after receiving notice of such decision appeal to the Minister, whose decision shall be final, and the licensing authority shall comply with the decision of the Minister. In any appeal any reference in this Order to anything being to the satisfaction of the licensing authority or subject to their approval shall be treated as if the reference were to the satisfaction or approval of the Minister. Pending the final determination of any appeal from a decision to suspend or revoke a licence, the licence shall continue to have effect.

10. A licensing authority may delegate to a committee of the authority, with or without restrictions or conditions as they think fit, all or any of their powers under this Order.

11. Two or more licensing authorities may concur in appointing out of their respective bodies a Joint Committee for carrying out their duties under this Order and in conferring with or without restrictions on any such committee any powers which the appointing authorities might exercise for the purpose, and the provisions of Sections 57 and 58 of the Local Government Act, 1894, (*) in regard to joint-committees shall, with the necessary modifications, apply to any joint committee so appointed.

12. Notwithstanding anything contained in this Order, a licence permitting a producer to sell milk produced by him as "Certified" shall entitle him to sell such milk as "Grade A (Tuberculin tested)" or as "Grade A."

13. Where milk has been sold by a dealer who is licensed in Scotland to sell milk under any special designation, this Order shall apply to the subsequent sale of that milk in England, or Wales in the same manner as if such dealer had sold by virtue of a licence granted under the provisions of this Order.

(*) 56–7 V. c. 73.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

Food and Drugs Analysis.

Estimation of Starch in Barley and Wheat. A. R. Ling, E. H. Callow and W. J. Price. (*J. Inst. Brew.*, 1922, **28**, 838–853.)—The development of the following method, based upon previous work of Brown and Morris (*J. Chem. Soc.*, 1885, **47**, 527) and of H. T. Brown (*T. Guinness Res. Lab.*, 1903, **1**, 88), is described in detail. Approximately 5 grms. of the finely-ground grain are extracted in a Soxhlet apparatus with 50 per cent. alcohol for 3 hours, by which means sugars, fats and a portion of the proteins are removed. The contents of the thimble, after draining, are washed into a beaker with about 100 c.c. of water and heated to boiling, with continuous stirring, for 10 minutes. After cooling the beaker to 57° C. and immersing it in a water-bath at that temperature, 10 c.c. of cold malt extract are added, and the mixture is stirred at intervals of 5 minutes for one hour. The contents of the beaker are then heated to boiling and filtered into a 200 c.c. graduated flask, the residue thoroughly washed, and the filtrate and washings diluted to 200 c.c. After thorough mixing, 30 c.c. of this solution are diluted to 100 c.c., and the diluted solution is titrated against 10 c.c. of mixed Fehling's solution (ANALYST, 1905, **30**, 182; 1908, **33**, 160). The result of the titration is corrected for the reducing power of the malt extract by means of a blank estimation with 10 c.c. of the extract alone. The malt extract is prepared by adding 100 grms. of finely-ground fresh malt of known diastatic power (Lintner) to 250 c.c. of water, at 15 to 20° C., stirring the mixture occasionally for 2 hours, and filtering it until bright. The percentage of starch present is equivalent to

$$94{\cdot}73 \times \frac{\text{percentage of apparent maltose produced from sample.}}{\text{percentage of apparent maltose produced from pure starch.}}$$

The apparent maltose formed from 100 parts of pure starch by malts of various diastatic powers is tabulated below:

D.P.	Maltose	D.P.	Maltose	D.P.	Maltose
22	80·6	50	81·0	80	83·25
30	80·75	60	81·25	90	85·0
40	80·85	70	82·0	100	87·25

Results obtained with similar samples of barley and wheat by using malts of widely varying diastatic power show excellent agreement. A curve is given showing the relation between the diastatic power of the malt used and the percentage of apparent maltose produced. T. J. W.

Micro-Estimation of Lactose. G. Fontès and L. Thivolle. (*Compt. rend. Soc. Biol.*, 1922, 164; *Ann. Chim. anal.*, 1922, **11**, 341.)—One c.c., or even

0·1 c.c. of milk is accurately measured by means of a pipette into a hard glass tube marked at 50 c.c. (If 0·1 c.c. of milk is taken, capillary tubing must be used for the pipette, and 0·1 c.c. of milk should occupy 6 to 10 cm. of the length of the tube.) The pipette is washed with 5 or 6 c.c. of water, which are added to the milk. One c.c. of mercuric nitrate solution (Patein's reagent, as modified by Denigès, diluted to 1 per cent.) is used for clearing the mixture, and 1 drop of sodium hydroxide solution (sp. gr. 1·36) diluted to 10 per cent., and 4 drops of glacial acetic acid diluted to 10 per cent. are then added, together with sufficient distilled water to bring the volume to 50 c.c. After filtration of the liquids the mercury is eliminated by means of copper turnings, and 1 or 2 c.c. of the filtrate are treated with 2 c.c. of Fehling's solution, and the mixture centrifuged. The tube is then kept in a saturated solution of calcium chloride at 120° C. for 6 minutes. From 0·1 to 2·5 mgrms. of lactose can thus be estimated, the amount of reduction being compared colorimetrically with that given by a solution containing 1 mgrm. of pure lactose. D. G. H.

Detection of Extract of *Atractylis gummifera* in Liquorice Extract. U. Giuffrè. (*Giorn. Chim. Ind. Appl.*, 1922, **4**, 460–461.)—Alkaline hydrolysis of potassium atractylate, the poisonous glucoside of *Atractylis gummifera*, yields valeric acid, sulphuric acid, a carbohydrate and atractyligenin. Adulteration of liquorice extract with *Atractylis* may, therefore, be detected as follows:—A dense solution of 50 grms. of the extract in a little water is treated with about 500 c.c. of alcohol, and the solution mixed with animal charcoal, allowed to stand, filtered and reduced to a small volume by distillation. The residue is diluted with a little water, rendered distinctly alkaline by means of sodium hydroxide, boiled for about 30 minutes in a reflux apparatus, cooled, acidified with dilute sulphuric acid and distilled in a current of steam to a small volume. The presence of valeric acid in the distillate is indicated by the odour. The residue left on distillation is saturated with ammonium sulphate, boiled for 15 minutes with alcohol and animal charcoal and filtered. The filtrate is concentrated and mixed with water, the precipitated atractyligenin being further purified by boiling with alcohol and charcoal. A drop of the filtered liquid is evaporated to dryness on a watch glass over a water-bath, and the cold residue taken up in a few drops of concentrated sulphuric acid; a yellowish coloration is thus obtained, this changing to carmine on addition of a little aqueous piperonaldehyde, vanillin or other aromatic hydroxy-aldehyde. T. H. P.

Analysis of Santonin Tablets. M. Francois. (*J. Pharm. Chim.*, 1922, **26**, 339–341.)—Since, on the addition of alkali to the nearly insoluble santonin, a soluble santoninate is formed from which santonin is recovered by the addition of strong acid, the santonin in tablets may be estimated as follows:—Ten grms. of slaked lime are added to 10 tablets finely ground in a glass mortar, and the whole triturated for 15 minutes to give a homogeneous mixture, which is introduced into a 125 c.c. conical flask. Fifty c.c. of 95 per cent. alcohol are added, and the flask heated for one hour on a water bath under a reflux condenser at a little under

the boiling point of the liquid. The decanted liquid contains the santonin in solution as calcium santoninate. Three more digestions are made, 100 c.c. of water added to the filtered liquid, and the mixture distilled over a naked flame until 180 to 190 c.c. of distillate have been collected, corresponding to the whole of the alcohol. Ten drops of concentrated hydrochloric acid are added to the alcohol-free solution in a separating funnel, and the whole shaken up six times with six portions of 20 c.c. each of chloroform. The united chloroform washings are shaken with 20 c.c. of distilled water to remove any sugar, and filtered through a dry filter paper into a large weighed crystallising dish, left to evaporate in the dark, and the residue subsequently weighed.

The characteristic colour reaction of santonin may then be obtained by putting on a microscope slide about 1 mgrm. of the product (noting that it is formed of clearly striated rectangular plates), placing on it powdered potassium hydroxide to about the size of a grain of wheat, and warming the slide over a small flame until the potash melts. A striation of a carmine red colour results which, under the microscope, is seen to be due to crystals of santonin which have become red without melting. If the little nodule is then dissolved in 95 per cent. alcohol, the alcohol is coloured red. In a mixture of 0·1 grm. of santonin, 0·2 grm. of gum tragacanth and 10 grms. of powdered sugar the weight of santonin obtained was 0·0995 grm.

Analysis of Santonin Chocolate Tablets.—The method described above may be used, but, owing to the large amount of inert material present, slight modifications are necessary. On digesting with the lime and alcohol the alcohol becomes turbid owing to the cacao butter present. After removal of the alcohol, the residue must be well cooled and carefully filtered, in order to get rid of the fat, before acidifying and shaking with chloroform. From a mixture of 0·1 grm. of santonin in 10 grms. of chocolate, 0·102 grm. of santonin was recovered. D. G. H.

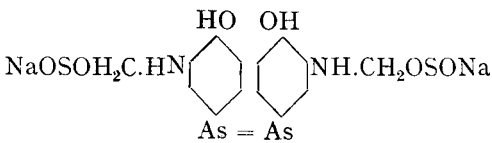
Estimation of Oxymethylanthraquinone Compounds in Drugs. E. Maurin. (*J. Pharm. Chim.*, 1922, **26**, 348–349.)—The two methods of (1) Tschirch (hydrolysis by means of dilute sulphuric acid and extraction with ether) and (2) Daëls (hydrolysis by means of dilute sulphuric acid in the presence of chloroform, which removes the products of hydrolysis as formed), the first colorimetric and the second gravimetric, are combined in the following way:—A mixture of 1 grm. of the powder (passing a No. 45 silk sieve), 25 c.c. of 20 per cent. sulphuric acid, and 100 c.c. of chloroform are boiled for 10 hours under a reflux condenser. The chloroform is separated, and the water solution washed with 20 c.c. of chloroform which is added to the first portion. About nine-tenths of the chloroform are then distilled off, and the residue shaken with 100 c.c. of 5 per cent. potassium hydroxide solution, which becomes coloured rose pink. Fifty c.c. more are used, and subsequently 25 c.c., if necessary. The combined alkali solutions are made up to 1 litre and compared in a colorimeter with an alkaline solution of 1 cgrm. of enodine in 1 litre. The results so obtained compare closely with those obtained by the Daëls' method. D. G. H.

Variation of Oxymethylanthraquinone Compounds in Alder and its Galenic Preparations. E. Maurin. (*J. Pharm. Chim.*, 1922, **26,** 349–350.)—The differences in the quantities of oxymethylanthraquinone compounds contained in the bark are suggested as a cause for the variability of the therapeutic action of these preparations, and the quantities present in bark of various ages, position, geographical distribution, etc., were determined by the method given above. Bark from twigs 3 or 4 years old was found to give the maximum of active principle.

D. G. H.

Compound of Antipyrin with Xanthydrol. R. Fabre. (*J. Pharm. Chim.*, 1922, **26,** 372–376.)—Antipyrin and xanthydrol unite, with elimination of a molecule of water, to form a compound, $C_{24}H_{20}O_2N_2$, melting on the Maquenne block at 178–179° C. Since ingested antipyrin passes rapidly and in considerable proportion into the urine, treatment of the latter with xanthydrol for the estimation of the urea yields dixanthylurea contaminated with the above compound, a high result being thus obtained. In such cases the urine (1 c.c.) should be subjected to a preliminary defecation with an acetic acid solution of potassium mercuric iodide (0·1 c.c.), which precipitates the antipyrin. T. H. P.

Examination of Neoarsphenamine. Constitution of the French Drugs. A. D. Macallum. (*J. Amer. Chem. Soc.*, 1922, **44,** 2578–2582.)—French neoarsphenamines are somewhat less potent therapeutically than the American preparations; their general type is a doubly substituted arsphenamine:



For their analysis, in addition to the usual estimation of arsenic and ultimate analysis, it is necessary to estimate the sulphoxalate and bisulphite groups; this is done by the following processes. (1) *Partial Reducing Power*: To 0·2 grm., dissolved in 10 c.c. of water and acidified with sulphuric acid, are added 5 c.c. of 0·1 *N* iodine solution, and the mixture titrated back after 3 minutes with 0·1 *N* thiosulphate solution; the number of c.c. of iodine solution multiplied by 5 = the P.R.P. (2) *Total Reducing Power*: To 0·1 grm. in 50 c.c. of water are added 100 c.c. of the iodine solution, and, after 3 minutes, 10 c.c. of 2 *N* sodium hydroxide solution, followed, after a further 3 minutes, by an excess of dilute sulphuric acid, and the mixture is titrated back with thiosulphate; the number of c.c. of iodine used multiplied by 10 = the T.R.P. (3) The percentage of arsenic × 5·172 gives the amount of iodine reduced by the arseno groups (if greater than the P.R.P. the presence of oxygenated arseno-groups is indicated). (4) The arsenic percentage × 10·02 indicates the total iodine required by the arsphenamine groups. (5) The P.R.P., less the iodine required by the arsenic, divided by 3·96 gives the percentage of sulphoxalate (as CH_2OSONa), and its iodine requirement is 1·5 times the above difference. (6) The percentage of methylenebisulphite (as $-CH_2OSO_2Na$)

is found by dividing the difference between the T.R.P. and the sum of the iodine requirements of the arsphenamine and the sulphoxalate by 3·41. The following is a typical result:—Arsenic, 20·22; nitrogen, 4·30; chlorine, nil; sulphur, 11·43; sodium, 7·52; sulphoxalate, 4·09; and bisulphite, 30·17 per cent. H. E. C.

Estimation of Nicotine in Tobacco and in Tobacco Smoke. M. Popp and J. Contzen. (*Chem. Zeit.*, 1922, **46**, 1001–1002.)—The extraction method of Rasmussen (*cf.* ANALYST, 1916, **41**, 208) and the steam distillation method of Mach (*Landw. Versuch.*, **45**, 40) for the estimation of the nicotine in tobacco have been examined; the distillation method is carried out by adding excess of alkali to the powdered tobacco and steam-distilling into 10 per cent. hydrochloric acid solution, from which the nicotine is precipitated with silicotungstic acid, and is to be preferred; but both methods yield accurate and concordant results, there being no pyridine to interfere. For the estimation of the nicotine in the smoke, a weighed quantity of the tobacco is smoked in a pipe and the nicotine absorbed by passing the smoke through wash bottles containing 10 per cent. hydrochloric acid. After the acid solution has been mixed and filtered, an aliquot part is made alkaline with sodium hydroxide solution and steam-distilled; the nicotine in the distillate is precipitated by means of 10 per cent. silicotungstic acid solution, dried at 120° C., and weighed. Tobacco and other plants yield, when burned, substances other than nicotine which are precipitated by silicotungstic acid, so that steam distillation after the absorption is essential. It was found that the brown sticky substance which collects in the absorption bottles does not retain nicotine and may be filtered off, and that three hydrochloric acid absorption bottles are sufficient to retain all the nicotine. The precipitation of pyridine with silicotungstic acid was also investigated, and it was found that quantities of pyridine above 25 mgrms. per 100 c.c. produce a precipitate in the presence of acetic or hydrochloric acids, but that the precipitation is never complete. Separation of nicotine from quantities of pyridine up to 0·5 grm. may be effected by acidifying the solution with acetic acid and steam-distilling; all the pyridine appears in the distillate, and the nicotine in the residue may be precipitated with silicotungstic acid. Statements that certain tobaccos contain their nicotine in a non-poisonous form are incorrect. Analyses of smoke from a number of different kinds of tobaccos smoked as cigars, cigarettes, and in pipes, show that about one half the nicotine in the tobacco appears in the smoke. H. E. C.

Biochemical, Bacteriological, etc.

Autolysis of Beef and Mutton. W. R. Fearon and D. L. Foster. (*Biochem. J.*, 1922, **16**, 564–571.)—The tissues were removed immediately after the death of the animal and separated from the connective tissue, fat, etc., after which they were minced, mixed with four times their volume of water and a small quantity of toluene, and incubated at a definite temperature. At regular intervals samples were removed, and the total nitrogen and the soluble nitrogen remaining

after precipitation with metaphosphoric or trichloracetic acid were estimated by Kjeldahl's method. The results were expressed as the percentage of soluble nitrogen compared with the total nitrogen. Ox and sheep liver both showed a rapid increase at 37° C. during 5 days, after which the values obtained were constant and identical for the two tissues. With muscle, the proportion of soluble nitrogen increased for about 8 days, afterwards remaining constant, but beef showed 5 per cent. less soluble nitrogen throughout. At temperatures of 6° C. and 0° C. the autolysis of beef and mutton muscle was considerably retarded, but the curves remained parallel as at 37° C. Three samples of ox muscle were frozen by (*a*) cooling in air to −8° C. for 1 day, (*b*) cooling to 0° C. for 20 hours and placing in cold store at −8° C., and (*c*) cooling in saturated brine to −8° C.; after which the estimation of nitrogen was carried out as above. The rates of autolysis varied greatly, and, after reaching equilibrium, the amounts of soluble nitrogen found were approximately 17, 15·5 and 12·5 per cent., respectively, whilst a corresponding unfrozen control showed a value of 13·5 per cent. No explanation is given of this variation, but the general belief that brine freezing causes little damage to the cell is confirmed. T. J. W.

Estimation of Carnosine. G. Hunter. (*Biochem. J.*, 1922, **16**, 640–654.)—A critical examination of the method previously described (*Biochem. J.*, 1921, **15**, 689) is given in detail, and the following modification is adopted:—To the solution of carnosine or muscle extract exactly half the volume of concentrated hydrochloric acid is added, and the mixture is heated to 90° C. for 75 minutes, the carnosine present being hydrolysed to histidine during this period. This solution, in addition to the original unhydrolysed sample, is treated with sodium carbonate and diazotised, as in the previous method, and both are compared with the colour standard. The ratio of the colour of the two solutions is termed the hydrolysis constant, and for a solution of pure carnosine is 0·73, whilst the values given for muscle extracts are: Cat, 0·71; ox, 0·76; and salmon, 0·79. The results obtained are vitiated by the presence of ammonium salts, sulphides, phenols and aldehydes, and in ox muscle about 3 per cent. of the colour is due to purines, and the total error is probably 5 per cent. Carnosine may be distinguished from histidine by Knoop's test, the former developing no colour with this reaction (see preceding abstract). The carnosine content of muscle varies considerably in different species of animals and in different individuals of the same species. The author points out that these results differ greatly from those obtained by Clifford (ANALYST, 1921, **46**, 507; 1922, **47**, 443), and indicate that the carnosine used by that worker was only of about 43 per cent. purity. T. J. W.

Changes Occurring in the Pectic Constituents of Stored Fruits. M. H. Carré. (*Biochem. J.*, 1922, **16**, 704–712.)—Estimations of the soluble pectin and protopectin (pectose) content of Lane's Prince Albert and Cox's Orange Pippin apples were carried out, the former by a method previously described (ANALYST, 1922, **47**, 263), and the latter by adding 100 c.c. of 0·05 *N* hydrochloric acid to the

residue after removal of the soluble pectin and heating it in an autoclave for 1 hour at 110° C. By this treatment the protopectin was rendered soluble and was readily removed by pressing and washing the insoluble residue, after which the estimation was carried out as in the preceding method. The results obtained show that in the early stages of development of the fruit little or no soluble pectin is found, but that this substance gradually increases in amount until the fruit is fully ripe, then remains constant for about 4 weeks, and decreases in amount when a general softening of the apple commences. This change proceeds similarly with both varieties of apples and is practically unaffected by extending the "picking over" for several weeks, or by storage at ordinary or low temperatures. The harder Prince Albert apples contain a larger amount of protopectin than do the softer Orange Pippins, and series of estimations indicate that the amounts of this substance and of soluble pectin present are roughly in inverse ratio, but during ripening an increase in the total pectic constituents occurs, due to a greater formation of soluble pectin than can be accounted for by the diminution of the protopectin. Preliminary experiments show that the increase in soluble pectin is due to enzymic action. T. J. W.

Mycological Identification of Inulin. A. Castellani and F. E. Taylor. (*Biochem. J.*, 1922, **16**, 655–658.)—A table is provided showing whether or no gas is generated by the growth of different species of *Monilia* and bacteria upon solutions of various carbohydrates and glucosides. Inulin yields gas only by inoculation with *M. macedoniensis* Cast, the other carbohydrates also giving gas by the growth of this organism being dextrose, lævulose, galactose and sucrose. A 1 per cent. solution of the substance under examination is prepared in sugar-free peptone water and transferred to two test tubes containing Durham tubes, in which it is sterilised, and, after cooling, one tube is inoculated with *M. macedoniensis*, the other with *M. tropicalis* Cast. The tubes are transferred to an incubator at 37° C. for 72 hours, when the presence of gas in the first tube, and its absence in the second, indicates the presence of inulin. In the case of mixtures the inulin may be detected by successive inoculations and cultivation of the same portion of solution with different organisms. From the table referred to above and a series of mycological formulæ, also given, it is possible to detect other carbohydrates either alone or in a mixture. T. J. W.

Action of Saponins on Yeast Cells. F. Boas. (*Ber. bot. Ges.*, 1922, **40**, 32–38; *Chem. Abstr.*, 1922, **16**, 3325.)—Different varieties of saponin vary in their influence on the fermentation of sucrose by yeast. In the case of highly active saponins fermentation is inhibited, owing to the destruction of the yeast plasma, whereas with less active saponins the rate of evolution of carbon dioxide is increased, owing to increased permeability of the plasma membrane. The action of different saponins, which is due to their effect on the colloidal state of the lipoid complex of the plasma membrane, may be correlated with their hæmolytic activity.

Yeast as a Source of Vitamin *B*. **C. Kennedy and L. S. Palmer.** (*J. Biol. Chem.*, 1922, **54**, 217–232.)—Yeast is generally considered to be a rich source of vitamin *B*, but experiments carried out by the authors on rats indicate that its value is over-estimated. The standard adopted for satisfactory results was the attainment of normal weight and physical well being of the rats, together with their ability to reproduce and rear their young at normal intervals. The actual number of yeast cells present in different commercial yeasts varies between the ratio limits of 1 and 26, and this factor does not appear to have been taken into account by previous workers whose results it is therefore impossible to correlate. Since the vitamin activity of the yeast does not correspond with the number of cells present, the authors assume that the yeast is not the active agent. The function of yeast as a growth promoter is partly dependent upon the manner in which it is fed to the animals. Thus less satisfactory results are obtained when the yeast is mixed with the basal diet than when the same weight is given separately. Although conditions were favourable for reproduction, young were produced in very few instances, and were never reared even when comparatively large amounts of yeast were given. The addition of 10 per cent. of yeast to the food ration added 25 to 50 mgrms. of nitrogen daily, but the growth produced was less satisfactory than when 11·4 per cent. of alcoholic wheat embryo extract, contributing about a fifth of the above weight of nitrogen, was used. It is considered a waste of labour to use highly purified casein, etc., in the preparation of a basal diet, and then to add to it such a complex mixture as yeast, especially in relatively large amounts. T. J. W.

Detection of Urobilin in Urine. **G. Rodillon.** (*J. Pharm. Chim.*, 1922, **26**, 379–381.)—Grimbert's method for detecting urobilin in urine may be simplified as follows:—The reagents used are: (A) 15 grms. of powdered zinc oxide, 250 c.c. of 95 per cent. (by vol.) alcohol, and 15 c.c. of glacial acetic acid, shaken frequently during 48 hours and then filtered. (B) Tincture of iodine diluted to ten times its volume with 95 per cent. alcohol. The urine is shaken vigorously in a test tube with an equal volume of (A), a drop of (B) being then mixed in. The liquid is then mixed with about one-tenth of its volume of chloroform by several inversions of the tube, the turbid alcoholic chloroform layer which settles being rendered clear by warming. If a ray of light is then allowed to penetrate this layer, an emerald-green fluorescence is observed if urobilin is present, and the layer will show a pink coloration if the proportion of urobilin is large. Albuminous urine should first be mixed with an equal volume of reagent (A) and filtered, and the filtrate treated as described above. T. H. P.

Agricultural Analysis.

Extraction of Copra Cake with Solvents. **A. P. West.** (*Philippine J. Sci.*, 1922, **20**, 509–517.)—Different solvents, purified by distillation, were found to dissolve varying amounts of oil and non-fatty materials from copra expeller cake. Ether, carbon tetrachloride, and benzene extracted 13·28–13·38

per cent. in 3 hours, chloroform and acetone 14·22–15·43 per cent., whilst ethyl alcohol (absolute and 95 per cent.), extracted 24·74–27·27, and methyl alcohol as much as 30·36 per cent. in the same time. Petroleum spirit, b. pt. 40°–55°, gave about the same result as ether, but over a long period the amount extracted was somewhat greater. After extraction of the fat with carbon tetrachloride the non-fatty material, subsequently dissolved by methyl alcohol, was in one case examined and found to be dark brown and viscous, like molasses. It contained free acids (possibly, amino acids) corresponding with 49 mgrms. of potassium hydroxide per grm. and required 145 mgrms. of potassium hydroxide per grm. for hydrolysis. It contained 13·16 per cent. of reducing sugars, and 1·26 per cent. of nitrogen, and had an iodine absorption of 6·6 per cent. D. G. H.

Organic Analysis.

Oxidation of Different Forms of Carbon and of Oils by means of Chromic Acid. D. Florentin. (*Bull. Soc. Chim.*, 1922, **31**, 1068–1072.)—When carbon in any form, whether free or combined in organic compounds, is oxidised by means of chromic and sulphuric acids there is invariably produced an appreciable quantity of carbon monoxide. This causes low results by all the methods which have been proposed for the wet oxidation of carbon in which the resulting carbon dioxide is absorbed in potassium hydroxide solution and weighed. As, however, the molecular volumes of the two oxides of carbon are the same, the error does not appear if the volume of gas is measured. The amount of the carbon monoxide produced varies with the experimental conditions and cannot be controlled, so that it is not possible to apply a correction. The only method of getting accurate results is to pass the gases evolved over red hot copper oxide before the potash absorption. The oxidation of carbon is preceded by the formation of a complex, and the production of carbon monoxide is a necessary result of the atomic structure of carbon. H. E. C.

Rôle of Chromic Oxide in the Sulpho-chromic Oxidation. L. J. Simon. (*Comptes rend.*, 1922, **175**, 768–770.)—Further investigation (*cf.* ANALYST, 1922, 405) shows that, in oxidation by mixtures of sulphuric acid and chromates, an important part is played by the chromium sesqui-oxide. If, as is usual, the organic material is mixed with 12 to 15 c.c. of concentrated sulphuric acid and either 4 grms. of chromic anhydride or 12 grms. of silver chromate and heated gradually to 100° C. and maintained at this temperature for 4 minutes, no gas is evolved from the reagents themselves; the same result is observed if 6 grms. of chrome alum, or a corresponding amount of the sesqui-oxide are present in addition. When, however, the heating at 100° C. is prolonged, sulphuric acid in presence of the sesqui-oxide is capable of decomposing chromic anhydride completely, the velocity of the decomposition being given by the parabolic formula, $v=at/(b+t)$, where a denotes the total volume of oxygen obtainable theoretically, t the time, and b the time required for the liberation of one-half of the theoretical amount of

oxygen. This velocity varies with the proportion of sesqui-oxide taken, and is lowered if the chromic anhydride is replaced by silver chromate.

Propionic acid is incompletely oxidised when heated for four minutes at 100° C. with the mixture of sulphuric acid and chromic anhydride, but on protraction of the heating the theoretical amount of gas is attained and then exceeded, free oxygen being liberated with the carbon dioxide. Acetic acid is not acted on under the normal conditions, but if the sesqui-oxide also is present, it undergoes partial oxidation, the extent of which is related to the proportion of sesqui-oxide present.

T. H. P.

New Reaction for Oxalic Acid. C. Müller. (*Chem. Trade J.*, 1922, **71**, 541.)—The solution containing free acid is evaporated almost to dryness in a test tube and a trace of pure resorcinol is added, after which 2 c.c. of sulphuric acid are allowed to run down the side of the tube. In the presence of oxalic acid a green or blue ring is formed and, on agitation, the solution becomes a deep azure blue, changing to violet on heating. This reaction appears to be characteristic for oxalic acid and its salts, since fourteen other acids occurring in plants yield either yellow, orange, pink, red or brown colorations when examined by the above test.

T. J. W.

Qualitative Test for Tannin. E. Atkinson and E. O. Hazleton. (*Biochem. J.*, 1922, **16**, 516–517.)—The reactions generally employed for the detection of tannins are not specific and, since the characteristic action of these compounds is that of combining with animal membranes to form leather, the authors have evolved the following test:—One grm. of the material is heated on a water-bath for 30 minutes with 50 c.c. of water, and 1 c.c. of the solution is poured on to a piece of moist gold-beater's skin, about 12 × 18 mm., pinned flat upon a surface of paraffin wax. After 30 minutes the tanned skin is washed for two minutes with water dripping at the rate of two drops per second, after which it is stained by 1 c.c. of 1 per cent. ferric chloride solution (which is allowed to act for 5 minutes) and is again washed and dried. By the use of this test the presence of tannin or gallotannin was demonstrated in a large number of substances possessing tanning properties, whilst negative results were obtained with phenol, gallic acid, pyrogallol, catechol, quinol, resorcinol, phloroglucinol, salicylic acid, protocatechuic acid and β-resorcylic acid. The combination between the tannin and the membrane is very permanent, since the stain produced may be repeatedly decolorised and restored by the alternate application of dilute hydrochloric acid and of ferric chloride solution.

T. J. W.

Determination of the True Specific Gravity of Coke. H. J. Ross. (*J. Ind. Eng. Chem.*, 1922, **14**, 1047–1049.)—Data are given showing the effect of the degree of fineness, nature of the liquid used, time of boiling and standing, and use of a partial vacuum on the results obtained in the determination of the specific gravity of coke. When a "60-mesh" coke is ground to pass a 200-mesh

sieve the specific gravity increases by 12·7 per cent., the greatest increase being found in the case of cokes made at a temperature below 800° C. As regards the liquid used, the results obtained by using benzene are 8 per cent. lower than those found when water is used. Smaller differences are produced by variations in the time of boiling and standing in the wetting liquid, and the use of a partial vacuum during boiling. Since the determination of the actual volume of coke substance in a given sample appears to be a difficult, if not impossible, matter, the author suggests the use of an arbitrary method in which water is employed as the wetting liquid, the period of boiling is thirty minutes, and the sample is ground to pass a 200-mesh sieve. W. P. S.

Results of Analyses of some American Woods. G. R. Ritter and L. C. Fleck. (*J. Ind. Eng. Chem.*, 1922, **14**, 1050–1054.)—Average results of analyses of certain American hard and soft woods are given in the following table; the figures express percentages on the material dried at 105° C.:

	Western Yellow Pine	Yellow Cedar	Incense Cedar	Redwood	Tanbark Oak	Mesquite	Balsa	Hickory
Ash	0·46	0·43	0·34	0·21	0·63	0·54	2·12	0·69
Soluble in cold water	4·09	2·47	3·64	7·36	4·10	12·62	1·77	4·78
hot water	5·05	3·11	5·38	9·86	5·60	15·09	2·79	5·57
ether	8·52	2·55	4·31	1·07	0·80	2·30	1·23	0·63
1% NaOH solution	20·30	13·41	17·69	20·00	23·96	28·52	20·37	19·04
Acetic acid	1·09	1·59	0·91	1·08	5·23	2·03	5·80	2·51
Methoxy content	4·49	5·25	6·24	5·21	5·74	5·55	5·68	5·63
Pentosan	7·35	7·87	10·65	7·80	19·59	13·96	17·65	18·82
Methyl-pentosan	1·62	3·42	1·35	2·75	None	0·70	0·86	0·80
Cellulose	57·41	53·86	41·60	48·45	58·03	45·48	54·15	56·22
Lignin	26·65	31·32	37·69	34·21	23·85	30·47	26·50	23·44

W. P. S.

Estimation of Phenol in Mixtures of Tar Acids. W. H. Hoffert. (*J. Soc. Chem. Ind.*, 1922, **41**, 334–337T.)—The following method, which allows of the direct estimation of phenol mixed in any proportion with any or all of the three cresols, depends on the observation that the depressions of the freezing point of phenol hydrate produced by equal weights of each of the cresols are identical. The method yields results in good agreement with those given by Fox and Barker's method (ANALYST, 1917, **42**, 329; 1918, **43**, 389; 1920, **45**, 309); it is more rapid and more suitable as a works' test than the latter and is practically unaffected by traces of moisture left in the phenol-cresol mixture.

If the mixture of tar acids contains more than 2 to 3 per cent. of neutral hydrocarbons and pyridine bases, a quantity of it is freed from these impurities, either by steam distillation of the solution in sodium hydroxide or by extraction of the solution with ether or benzene, as described by Fox and Barker (*loc. cit.*). When the tar acids are recovered by acidification of the alkaline solution with sulphuric acid, the acid layer should be extracted to recover any phenol present in the sodium sulphate solution. If a preliminary distillation test shows that the mixture of tar acids, thus treated, distils over entirely below 205° C., the estimation of the phenol may be proceeded with at once. If, however, the presence of more

than traces of xylenols and higher homologues is indicated, these should first be removed by a preliminary fractionation, with an efficient column, up to 203° C. If much phenol is present, the distillate obtained above 203° C. may still contain an appreciable quantity of phenol, but this is rarely the case; moreover, fractions with high phenol content are usually free, or almost free, from xylenols and higher fractions. To a weight a of the mixture of tar acids (X), obtained as above, and freed from water, is added such amount b of standard phenol freezing at 40·5° C. as will yield a mixture containing at least 55 per cent. of phenol, and 10 per cent. of its weight of water is then run in from a burette. From 12 to 15 grms. of this solution are used for a preliminary approximate determination of freezing point in an apparatus similar to that of Fox and Barker, and consisting of a thin-walled test-tube inserted into another slightly wider tube with a little dry calcium chloride at the bottom. The thermometer should be graduated in tenths of a degree from +20° to −10° C., the tube being placed in ice and salt and the liquid stirred with a copper wire stirrer until crystals of phenol hydrate appear; seeding with a crystal of the hydrate is advisable. The temperature at which the crystals just disappear having been read, the tube is again cooled to a few degrees below this temperature and the liquid seeded, stirred rapidly so as to obtain a fine cloud of crystals, placed in a bath 1 to 2° C. above the freezing point and stirred rapidly until the crystals just disappear. The percentage of phenol p corresponding with this freezing point is then read off from the curve (see below), the percentage of phenol x in the original mixture (X) being then $x = \frac{p(a+b) - 100b}{a}$. The curve is constructed from the following data:

Per cent. of phenol in mixture	100	95	90	85	80	75	70	65	60	55
Freezing point of hydrate (9·1 per cent. of water), °C.	16·0	14·25	12·25	10·25	8·1	5·8	3·4	0·75	−2·2	−5·2

To estimate phenol in crude carbolic acid, the sample should first be freed from neutral hydrocarbons and pyridine bases, if these amount to more than 2 to 3 per cent. A weighed quantity is then fractionated with an efficient column, the water being collected separately and salted out and the oil returned to the flask. The oil is fractionated to 202° C. (distillate A), the residue being again fractionated to 202° C. with about one-half of its volume of o-cresol (distillate B). The phenol in A and B, which are weighed, is then estimated by the hydrate method.

T. H. P.

Inorganic Analysis.

Conductivity Titrations by means of Barium Chloride. I. M. Kolthoff. (*Zeitsch. anal. Chem.*, 1922, **61**, 433–448.)—*Sulphate.* The titration of potassium sulphate with 0·1 N barium chloride solution gives low results (about 1 per cent.), but sodium sulphate can be titrated accurately. In presence of mineral acids and an aluminium salt the results are low, whilst ferric salt interferes in a more irregular manner. Nitrate causes high results in more concentrated (*e.g.* 0·035 N) sulphate solutions, but is almost harmless at greater dilutions (*e.g.* 0·0038 N). As in the

gravimetric sulphate estimation, the results are more or less low in presence of calcium; in very dilute solutions the negative error amounts to 12 to 14 per cent.; hence the small quantities of sulphate present in drinking waters cannot be estimated conductometrically. Accurate results are obtained with sulphate concentrations as low as 0·001 *N*, but the conductivity does not become constant for some minutes after the addition of the precipitant; alcohol accelerates the precipitation to a greater extent than an addition of solid barium sulphate. *Carbonate* can be titrated with accuracy, as well as *chromate*; *phosphate* cannot be estimated, and *pyrophosphate* titrations are about 4 per cent. low. *Organic acids*: Neutral sodium oxalate, tartrate, citrate, and malate solutions are titratable after addition of one-half the volume of strong alcohol. The titration of succinate, salicylate, and benzoate is not feasible. (*Cf.* ANALYST, **1922, 47,** 453.)

W. R. S.

Electrometric Standardising of Titanous Solutions. W. S. Hendrixson and L. M. Verbeck. (*J. Amer. Chem. Soc.*, 1922, **44,** 2382–2386.)—The use of titanous solutions in volumetric analysis may be extended by the application of the electrometric method; the solution is so strongly reducing in character that there is a well-marked change in the voltage when it is titrated against dichromate or permanganate in the usual electrometric apparatus. A solution of titanous sulphate in dilute sulphuric acid is preferred to the usual solution of the chloride, and may be titrated, either directly or through the medium of ferric iron, with dichromate or with permanganate, and it is immaterial which solution is run into the other. There is a sharp change of 0·9 volt from titanous ions in slight excess to a slight excess of permanganate, or of 0·6 volt in the case of dichromate, which fact may permit of the estimation of two substances of different oxidising or reducing powers in the same solution. It is shown also that titanous solutions may be readily standardised directly against permanganate or dichromate.

H. E. C.

Separation of Ferric Oxide and Alumina from Magnesia by the Nitrate Method. A. Charriou. (*Comptes rend.*, 1922, **175,** 693–695.)—The precipitation of magnesia along with ferric oxide and alumina is reduced to minimum proportions by the procedure already described for preventing the co-precipitation of lime (*cf.* ANALYST, 1922, **47,** 271). T. H. P.

Gravimetric Estimation of Nickel as Dioxide. W. Vaubel. (*Chem. Zeit.*, 1922, **46,** 978.)—Nickelous hydroxide, precipitated by sodium hydroxide as usual, is well washed, dried, ignited, and leached with water to remove the last of the alkali. The precipitate is again ignited, dissolved in nitric acid, the acid evaporated, and the dry residue heated for half an hour in an air-bath at 280° to 330° C. The black oxide is NiO_2. It is pointed out that the sesqui-oxide, which is sometimes said to be obtained by ignition of the nitrate, has not yet been prepared in the pure state. W. R. S.

Electrometric Titration of Nickel with Silver Nitrate. E. Müller and H. Lauterbach. (*Zeitsch. anal. Chem.*, 1922, **61**, 457–464.)—Moore's cyanide titration of nickel can be modified in the following manner:—The neutral solution is not made ammoniacal, but treated with a measured excess of standard cyanide solution; the excess of the latter is measured with standard silver solution, the end-point being marked by the appearance of a cloudiness of silver cyanide. The modified process can be carried out electrometrically with a silver indicator electrode and a normal calomel electrode. The rheostat being adjusted to 0·075 volt, the mechanically stirred solution is titrated with silver nitrate solution until the galvanometer indicates zero. If b = c.c. of silver nitrate solution, a = c.c. of cyanide solution, and m = grms. of silver per c.c. of silver solution, the quantity of nickel is given by the expression $\frac{58{\cdot}68(a-b)m}{2\times 107{\cdot}88}$. W. R. S.

New Method for the Gravimetric Estimation of Germanium. J. H. Müller. (*J. Amer. Chem. Soc.*, 1922, **44**, 2493–2498.)—The usual estimation of germanium as oxide after treating the sulphide with nitric acid is open to serious errors. Germanium is quantitatively precipitated by magnesium sulphate in ammoniacal solution as magnesium orthogermanate, Mg_2GeO_4, which is not changed by strong ignition. For the estimation there is added to the germanic acid solution excess of N solution of magnesium sulphate (the chloride should not be used) and an equal volume of $2N$ ammonium sulphate solution, then 15 to 20 c.c. of 0·890 ammonia solution, and the mixture is well stirred, heated to boiling for a few minutes and allowed to stand for about 12 hours before filtering. The precipitate is washed with water containing 10 per cent. of ammonia solution, and may be ignited with the filter paper over a strong burner; if a platinum crucible is used, the filter paper should be burnt separately. When the germanium has been separated from other metals as sulphide the simplest method of obtaining the required ammoniacal solution of the oxide consists in the addition to the sulphide of a slight excess of ammonia and hydrogen peroxide; excess of the latter must be subsequently destroyed by boiling. When arsenic accompanies the germanium, the germanium sulphide is extracted from the mixed sulphides by means of hydrofluoric acid, evaporated with sulphuric acid to remove *all* the hydrofluoric acid, and then treated with magnesium sulphate and excess of ammonia as before. The solubility of germanium sulphate in 10 per cent. ammonium sulphate solution is 0·00013 grm. per c.c., but is lowered by the magnesium sulphate; in dilute ammonia solution the solubility is only 0·0002 grm. per c.c., so that it is essential carefully to control the concentration of the solution and the reagents. For quantities of germanium oxide between 0·5 and 0·2 grm. per 100 c.c. it is best to add 20 to 25 c.c. of 2 N ammonium sulphate solution and 15 to 20 c.c. of N magnesium sulphate solution, and then the excess of ammonia. The method is accurate for quantities from 0·5 to 0·0002 grm. H. E. C.

Preparation of Bismuth-Sodium Thiosulphate and its Use in the Estimation of Potassium. V. Cuisinier. (*Bull. Soc. Chim.*, 1922, **31**, 1064–1068.)—The best method for the preparation of the double thiosulphate of sodium and bismuth is to dissolve 10 grms. of bismuth sub-nitrate in 10 c.c. of hydrochloric acid, with the aid of heat, to cool the solution and make the volume up to 100 c.c. with 95 per cent. alcohol, and to this add 20 grms. of sodium thiosulphate dissolved in 100 c.c. of water. The mixture is then diluted with five times its volume of 95 per cent. alcohol, with the addition of a drop of hydrochloric acid, if necessary, to keep the mixture clear; after about a quarter of an hour the double salt separates out as yellow crystals which are filtered off, washed with acidified alcohol, and dried over sulphuric acid. The potassium compound is almost completely insoluble in alcohol and is the basis of Carnot's method for the estimation of potassium, in which method the double salt bismuth-potassium thiosulphate is separated and titrated with standard iodine solution. Critical examination of the method shows, however, that, even under identical and carefully defined conditions, the results obtained are seriously too high and are not concordant. It is concluded that the method is unsuitable for the estimation of potassium salts.

H. E. C.

Volumetric Estimation of Potassium. Macheleidt. (*Woch. Bran.*, 1922, **39**, 23–24; *Chem. Abstr.*, 1922, **16**, 3044.)—A standard solution of sodium hydrogen tartrate is made by dissolving 60 grms. of tartaric acid and 16 grms. of sodium hydroxide in water and diluting the liquid to a litre. Six grms. of potassium hydrogen tartrate are added and the solution shaken for several hours, after which 30 c.c. are filtered and titrated with 0·1 *N* barium hydroxide solution. A second portion of 30 c.c. is shaken for 1 to 2 hours with 0·5 to 0·75 grm. of the salt mixture to be tested, the liquid then filtered into a weighed basin, and, without washing the filter, titrated with the barium hydroxide solution. The solution is weighed before and after filtration and allowance made for the loss. The difference between the two titrations is calculated into potassium oxide.

Estimation of Sulphur in Iron and Steel. F. Nikolai. (*Chem. Zeit.*, 1922, **46**, 1025–1026.)—A weighed quantity (0·25 grm.) of fine drillings or borings is placed in a minute glass tube closed at one end. This is introduced into a round flask (capacity about 13 c.c.) provided with a ground-in glass tube bent at an acute angle, the ascending part of which (12 cm. long) acts as a reflux condenser; the descending part (20 cm. long) terminates in a drawn-out jet, and reaches to the bottom of a 70 c.c. cylinder half full of 2 per cent. sodium hydroxide solution. The flask contains 7 c.c. of hydrobromic acid (sp. gr. 1·48). After the flask has been closed, the acid is brought into contact with the metal, and gradually heated; boiling is continued until solution is complete. The cylinder is then lowered and the tube rinsed down. The liquid is treated with 10 c.c. of acetic acid (250 c.c. of glacial acid per litre) and starch solution, and the sodium sulphide titrated at once with 0·066 *N* iodine solution contained in a small burette; not more than 4 c.c. are required in the titration. The method is claimed to be accurate and very

quick (20 to 25 minutes); the results are higher than those obtained by the usual method. This is explained by the more complete conversion of the sulphur into hydrogen sulphide, brought about by the hydrobromic acid. W. R. S.

Estimation of Selenium. L. Losana. (*Giorn. Chim. Ind. Appl.*, 1922, **4**, 464–466.)—The method devised for the estimation of sulphur (ANALYST, 1922, **47**, 365, 492) serves also for estimating selenium, a larger excess of iron being used and the air excluded as completely as possible during the reduction. The concentration of the hydrochloric acid used for decomposing the selenide should be not less than 40 to 50 per cent., and the decomposition is best carried out in a current of hydrogen purified by means of permanganate, although carbon dioxide may be employed. The actual amount of selenium taken for analysis should not exceed 0·05 grm.

If both sulphur and selenium are present, the gases from the decomposition of the sulphide and selenide are driven by a very gentle current of hydrogen through two absorption tubes, the first containing zinc chloride solution acidified with hydrochloric acid just sufficiently to prevent precipitation of the zinc sulphide, and the second the usual zinc acetate solution. If it is suspected that some of the hydrogen sulphide is retained in the first tube, the latter is immersed for a time in a bath at 40° to 50° C. while a rapid stream of gas is passed through the apparatus. With little sulphur and much selenium it is advisable to insert a second small tube charged with zinc chloride solution. The zinc chloride and zinc acetate solutions are treated separately with iodine and the excess titrated with thiosulphate solution; if any selenium has penetrated to the zinc acetate tube, this will show the red colour of selenium when treated with iodine solution. T. H. P.

Volumetric Estimation of Nitrous and Arsenious Acids. A. Klemenc. (*Zeitsch. anal. Chem.*, 1922, **61**, 448–454.)—The following modification of Lunge's permanganate titration of nitrous acid is recommended:—A measured excess of 0·1 *N* permanganate solution acidified with one-third the volume of 60 per cent. sulphuric acid is introduced into a round flask having a ground glass stopper through which pass a thistle funnel, the tube of which reaches nearly to the bottom of the flask, and a side tube. Both the side tube and the funnel are provided with taps. The flask is filled with carbon dioxide, exhausted slightly through the side tube, and the nitrite solution admitted through the thistle funnel, which is afterwards rinsed with boiled water. After warming the flask to 40° C. for 5 minutes, air is admitted, the stopper removed, and the excess of permanganate titrated with oxalic acid. For the estimation of nitrous and arsenious acids in presence of each other, a portion of the solution is introduced into the above-described apparatus containing the same mixture of a known excess of permanganate solution and sulphuric acid. The titration, which is conducted in the same manner as that of nitrous acid, gives the sum of the two acids. Another portion of the solution is treated with bicarbonate, and the arsenious acid estimated iodimetrically, nitrous acid not interfering. W. R. S.

Ferrous Hydroxide as Reducing Agent in the Estimation of Nitrites and Nitrates. S. Miyomato. (*J. Chem. Soc.* (*Japan*), 1922, **43**, 397–438; *Chem. Abstr.*, 1922, **16**, 397–438.)—*Nitrite*: From 0·1 to 0·3 grm. of the nitrite is mixed in a litre flask with 15 grms. of ferrous sulphate, the mixture heated with 200 c.c. of a saturated solution of potassium hydroxide, and the resulting ammonia distilled into a measured quantity of standard acid, the excess of which is then titrated. The reaction is complete in 30 to 45 minutes. *Nitrate*: More time is required to complete the reduction of a nitrate than of a nitrite, and it is best to use two condensers, the first serving to prevent too great a concentration of the potassium hydroxide solution. The reduction takes about $3\frac{1}{2}$ hours, and it is advisable, during the heating, to conduct an inert gas through the solution.

Physical Methods, Apparatus, etc.

Separation of Isotopes by Distillation and Analogous Processes. R. S. Mulliken. (*J. Amer. Chem., Soc.*, 1922, **44**, 2387–2390.)—Mercury may be separated into its isotopes by distillation under conditions which make the process as nearly as possible irreversible. The increase in atomic weight of the distillate is given by the formula $\triangle A = EB \log C$, where E is the efficiency, B the separation coefficient, and C the cut (for definitions of the latter two terms *cf. J. Amer. Chem. Soc.*, 1922, 37; abst. *J. Chem. Soc.*, 1922, ii., 295). B has a value 0·0063, and by using a 300 c.c. flask, with the neck bent over and sealed to a water-jacketed condenser, and distilling at the rate of 15 c.c. per hour, under a pressure of less than 0·001 mm., an efficiency of 90 per cent. has been obtained. An efficiency of about 50 per cent. is easily obtained with pressures up to about 0·1 mm.; a film of dirt is found to increase the efficiency, but the mercury must be thoroughly purified by nitric acid before the distillation. It is possible that non-isotopic mixtures can be similarly separated by distillation under irreversible conditions. The electrolytic method failed to effect any separation of isotopes, probably owing to the difficulty of obtaining non-equilibrium conditions at the boundary between two condensed phases. H. E. C.

Magnetic Analysis of Silicates and Silicic Acids. P. Pascal. (*Compt. rend.*, 1922, **175**, 814–816.)—By determining the molecular magnetic susceptibility of the two ethyl silicates, and then subtracting the quantity contributed by the ethyl radicle, the constants for Si and SiO_2 are obtained. From these it follows that the value of the oxygen in the molecule is the same as that for $-OH$ groups in ethers, and that these compounds follow the ordinary additive law. From the data thus obtained the structural formulæ of the silicic acids and their susceptibility constants are deduced. By the application of these figures to the values obtained experimentally for hydrated silica gels and granulated silica prepared from silicon tetrachloride and water, the conclusions of Van Bemmelen and of Le Chatelier that these substances are silica hydrates and do not contain any definite silicic acids, is proved quantitatively. H. E. C.

Determination of the Molecular Weight of Substances in Alcoholic Solution from the Elevation of the Flash Point. R. Wright. (*J. Chem. Soc.*, 1922, **121**, 2247–2250.)—The elevation of the flash point by a non-volatile solute follows the same laws as the elevation of the boiling point, the constant being given by the expression $K = 0{\cdot}02T^2/L$, which for ethyl alcohol is 7·7, and the flash point of the alcohol is 17·4° C. Abel's apparatus will not give results closer than about 1°, but the following simple apparatus is suitable for determinations of the required accuracy, namely, within about 0·05°. A tinned copper cylinder, 55 mm. in diameter and 75 mm. high, is fitted with two tubes, one of which carries a thermometer graduated in tenths of a degree, and the other is 20 mm. long and of 4 mm. bore, and is connected by a rubber tube with a short silica tube to act as a jet. A copper tube is wound round the outside of the cylinder in spiral form, the last round entering the bottom of the cylinder and being pierced with a number of fine holes, so that on passing air through the spiral it serves as an air heater and stirs the liquid. For the determination of the flash point 100 c.c. of the solvent are placed in the vessel (which is warmed very slowly in a water bath) and stirred, while short gusts of dried air are admitted through the spiral at intervals. From time to time a flame is held close to the jet, and when the flash point is reached the flame travels back, occasioning a slight explosion in the vessel. The temperature must be quite constant, and the products of previous combustion must be removed by means of a few gusts of air before applying the flame to the jet. The lowest temperature recorded is taken as the flash point. The above process is then repeated with 100 c.c. of the solvent containing from 2 to 7 grms. of the solute; the results are comparable in accuracy with those of the boiling point method. When alcohol is used as solvent variations of atmospheric pressure are found to make no difference to the flash point; solvents, such as benzene, which burn with a smoky flame are unsuitable for use with this method.

H. E. C.

Reviews.

ANNUAL TABLES OF CONSTANTS AND NUMERICAL DATA: CHEMICAL, PHYSICAL AND TECHNOLOGICAL. Publisher for British Empire: The Cambridge University Press, Fetter Lane, London, E.C.4. Price of Volume IV. (in two parts) complete £7.

The publication of the Annual Tables of Constants, which was discontinued during the war, is being resumed; the Committee is publishing Volume IV., in which are collected numerical data gathered from all scientific and technical papers from 1913 to 1916 inclusive.

The work, which is one of great magnitude, is now under the high authority of the "International Union of Pure and Applied Chemistry" and of the "International Research Council."

In its task the Committee had to overcome great difficulties; these have not yet been totally removed, and may yet imperil an organisation which scientists and engineers consider as an absolutely necessary one.

In view of supporting the Committee, the "Union of Pure and Applied Chemistry" decided to form an "International Fund." Both France and the United States gave their adhesion in June, 1922; their subscription amounts to half the necessary sum. It is greatly to be hoped that the various countries in which an effective scientific and technical development has been reached, will join without delay; their agreement would enable the Committee to publish Volume V., the matter for which is ready at hand. This book is to contain numerical data collected from 1917 to 1922 inclusive.

The importance of such numerical data is rendered clearly manifest day by day. Besides the names of practically all the scientific establishments of the world, the lists of subscribers contain those of a considerable number of firms dealing with the most varied branches of industry: Chemistry, Metallurgy, Electricity, Electro-Chemistry, Mechanics, etc.

The enclosed list* gives an idea of the rate at which the Annual Tables of Constants circulate among men who know the value of all numerical information, and who are aware of the technical improvements to be derived from it, and of the financial advantages brought in its train.

They are also aware that such information cannot be gathered by the ordinary bibliographic method, but only by carefully studying every separate scientific and technical periodical, page by page.

The task of the Committee is a particularly heavy one, considering the present circumstances throughout the whole world. The best way to facilitate it is to bring the Committee's valuable information to the notice of scientists who are not yet acquainted with its existence. When the Tables count among their subscribers all those who *should subscribe*, in their own interests, then only will the future of the said Tables be secured.

For the International Committee,

CH. MARIE, *General Secretary*.

9 Rue de Bagneux, Paris VIème.

A SYSTEMATIC QUALITATIVE CHEMICAL ANALYSIS. By G. W. SEARS, Ph.D. Pp. vi.+119. New York: Wiley & Sons; London: Chapman & Hall. 1922. Price 8s. 6d. net.

The volume deals with the subject of qualitative analysis in a somewhat disjointed manner. There are many indications which show that insufficient attention has been given both to its compilation and publication. Certain aspects of the book, however, will commend themselves to teachers and students.

It is divided into three sections and an appendix. Part I., 21 pages, is an epitome of the physico-chemical principles which are of fundamental importance in analytical processes. It would have been an advantage had the bearing of these principles on qualitative analysis been shown more fully. This is especially the

* A list of subscribers was forwarded with this notice.—EDITOR.

case in the treatment of the law of "Mass Action," in which the two examples given deal with its application to dissociation of electrolytes, and none to reactions in general. Laboratory suggestions take up two pages and deal only with filtration, wash bottles and the recording of results. Suggestions might well have been given on such topics as the washing of precipitates, ensuring complete precipitation, and occlusion by gelatinous precipitates. On page 19, it is stated that "Qualitative Analysis has to do with both dry and wet reactions," and that as "dry reactions are those used largely in blow-pipe analysis, they will not be considered." This, undoubtedly, is a weakness. Flame tests, nevertheless, are included with the wet tests.

Part II. begins with the preparation of solutions, but not a word of explanation is given of the principles underlying the general scheme of analysis. The subject matter of analysis is given in Part II., 62 pages, on "Kations," and Part III., 23 pages, on "Anions." In the former section the matter is presented in a sound manner from the standpoint of the teacher. Before proceeding to consider each new group separation, certain experiments are to be performed on known solutions containing kations which are to be separated subsequently. By so doing, the student is led to discover a procedure for separating mixtures of these kations. Then follows a detailed account, each stage of which is numbered, of the method of separation of the members of the group in question. The frequent references made to these numbered operations, tend to make reading difficult and consequently detract from the clearness of the descriptions. The skeleton tables which are given, however, help to make the separations clear. After each group separation comes a discussion of the reactions involved, on the lines of the conceptions put forward in Part I. These explanations are, as a rule, remarkably good, but two cases appear unsatisfactory. On page 33, the solubility product of bismuth oxychloride is represented as $C_{Bi}^{+++}.C_{O}^{--}.C_{Cl}^{+}=K$ and the concentration of oxygen ions is afterwards discussed. It is well known that gelatinous precipitates may carry down with them other substances from solution, yet on page 71 it is stated that in the presence of much chromium, magnesium may be precipitated completely as $Mg(CrO_2)_2$. Bismuth hydroxide as encountered in analysis does not conform with the formula BiOOH, which is used throughout the text. In Part III., the author has described a new method for "Anion" analysis. The procedure involves the use of only one solution and does not require the previous removal of kations. As many of the reactions are the same as those used in the course of metal analysis, he has considered it unnecessary to include preliminary experiments.

Throughout the volume, only those tests are given which are required in the schemes described. The scope, and therefore the usefulness of the book, are seriously curtailed by the fact that neither alternative tables nor alternative tests are given. For example, dimethylglyoxime has become an efficient and common reagent for the presence of nickel, yet other tests might have been included with advantage.

In conclusion, reference must be made to the numerous errors made in reproducing formulæ, ten of which were counted, *e.g.* $Zn(NO)_3$, CH_5OH, $(AlOH)_3$, $(Na_4)SnS_3$, $CaCO_4$, $K_3Cr_2O_7$. HUBERT T. S. BRITTON.

LABORATORY MANUAL OF COLLOID CHEMISTRY. By HARRY N. HOLMES, Ph.D. (Professor of Chemistry, Oberlin College (U.S.A.), Chairman of the National Research Council's Colloid Committee). Pp. xii.+127. 31 Illustrations. New York: Wiley & Sons, Inc.; London: Chapman & Hall. 1922. Price 10s. net.

Though numerous text-books are available which deal with the theoretical principles of colloid chemistry, the practical or laboratory aspect of the subject has been neglected. Recently, and in turn, there have appeared an English and a German laboratory manual, and now follows this American work, written at the request of the Colloid Committee of the National Research Council.

Such pioneer works are to be welcomed. They break new ground and meet a much-needed want in teaching students the practical side of a subject, technique of which is considerably removed from that of the more familiar laboratory exercises. Again, technical chemists in all branches are witnessing to the need of a sound working knowledge of colloid chemistry, and theory alone is not sufficient.

Professor Holmes has covered a wide field, and his 186 experiments include special contributions by such authorities as Bancroft, Fischer, Parsons, Spear, Sheppard, Mathews, Wilson, and Alexander. The experiments are well set out, and deal in order with:—Suspensions (coarse and fine); dialysis and diffusion; condensation methods of preparation; dispersion methods of preparation; coagulation; protective colloids; solvated colloids; surface tension; emulsions; viscosity; adsorption from solution; adsorption of gases; reactions in gels.; experiments with the ultramicroscope; soils and clays; and "special topics."

A running commentary on the theory underlying the experiments is provided, but this is too frequently inclined to be "scrappy." Incidentally, the explanation of experiment 3 (p. I.) follows immediately after experiment 6 (p. 2), and the graph referred to on p. 106 (experiment 163) is missing. Numerous references to the literature mark a valuable feature of the book.

The choice of the experiments—qualitative and quantitative—has been well made, though, naturally, where there is so much to choose from, different workers will hold different opinions as to the ideal selection. Specially noteworthy are the sections dealing with the measurement of surface tension (du Nouy apparatus), the adsorption of gases, and reactions in gels, the latter culled from Professor Holmes' own investigations.

The reviewer's opinion is that students following out this course of experiments will need careful guidance on the theoretical side, owing to the fact that the experiments illustrate various (and in some cases conflicting) theories. Thus Professor Holmes refers to "the brilliant work of Martin H. Fischer on the colloid chemistry of soaps brought together in a recently published book, "Soaps and Proteins"; experiments 71–76 are taken from that book, which again on p. 120 is referred to as "a brilliant production." The student would gather that herein lies the last word on this particular branch, whereas Fischer's theories of solvated colloids are called in question by some equally eminent authorities. Similarly,

the solvated colloid theory of emulsifying efficiency (p. 58) is only very partially true.

Professor Holmes has produced a well-written manual for students of elementary colloid chemistry, and it should prove of great utility in our universities and colleges, where lecture courses on the subject are now available, but where laboratory work has been at a minimum.

The book is printed on very good paper, and the binding and appearance are unusually good. WILLIAM CLAYTON.

AN INTRODUCTION TO THE CHEMISTRY OF PLANT PRODUCTS. Vol. II. METABOLIC PROCESSES. By PAUL HAAS, D.Sc., Ph.D., and T. G. HILL, A.R.C.S. Pp. viii.+140. London: Longmans, Green & Co. 1922. Price 7s. 6d. net.

The aim of the authors, as stated in their preface, is to give such an account of the metabolic processes of plants as may form a basis for further study. A text book fulfilling such a purpose would indeed fill a gap among English scientific text books, which tend to deal with the subject of biochemistry mainly from the standpoint of animal physiology, leaving the botanist to fish for himself in a sea of original papers.

The contents table of the book is evidence that a wide field of plant physiology has been covered, and the large number of references—in itself a useful contribution to scientific literature—evinces the erudition of the authors. Unfortunately the very confused style of writing detracts from the usefulness of the volume; in some chapters (*e.g.* pp. 1–4) it resembles sketchy lecture notes, whilst in others it suggests abbreviated abstracts suited to a card index of original literature for the use of the compiler. Generally speaking, it is little adapted to assist the student in obtaining any clear picture of his subject.

The physiology of fats was dealt with at some length in Vol. I., the short chapter in Vol. II. being supplementary to this. It contains, unfortunately, one highly misleading statement (p. 13): "The salient feature in the germination of a fat-containing seed is the conversion of the fat into carbohydrate. . . . The change is effected by the activity of lipase which hydrolyses the fat into glycerol and fatty acid."

Chapter IV., on the synthesis of proteins, is remarkable for the relative space allotted to the various sections of the subject. Out of a total of ten pages one only is devoted to protein formation by micro-organisms, and the subject of the fixation of atmospheric nitrogen is completely omitted. On the other hand, $3\frac{1}{2}$ pages are devoted to the very recent (July, 1922) work of Baly, Heilbron and Hudson, the experimental evidence for much of which is yet unpublished.

The chapter on respiration contains a good summary of many views on cell oxidations, but one may perhaps be forgiven for mentioning that to represent the action of catalase on hydrogen peroxide by the equation $H_2O_2 = H_2O + O$ is misleading, as it ignores the salient point that molecular (and not active) oxygen is liberated by the action of the enzyme. The subsequent statement (p. 69): "This cycle of changes does not occur in anaerobic oxidations, which may explain the

absence of catalase under these conditions," surely contains inversion of cause and effect. Page 71 contains two curious remarks: (1) "Fats carbohydrate proteins *and protoplasm** may be physiologically consumed." (2) "Respiration may be distinguished as aerobic, anaerobic and *facultative anaerobic**."

To write the chapter on "Growth" must have been a matter of some difficulty to the authors, owing to the great confusion of data and the fluctuating nature of the evidence on this subject. They have not attempted to produce a consistent story; doubtless to do so at present is impossible. The method they have adopted is to give brief abstracts (chiefly selected with slight verbal alterations from the authors' own summaries) of a number of papers, without much attempt at elucidation. The inclusion of an account of vitamins in this chapter is hard to account for, particularly since the stimulating effect of yeast extract on the growth of micro-organisms finds no place in it.

The statement (p. 134) that the third (or anti-scorbutic) vitamin may, or may not, be identical with one of the other two is peculiarly unfortunate, since, among much that is uncertain in our knowledge of vitamins, the complete dissimilarity of vitamin *C* from vitamins *A* and *B*, in distribution as well as in chemical and physiological properties, has been established beyond all question.

M. STEPHENSON.

* The italics are the reviewer's—EDITOR.

Publications Received.

THE THEORY OF ALLOTROPY. By A. SMITS. (Text Books of Physical Chemistry.) Pp. xiii+397. London: Longmans, Green & Co. 1922. Price 21s. net.

SYNTHETIC COLOURING MATTERS. DYESTUFFS DERIVED FROM PYRIDINE, QUINOLINE, ACRIDINE, AND XANTHENE. By J. T. HEWITT. (Monographs on Industrial Chemistry.) Pp. xi.+405. London: Longmans, Green & Co. 1922. Price 14s. net.

FLAVOURING MATERIALS: NATURAL AND SYNTHETIC. By A. CLARKE. Pp. xxi.+166. London: H. Frowde and Hodder & Stoughton. (Oxford Technical Publications.) 1922. Price 8s. 6d. net.

THE INLAND LAKES OF WISCONSIN.—THE PLANKTON. I. Its Quantity and Chemical Composition. By E. A. BIRGE and C. JUDAY. (Wisconsin Geological and Natural History Survey.) 1922. Published by the State, Madison, Wisconsin.

THE THEORY OF EMULSIONS AND EMULSIFICATION. By W. CLAYTON. Pp. 160+vi. London: J. & A. Churchill. 1923. Price 9s. 6d. net.

BLEACHING POWDER AND ITS ACTION IN BLEACHING. By R. L. TAYLOR. Pp. 79. Manchester: John Heywood & Co. 1922. Price 4s. 6d.

A DICTIONARY OF APPLIED CHEMISTRY. Edited by Sir E. Thorpe. Vol. IV. L—Oxydisilin. Pp. viii.+740. London: Longmans, Green & Co. 1922. Price 60s. net.

World's Dairy Congress.

THE Ministry of Agriculture and Fisheries has issued a circular letter giving preliminary information about the international dairy meeting to be held in October, 1923, in the United States of America, under the title of the World's Dairy Congress. The initiative of organising the meeting has been taken by the United States Government, and the Congress will be conducted by the World's Dairy Association, with the co-operation of the U.S. Dept. of Agriculture and the International Dairy Federation.

The Congress will include representatives from many nations for the purpose of seeing and hearing the latest advances in the field of dairying. It will be concerned with four main groups of interests: (*a*) Research and education; (*b*) industry and economics; (*c*) regulation and control; and (*d*) national health.

The Ministry of Agriculture and the Ministry of Health are in full sympathy with the objects of the Congress, and are prepared to support an effort to arrange for an adequate representation at the Congress of the dairying interests—educational, commercial and hygienic—in England and Wales. With this object a World's Dairy Congress Committee has been formed to act as a link between the Congress Association and the dairying industry (including "public health") in this country. Sir Daniel Hall has been elected chairman of this Committee, and Sir Douglas Newton, M.P., deputy chairman, and an officer of the Ministry of Agriculture is acting as secretary to the Committee. The Committee is charged (*a*) with the selection of a body of delegates to attend the Congress, and (*b*) with the arrangement of suitable papers to be read at the Congress.

Apart from action taken by the Committee, certain bodies may wish to send representatives to the Congress and to pay their expenses. There may also be individuals identified with the industry who can attend the Congress at their own expense. To secure adequate representation, however, the Committee makes an appeal for funds to enable it to send persons who cannot pay their own expenses, and so ensure that every important aspect of the dairying industry in this country is represented in the delegation from this country.

The Committee would also be glad to receive suggestions from societies of the names of persons who might be invited to write papers for the Congress on some part of the subject, and especially papers dealing with *new achievements* in any branch of the dairy industry. Papers of general rather than local application are desired.

Important Notice.—The Hon. Secretary of our Society would be glad to have the name of any qualified member who would be able to go to America, at his own expense, and to represent the Society at the Congress. Names should be submitted not later than January 31. The Hon. Secretary would also be obliged if those who wish to send papers to be read at the Congress would communicate with him as soon as possible.