JULY, 1928. Vol. LIII., No. 628.

THE ANALYST

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

Cacao Tannin and Its Determination.

By H. R. JENSEN, M.Sc., F.I.C.

(*Read at the Meeting, March* 7, 1928.)

Although investigations have been made by the industries concerned, apparently no scientific work of importance has been published describing the tannin of cacao. Even technical workers have been content to refer vaguely to "astringent principles," and the values for these have been recorded as too low.

It is, of course, common knowledge that chocolate, on occasion, can be particularly bitter and astringent, by reason of exceptional nibs or process. It is, therefore, of special interest to be able to measure such unpalatable substances, both in the original cacao beans and at the various stages of chocolate manufacture.

It has long been accepted that the oxidations during fermentation of the beans, and the colour development during roasting—the latter perhaps owing to tannin dehydration to insoluble phlobaphenes—result, in each case, in a reduction of tannin and of astringency.

An aqueous extract of fermented cacao nib, both raw and roast, gives all the reactions of a typical phlobatannin (catechol tannin). That is, a brownish-green colour is formed with ferric chloride, a red precipitate is given with acid and formaldehyde, and the typical lead and bromine reactions are obtained. With such phlobatannins there always occur related compounds, the crystalline catechins and the coloured amorphous phlobaphene derivatives—the latter of various degrees of hydration and solubility. It may be possible to adapt Mitchell's iron reagent with the hard water and ammonium acetate modification (Glasstone, Analyst, 1925, **50**, 49) for colorimetric measurements, with a standard phlobatannin, but the interference of strong colour and of catechin will have to be overcome.

Dr. Nierenstein has informed the author privately that unfermented Trinidad, African and Javan cacao do, in fact, contain catechin—the same in each case, but distinct from the acacatechin of cutch. The same worker also states that no catechin remains in the fully fermented beans, and that the catechin concerned is quickly converted into tannin by hot water.

Since both catechins, and, at least, the sparingly soluble phlobaphenes have probably little astringent bitterness, it is desirable to use the most selective method possible for tannin determination, more particularly to the exclusion of any unconverted catechin. For this purpose the best method available is precipitation by cinchonine, as applied originally by A. C. Chapman to hops (ANALYST, **1909, 34, 372**), and since by H. L. Smith to tea (ANALYST, **1913, 38**, 312), and D. Hooper to catechin (ANALYST, 1925, **50**, 162), etc. In the case of cacao, however, this involves finding the best and most convenient method of extraction, the best conditions for precipitation, and also an exact Dumas determination of the nitrogen of such precipitates for calculating the tannin equivalent. In applying the Dumas method to these compounds admixture with lead chromate was found necessary, and the gases were passed over hot fused chromate. Cinchonine itself, even after a 5 hour Kjeldahl digestion, has only 60 per cent. of its nitrogen converted.

EXTRACTION OF TANNIN.—With many products an extraction temperature of 60°–90° C. with water is found to be necessary. Although cold water dissolves the purest tannin from cacao, it is impracticable to extract even fat-free nib completely other than by hot water.

De-fatting with an organic solvent is to be avoided in any case. No doubt a small but unavoidable error occurs from solubility of a little phlobaphene in tannin and its adsorption on precipitation. It was not found practicable to extract a given weight to exhaustion by a large volume of water, since the evaporation required caused decompositions, with subsequent failure in the precipitation. Moreover, an extraction with alcohol dissolves less than half the tannin present. As extraction at 65°–80° C. gave decidedly low results, even after an hour's decoction, a temperature of 95°–99° C. was used, finely ground cacao mass (fat-containing) being extracted for 30 minutes. For the extractions, uniform additions of water were made, and direct aliquot portions were then taken, typical volume corrections being made for the "soluble extractive" as determined by experiment. In every case 25 grms. of normal fully roasted unalkalised cacao nib or mass were heated with 460 c.c. of water mixed with about 37 c.c. of 0·1 *N* sodium hydroxide solution. For the determination 50 c.c. portions of the filtrate were treated with the reagent and set aside for 4 to 18 hours.

It was found that the highest results were obtained after a neutralisation by sodium hydroxide of the natural free acidity of the nib. Even so, the working P_H value may still be assumed to be smaller than 7, *i.e.* slightly acid. The estimated figure, which was increased thus by as much as 30 per cent., can be explained.

1. By a reduction in the solubility of the cinchonine tannate, which Hooper has already recorded as freely soluble in dilute acetic acid.

2. By a reduction of possible conversions to phlobaphene during hot acid extractions.
3. By making the extraction of tannin from the tissues more rapid.

It was found that neutralisation, after extraction, caused a smaller increase of the tannin value than before extraction.

The precipitations were found to be more complete, and the precipitates better aggregated, and thus more rapidly filterable when the ratio of cinchonine solution to tannin was greater than that used by other workers (*viz.* 150 c.c. of saturated solution to 2·5 grms. of nib). This also permits of filtration after four hours instead of waiting overnight. Otherwise a standard procedure was followed as to washing and drying.

The collection of the bulky precipitates on counterpoised filter papers was found to be most rapid and sufficiently accurate, and the papers were dried in the air or in an exsiccator, and finally at 105° C. until constant in weight.

The troublesome removal of theobromine by chloroform before precipitation appears to be necessary in exceptional cases.

The following abbreviated table gives the results obtained with different kinds of cacao, etc.:

TANNIN MEASUREMENTS IN VARIOUS CACAO NIBS, ETC.

All extracted at the standard time of ½ hour, in a water-bath, and treated with the normal 150 c.c. of cinchonine solution. The precipitate filtered after 18 hours. The determinations were made, as before, on 2·5 grms. of nib.

		Weight of precipitate	Cinchonine tannate	Equivalent tannin
		Grms.	Per Cent.	Per Cent.
(1)	Grenada mass	0·268	10·72	5·72
(2)	,, ,, ...	0·26	10·4	5·56
(3)	Ariba mass	0·267	10·68	5·7
(4)	,, ,, ...	0·242	9·68	5·17
(5)	Trinidad mass	0·265	10·58	5·65
(6)	,, ,, ...	0·267	10·68	5·7

Nos. 2, 4 and 6 were after extraction of theobromine with chloroform.

(7)	Caracas mass	0·295	11·82	6·31
(8)	Ceylon mass	0·281	11·3	6·03
(9)	Chocolate	0·336	14·2	7·58 (in nib)
(10)	Forastero shell (blend) powder	0·204		
	(on 1·25 grm. shell) ..	0·21	16·4	8·76
	(neutralised)	0·20		
(11)	Accra nib A	—	10·3, 10·68	5·51, 5·7
(12)	Accra mass B	—	9·84, 9·08	5·25, 4·85
(13)	Accra mass C	—	12·1	6·5
(14)	Accra nib 4	—	10·8	5·77

Many determinations were repeated, with a variation not exceeding ± 1·7 per cent. Examination of many of the filtrates after precipitation, by the gold-beater's skin test, indicated the possibility that 3 per cent. of soluble tannin matter might still be undetermined.

PROPORTION OF TANNIN IN CACAOS.—The results summarised indicate that the water-soluble tannin content for eleven samples of full-roast cacao nib ranged from 5·2 to 6·5 per cent. (average 5·9 per cent.). This is equivalent to an average tannin content of 13·8 per cent. in the fat-free and dry cacao.

It was surprising to find that fine mild Ceylon cacao (Criollo), with 6 per cent. tannin, did not contain less than the stronger Accra and Trinidad cacaos (Forastero).

It was also noticeable that the highest recorded tannin figure was obtained with a well-known chocolate. This may be a result of an alkalisation before roasting decreasing the conversion of tannin to phlobaphene. In this connection it was interesting to find that the tannin content of an Accra nib was reduced by 16·4 per cent., as the result of a full straight roast.

Compared with the 15–16 per cent. tannin present in tea, cacao tastes less astringent than would be expected. No doubt milk and fat modify such effects on the palate, and, of course, tea contains a gallotannin. The tannin of cacao may be variably combined with the theobromine, with modification of its taste, but repeated tests with Molisch's reagent, after hydrolysis with hydrochloric acid, gave no indication that it was in glucosidic combination.

A mixed cacao husk was found to contain 8·76 per cent. of tannin, which is a much lower equivalent than that present in fat free-nib.

The nitrogen content of the precipitates prepared by the standard alkalisation was found to average 4·49 per cent. (4·41–4·58 per cent.), which is similar to the value 4·3 found for the hop and tea tannin compounds. The change of the fawn colour of the precipitates, during heat-drying, to a dark brownish purple suggests a partial change to phlobaphene bodies. The cinchonine compound is soluble in alcohol, and is decomposed by alkali and chloroform, and the cinchonine therefrom may be used to check its composition. The tannin matter so extracted, when purified by conversion into the lead salt, and treatment with hydrogen sulphide and alcohol, and the product acetylated for two hours with acetic anhydride, yielded an acetyl derivative melting at 160° C. This melting point closely agrees with that of a phlobaphene acetyl; possibly it is converted into this compound in the process.

The author desires to thank Mr. D. A. Osmond, M.Sc., for his assistance with the concluding analytical work here added.

Determination of the Colour-Producing Constituents of the Cacao Bean.

By WILLIAM BENNETT ADAM, M.A., A.I.C.

(*Read at the Meeting, April* 4, 1928.)

The nature of the changes which take place during the fermentation of the cacao bean has engaged the attention of a number of workers, but no very satisfactory explanation to account for the alteration in colour and flavour has yet been put forward. It is proposed in the present paper briefly to describe a method of determining the two principal substances responsible for the production of the red and brown colouring matters, and to show the extent of the alteration of these parent substances during the process of fermentation.

It should be mentioned that these colour changes vary considerably in cacao obtained from different sources. At one end of the scale is the true Criollo bean of Java and Ceylon, which is almost colourless when first removed from the pod, but changes during fermentation to a light brown. At the other is the Forastero bean of West Africa, which is of a bright slaty blue colour when unfermented, but the colour of which gradually changes, during a period of fermentation of about a week, to a deep red brown.

A number of explanations for these changes in colour have been put forward. Hilger (*Apoth. Zeit.*, 1898, 389) concluded that the colouring matter extracted in the ordinary way was a mixture of non-nitrogenous cacao-red, with some glucoside. He isolated a substance which he termed "cacao-ol." Schweitzer (*Pharm. Zeit.*, 1892, 469) compared the colour changes during the fermentation of cacao with those which take place in the oak and kola-nut, and suggested that they were due to the decomposition of a glucoside "cacaonine," which breaks down yielding cacao red, glucose, and theobromine. L. Reutter (*Compt. rend.*, 1913, **156**, 1842) described three nitrogenous substances, "cacaorine," "cacao-red," and "cacao-brown," and assigned formulae to them. In a publication of the Agricultural Station at Java in 1909, Ultee and Van Dorssen investigated more fully the so-called "cacao-ol" of Hilger, and mentioned the existence of a caffeine compound of this substance.

There is little room for doubt that in certain cases mentioned above the substances described were in reality mixtures of red alteration products of tannin substances, with traces of the xanthine bases which are present in the cacao bean.

By using a suitable sequence of solvents certain well-defined substances have now been isolated. It has been found that there are present in the unfermented cacao bean (*a*) a substance belonging to the catechin series; (*b*) a catechutannin; and (*c*) a compound of the cacao catechin with caffeine.* The red and brown

* A detailed description of the chemistry of this cacao catechin will be published elsewhere in due course.

colouring matters are complex alteration products of the catechin and tannin originally present in the unfermented bean.

Methods of determining the catechin-like substance and the cacao tannin have been elaborated, and quite consistent results have been obtained in the classes of cacao which have been examined. The determination of the cacao catechin was based on the colorimetric method devised by Mitchell (ANALYST, 1923, **48**, 2), and investigated by Price (ANALYST, 1924, **49**, 361), with the modifications suggested by Glasstone (ANALYST, 1925, **50**, 49). For the determination of the cacao tannin the method put forward by Hooper (ANALYST, 1925, **50**, 162) for the determination of tannin in cutch and gambier was employed.

EXTRACTION OF CACAO CATECHIN.—The raw material used in the preliminary investigation was a specially prepared sample of unfermented cacao beans from West Africa, of the class known as Accra. The beans had been removed from the pods, dropped into boiling water to destroy enzymes, and dried.

The shell was removed by hand, the raw nib ground in a mechanical mortar, and a weighed quantity (about 150 grms.) extracted in a Soxhlet apparatus with petroleum spirit. The material was re-ground, a known weight of acid-treated white sand being added to ensure complete removal of the fat. The extraction was continued for two days, when the powder was removed, ground once more, and extracted for five days with chloroform. This treatment was found to be necessary in order completely to remove the last traces of fat and the xanthine bases. No trace of catechin or tannin was found in the petroleum spirit and chloroform extracts.

The final stage is the removal of the catechin by extraction with ether. By the use of this solvent the catechin and tannin may be separated, but, owing to the small solubility of the former in ether, the complete extraction is a somewhat lengthy process.

The cacao catechin thus obtained, when purified with lead acetate, is a white crystalline substance with m.pt. 229° C.

When determining the catechin by this method it was found that the ethereal extraction had to be carried out with great care, in particular to avoid too vigorous boiling of the solvent. After five or six days' gentle extraction, most of the catechin was removed, but, in some cases, slight traces were still obtained even after sixteen days. The liquid in the flask was removed several times during the early stages to prevent the catechin drying out above the level of a solvent.

The ethereal extract was evaporated gently, and warm water added. At this stage the aqueous solution was found to be free from tannin and nitrogenous bodies. This was confirmed in several tests by the addition of cinchonine sulphate, but it should be pointed out that the colorimetric determination cannot be carried out with cinchonine sulphate in the solution, as the presence of this alkaloid salt causes the precipitation of the iron of the ferrous tartrate reagent. The aqueous solution of cacao catechin thus obtained is nearly colourless, or faintly pink, and may be compared colorimetrically with a standard solution prepared from a

specimen of pure crystalline cacao catechin, by means of the ferrous tartrate reagent, with ammonium acetate as buffer.

The following table shows the rate of extraction of cacao catechin by ether from a number of samples of unfermented cacao beans. These beans were picked individually from samples of unfermented and very poorly fermented cacao specially shipped from West Africa for this purpose. All beans showed the characteristic slate-blue tint of the cotyledons.

	No. of days' extraction with ether.					
Sample No.	3 Per Cent.	5 Per Cent.	9 Per Cent.	12 Per Cent.	16 Per Cent.	Remarks.
1	0·40	0·46	0·60	0·62	—	Very poorly fermented
2	0·34	0·42	0·53	0·55	—	do.
3	0·42	0·58	0·67	0·69	0·69	do.
4	0·40	0·53	0·60	0·65	0·66	do.
5	0·41	0·57	0·62	0·67	0·69	do.
Special	0·48	0·63	0·76	0·80	0·81	Totally unfermented

The percentages were calculated on the shelled material.

DETERMINATION OF CACAO CATECHIN.—Solutions of strengths varying between 0·10 and 0·15 per cent. of anhydrous catechin were accurately prepared. To 85 c.c. of water in the colorimetric tubes was added 1 c.c. of the solution for estimation, 8 c.c. of 10 per cent. ammonium acetate, and 2 c.c. of Mitchell's ferrous tartrate reagent, and the whole was made up to the 100 c.c. mark. The solutions in the tubes were matched in the usual manner, and the ratio of concentrations calculated.

It may be mentioned that this method yields excellent results in the comparison of gambier catechin with aca-catechin.

Owing to the difficulty of obtaining cacao catechin in sufficient quantity to meet the requirements as a standard for the considerable number of colorimetric comparisons demanded by the method of extraction outlined above, it had been hoped that gambier catechin might serve as a substitute, when once the colorimetric relationship between the two catechins had been established. It was found in practice, however, that when this comparison was made, the tint produced by the cacao catechin was slightly bluer than that obtained with gambier catechin. The use of gambier catechin as a standard is therefore open to serious errors.

All the determinations of catechin present in the cacao beans were consequently made by comparison with specimens of pure crystalline cacao catechin as standard. These specimens were isolated from a number of samples of cacao material, and agreed satisfactorily one with another when compared by this method.

The cacao catechin, extracted as above, should represent the total catechin present in the bean, as the caffeine compound was found to be unstable under the action of boiling chloroform, and to break down into its constituents, catechin and caffeine. No corresponding theobromine compound could be isolated or synthesised.

CACAO TANNIN.—The dried powder, after the stage of extraction with ether, was shaken with a suitable volume of water at 60° C., and filtered, and to the filtrate was added an equal volume of a saturated solution of cinchonine sulphate. After the coagulation of the precipitate, the solution was filtered through a weighed alundum crucible, washed with a dilute cinchonine sulphate solution, and dried, first in air, and then to constant weight in a steam oven.

As cacao catechin does not give a precipitate with cinchonine sulphate, unless it has been boiled for an appreciable time in slightly acid or alkaline solution, the determination of the tannin may be carried out even if the catechin has not previously been removed. In considering the figures for cacao tannin obtained from fermented cacao beans, however, it should be borne in mind that a certain proportion of the total precipitate may be due to tannin-like alteration products of cacao catechin, which have been formed by oxidation and condensation during the period of fermentation.

ANALYTICAL FIGURES.

Class of Cacao Bean	Catechin. Per Cent.	Tannin. Per Cent.	Degree of Fermentation.
West African	0·80	2·37	Unfermented
do.	0·62	—	Very poorly fermented
do.	Nil	1·88	Fermented
Guayaquil (Summer Arriba)	Nil	1·70	Fermented
do. (Epoca Arriba) ..	0·75	—	Very poorly fermented
do. (Machala) ..	—	3·48	Very poorly fermented
Trinidad	Nil	1·99	Fermented
Costa Rica	Nil	1·87	Fermented
Bahia	Nil	1·92	Fermented
Java	Nil	1·97	Fermented

From the analytical results set out above, it will be seen that the cacao catechin undergoes alteration during the process of fermentation, and in the completely fermented bean no catechin is to be found. The alteration of the tannin appears to be less drastic, and a fairly constant value of about 1·90 per cent. was obtained in all beans where the fermentation had been completed. The tannin content of the two main classes of cacao beans—Criollo and Forastero—appears to differ very slightly.

The author's thanks are due to Dr. Nierenstein for the interest he has shown in the work, and the advice he has given; also to Messrs. J. S. Fry & Sons for permission to publish these results.

RESEARCH LABORATORIES,
J. S. FRY & SONS, LTD.,
BRISTOL.

A New Method for the Colorimetric Determination of Small Quantities of Antimony, and their Separation from Tin.*

By S. G. CLARKE, B.Sc., A.I.C.

(*Read at the Meeting, April* 4, 1928.)

In this paper is described a method for the determination of the small amounts of antimony in high-grade tin, based on the separation of the antimony by Reinsch's reaction (deposition on metallic copper from a halide solution). In this is incorporated a new method for the determination of antimony applicable to small quantities of this element, which is based on the golden-yellow colour of the compound formed by its reaction in solution with pyridine and an iodide; this colour is susceptible of colorimetric comparison with a standard. This method is applied to the estimation of antimony in the solution obtained by stripping the deposited film with sodium peroxide. It was developed on account of the failure of the usual colorimetric method, namely that employing the colloidal sulphide colour, to deal with this particular estimation; this point is discussed later in the paper.

The determination of antimony in fairly pure tin does not seem to be generally carried out by other than the usual classical methods applicable to the higher amounts of this element present as, for example, in some white bearing metals, or in the rather impure class of tin (of about 99 per cent. purity) which may contain several tenths per cent. of antimony. When dealing with the much smaller amounts present in tin of up to 99·9 per cent. purity, the question of a suitable method assumes some importance, as the application of the older methods would be attended with serious, if not insurmountable, difficulties. As an instance of this may be quoted F. W. Clarke's well-known method for separating tin and antimony, which depends on the precipitation of pentavalent antimony with hydrogen sulphide in presence of approximately 5 grms. of oxalic acid for every decigram of mixed metals, the tin being retained in solution. Now if it be desired to obtain sufficient antimony suphide for subsequent gravimetric or volumetric determination from a "pure" tin, a large weight of the tin would have to be taken, corresponding with an unmanageable quantity of oxalic acid. Even had this separation been accomplished, there would still remain a tedious separation of the antimony from, roughly, an equivalent amount of other elements—arsenic, copper, lead bismuth—which are almost always present in the purest samples of tin.

Other Methods of Determination.—Reference should be made here to a method which seems to be used to some extent, in which the sample of tin is treated with hydrochloric acid until all but a small amount of black undissolved

* Communication from the Research Department, Woolwich.

material remains; this is filtered off, washed, dissolved in hydrochloric acid containing bromine, and submitted to a standard volumetric process for the determination of antimony. This method is suitable only as a sorting test, for, in addition to the errors in the volumetric process caused by the other impurities in the tin, and also the slight solubility of antimony in strong hydrochloric acid in presence of air (this would be minimised if filtration were done before quite all the tin had dissolved), the loss of antimony as stibine is neglected. This point has been examined by dissolving, in an all-glass apparatus, tin of known small antimony content (of the order 0·01 per cent.) in hydrochloric acid, passing a stream of carbon dioxide to sweep out the gas as evolved; this was bubbled through dilute hydrochloric acid containing bromine, to absorb any stibine. Upon determining the antimony in this solution by the method to be described, it was found that, although the actual quantity varied from one experiment to another, yet from about one-tenth to one-fifth of the total antimony in the sample had passed over in the gas.

Practically the only publication setting out a definite method for the determination of small quantities of antimony in tin is the *Mitteilung des Chemiker-Fachausschusses der Deutscher Metallhütten- und Bergleute, e.V.*, Berlin (1924). This process is merely a modification, with certain refinements, of the one previously mentioned. The source of error due to the solubility of antimony in hydrochloric acid is obviated by digestion with finely divided iron; but the important factor of loss of antimony as hydride has been neglected, as the method of solution of the tin recommended is to use strong hydrochloric acid in a flask closed by a Bunsen valve. This method has been modified in this laboratory by dissolving the sample in hydrochloric acid containing bromine, precipitating the antimony on iron powder according to the directions of Järvinen (*Z. anal. Chem.*, 1923, **63**, 184) and determining it after careful separations by the volumetric bromate process. In this form it has been used with success on tin containing fairly considerable amounts of antimony. With lower amounts, however, its accuracy has not been tested; further, it would necessitate taking a large sample—50 to 100 grms.—a quantity which was not available in some cases where an accurate determination was desired of amounts of the order 0·003 per cent.

THE NEW METHOD.—The method worked out for determining antimony in amounts of the order of 0·001 to 0·05 per cent. in tin is as follows:—Five grms.* of the tin are dissolved, in a conical flask of 750 c.c. capacity, in 50 c.c. of strong hydrochloric acid, sufficient bromine being added to have an excess present while the tin is dissolving; 10 grms. of oxalic acid are added, followed by about 350 c.c. of water. The liquid is gently boiled, the oxalic acid will have then dissolved, yielding a solution still tinged with bromine. A small quantity of sodium hypophosphite (about 0·5 grm.) is added to reduce all free bromine, and render the solution quite colourless. A strip of copper foil, about 15 × 2 cm., which has been coiled into a flat, open spiral, is cleaned by warming in dilute nitric acid (sp. gr. 1·2),

* With a tin of moderate purity, of the order of 0·05 per cent. of antimony, a 1 grm. sample should be taken, as indicated over Table I.

well washed, and dropped into the solution; it should stand upright on the bottom of the flask. The boiling is continued for 1½ to 2 hours, after which the coil is removed from the flask by means of a hooked glass rod and washed successively by a gentle stream of warm 5 per cent. hydrochloric acid, and of water. It is placed in a small beaker of diameter not much greater than that of the coil, covered with water, and about 1 grm. of sodium peroxide added; the whole is warmed until the deposit is dissolved and the copper is well darkened by oxidation; the solution is poured off the coil, which is then rinsed with water. It is recommended that the coil be now tested by immersion in hydrochloric acid (1:1), when the oxide stain dissolves and should leave a perfectly bright copper surface; any deposit remaining after this treatment is an indication that the antimony film had not been completely removed and that the sodium peroxide treatment should be repeated.

A current of hydrogen sulphide is passed for about 15 seconds into the solution containing the antimony; this is then set aside on a water-bath until the somewhat small precipitate of copper sulphide, etc., has coagulated; about 15 minutes are necessary. The liquid is now passed through a small filter, and this is washed lightly with a dilute solution of an electrolyte—ammonium nitrate or sodium sulphate. To the filtrate 5 c.c. concentrated sulphuric acid are added, and, after the addition of a few drops of nitric acid near the end stage, the solution is evaporated just to fuming ; after cooling, 15 c.c. of water are added, and a clear solution is obtained. (Some sodium peroxide contains silica, but the amount is usually small, rendering filtration of this solution unnecessary.) The antimony now contained in this approximately 20 c.c. of solution is determined colorimetrically as follows:

Into a 100 c.c. Nessler glass are put reagents in the order named:—Ten c.c. of 1 per cent. gum arabic, 5 c.c. of 20 per cent. potassium iodide, 1 c.c. of 10 per cent. aqueous pyridine, 1 c.c. of a dilute solution of sulphur dioxide (one-tenth saturated), 60 c.c. of cold dilute (1:3) sulphuric acid. The solution obtained as described above is now added, the beaker being rinsed with not more than 5 c.c. of water, and the whole is well stirred with a glass rod. Standard antimony solution (0·0001 grm./c.c.) is run into another Nessler glass containing similar quantities of reagents (except that 80 c.c. of 1:3 sulphuric acid are used instead of 60 c.c.), until the colours match after the solution has been well stirred. A final adjustment is made just before the final match is obtained, by adding a small quantity of water to make the volumes in the Nessler glasses equal. The colorimetric comparison is made by viewing the tubes vertically over a white tile inclined at an angle to act as a light reflector.

The standard antimony solution contains 0·2764 grm. of tartar emetic in 1000 c.c. of 10 per cent. of sulphuric acid.

If more than 10 c.c. of this standard solution have to be added, the colour is too deep for accurate comparison; if the amount of antimony present is still greater, a turbidity is produced. In this case 20 c.c. of solution are withdrawn from the Nessler glass into another one, similar quantities of reagent are added as

in the first instance; any turbidity thereupon disappears, and the colour is matched with a fresh standard.

Test results obtained by this method are shown in the tables. In Table I 5 grms. of "Chempur" tin were taken and varying amounts of antimony added.

TABLE I.

	Tin taken. Grms.	Antimony added. Grm.	Antimony added. Per Cent.	Antimony found. Gross. Grm.	Antimony found. Nett. Grm.	Antimony found. Per Cent.
1	5·0	nil	blank	0·00012	—	0·0024
2	5·0	0·00025	0·0050	0·00037	0·00025	0·0050
3	5·0	0·00050	0·0100	0·00066	0·00054	0·0108
4	5·0	0·00075	0·0150	0·00087	0·00075	0·0150
5	5·0	0·00100	0·0200	0·00110	0·00098	0·0198
6	5·0	0·00200	0·0400	0·00200	0·00188	0·0376
7	5·0	0·00300	0·060	0·00305	0·00293	0·058

In experiments 6 and 7 an aliquot portion of the colorimetric solution was taken, as described.

When a tin of moderate purity is being analysed it is sufficient and quite accurate to work on a 1 grm. sample; 2 grms. of oxalic acid are used in this case instead of 10 grms. as above. Table II shows results obtained with rather higher amounts of antimony; comparatively large amounts of arsenic and bismuth were added to verify the non-interference of these elements, as the "Chempur" tin used was very pure in this respect.

TABLE II.

	Tin taken. Grm.	Antimony. Grm.	Antimony. Per Cent.	Arsenic added. Grm.	Arsenic added. Per Cent.	Bismuth. Grm.	Bismuth. Per Cent.	Antimony found. Gross. Grm.	Antimony found. Nett.* Grm.	Antimony found. Per Cent.
1	1·0	0·00010	0·010	0·00113	0·113	0·0005	0·05	0·00014	0·00012	0·012
2	1·0	0·00020	0·020	Nil	—	0·0005	0·05	0·00022	0·00020	0·020
3	1·0	0·00040	0·040	Nil	—	0·0005	0·05	0·00041	0·00039	0·039
4	1·0	0·00075	0·075	0·00113	0·113	0·0005	0·05	0·00073	0·00071	0·071

* Value obtained by deducting proportional blank to that shown in Table I.

In experiment 4 a small amount of the deposited film became detached during boiling.

As a matter of interest, a few results were obtained on very low amounts (Table III). A 10 grm. sample of the purest tin available was used, and the amount of oxalic acid increased to 20 grms.

TABLE III.

Tin taken. Grms.	Antimony added. Grm.	Antimony added. Per Cent.	Antimony found. Gross. Grm.	Antimony found. Nett. Grm.	Antimony found. Per Cent.
10·0	Nil (blank)		0·00014	—	0·0014
10·0	0·00005	0·0005	0·00019	0·00005	0·0005
10·0	0·00010	0·0010	0·00024	0·00010	3·0010

These results show that amounts of antimony as low as five-hundredths of a milligram may be successfully separated and determined by this process.

As regards the amounts of antimony normally present in tin, a sample weight for the determinations should be taken in proportion to its quality; for high grade tin 5 grms. are suitable, whilst for the more impure varieties not more than 1 grm. should be used; otherwise difficulties may occur due to the antimony-arsenic film detaching from the copper during boiling.

The following notes on the chemistry of this process may make some points more clear.

THE SEPARATION.—The use of the Reinsch reaction for separating antimony from tin was indicated by the work of Evans on the determination of small quantities of antimony in copper (ANALYST, 1922, **47**, 1) and in lead (ANALYST, 1927, **52**, 565). In both these cases, after a preliminary separation of the main metal, and of the impurity, arsenic, by precipitating this in hydrochloric acid solution by hypophosphite, antimony is precipitated on copper by the Reinsch method. It is determined, after stripping with sodium peroxide and separating the small quantities of bismuth and copper accompanying it by means of zinc sulphide, by the colour in acid solution of its sulphide retained in colloidal solution by gum arabic. The success of this method depends on there being available, in sodium hypophosphite, a means of completely separating arsenic prior to applying the Reinsch method for precipitating antimony; otherwise arsenic would accompany the antimony throughout to the colorimetric stage of the process, where its sulphide colour would develop and produce a high result. In attempts to apply Evans's method to the determination which is the subject of this paper, it was found that arsenic could not be separated quantitatively from tin in this way; (on adding sodium hypophosphite to the boiling solution a distinct precipitation of arsenic was seen, but before it could be filtered off it had re-entered solution by reducing some tin to the divalent condition). It would, no doubt, be possible to separate arsenic in this way if all the tin were in the stannous state, but this is not practicable in the present case.

No difficulty was experienced in depositing all the antimony, together with the arsenic, on copper. The fact that antimony metal goes into solution when boiled with stannic chloride, reducing an equivalent amount of tin, would render the deposition of antimony on copper in presence of excess of stannic tin very surprising, were it not borne in mind that the deposit consists largely of a compound of antimony and copper, Cu_2Sb; arsenic deposits contain the compound Cu_5As_2. (Evans, ANALYST, 1923, **48**, 1).

There is certain evidence upon a point mentioned in Reinsch's original paper (*J. prakt. Chem.*, 1841, **24**, 244) that a small amount of tin accompanies antimony when this is deposited from a solution containing considerable amounts of the former metal; high results were obtained when antimony, deposited in presence of tin free from arsenic, was determined by the colorimetric sulphide method.

The colorimetric method used in this paper was therefore developed to give a direct determination of antimony in presence of co-deposited arsenic and tin.

THE COLORIMETRIC METHOD.—It had previously been stated by Caille and Viel (*Compt. rend.*, 1923, **176**, 1156, 1759) that many alkaloids and organic bases yield highly coloured insoluble iodo-antimonites and iodobismuthites of the general formula B, HI, MI_3 (B=organic base, M=antimony or bismuth); in fact, they recommended a solution of 1 grm. of antipyrine with 2 grms. of potassium iodide in 30 c.c. of water for detecting antimony in certain biological liquids, claiming to detect as little as 0·025 mgrm. when present in hydrochloric acid of 20 per cent. strength. These authors paid no attention to the use of their reagent for quantitative purposes. I attempted to apply this reagent quantitatively, using gum arabic to prevent the insoluble antimony compound rapidly settling out of solution, with a view to comparing the colours of the turbidities produced with standards. No satisfactory method could be evolved, for the reasons that the intensity and actual tint of the yellow turbidity were dependent to a high degree on conditions such as amount of acid present, volume of solution, amount of reagent added, presence of neutral salts; the colour also changed rapidly with time owing to change in grain-size of the turbidity and slight absorption of iodine. Further, as a clear solution was not obtainable, the simple method of colour comparison in Nessler glasses could not be used.

By the substitution of pyridine for antipyrine, however, a more soluble antimony compound is produced, yielding a clear yellow solution, and the conditions were determined under which an accurate estimation may be made in presence of moderate amounts of arsenic and tin.

It was found that almost any relative amounts of pyridine, potassium iodide and mineral acid, would, providing they were kept in fairly concentrated solution, yield a bright yellow turbidity with small amounts of antimony. Attention, however, was directed towards the production of the maximum intensity of colour for a given amount of antimony, beyond which further addition of reagent would produce no change, and to assure this being reproducible. The study was made at a standard volume of 100 c.c. in Nessler glasses.

Gum Arabic.—Ten c.c. of 1 per cent. solution are necessary, for the amounts of antimony covered by this method, to give a clear solution free of opalescence.

Potassium Iodide.—The amount necessary was found to be inversely proportional to the amount of acid. High concentrations of this salt were avoided, both on account of cost and because separation of iodine is likely to occur through atmospheric oxidation. Five c.c. of 20 per cent. solution proved an optimum amount; with much less than this the maximum colour does not develop.

Acid.—A series of experiments were made over a range of sulphuric acid concentration, the quantities of antimony and other reagents being kept the same. It was found that a straight line relationship did not exist between the intensity of colour and the acid concentration; both increase together until, at a strength corresponding approximately to 75 c.c. 1:3 sulphuric acid in 100 c.c., the colour reaches a steady maximum. The presence of alkali sulphates does not influence the colour.

Hydrochloric acid is undesirable in this determination, as it causes a serious diminution of the colour; notable amounts of alkali chlorides entirely bleach it. This acid must therefore be removed before the colorimetric determination is attempted. I have carried out some experiments which show that this may be done without loss of antimony by evaporating with sulphuric acid till fumes of sulphur trioxide appear, nitric acid having been added previously in quantity roughly equivalent to the hydrochloric acid present. A slight loss of antimony occurs if the nitric acid is omitted. The following figures may be quoted:

	Antimony taken. Grm.	Antimony found. Grm.
(*a*) Without nitric acid	0·00025	0·00022
	0·00050	0·00045
(*b*) With nitric acid	0·00025	0·00025
	0·00050	0·00049

With weak acids, like acetic acid, the colorimetric reaction does not take place.

Pyridine.—Quite a small amount is required for the development of maximum colour, but the amount must not be increased excessively, as too much has a bleaching effect; 1 c.c. of a 10 per cent. aqueous solution is satisfactory.

Sulphur Dioxide.—A small addition of this is necessary, as in the colorimetric iodide bismuth estimation, to counteract the oxidation of the iodide by the air. The amount must, however, be kept strictly limited, on account of its well-known property, when in increased quantity, of actually causing a separation of iodine. Not more should be used than 1 c.c. of a solution made by diluting a saturated aqueous solution of sulphur dioxide to one-tenth its strength.

Effect of other Metals.—Tin and arsenic in the moderate amounts examined (several centigrams) produce no colour with the reagent and do not interfere. Bismuth and several of the heavy metals give precipitates, generally coloured. It is notable that zinc gives a white crystalline precipitate; the zinc sulphide method of separating antimony from bismuth and copper cannot, therefore, be used in conjunction with this colorimetric process.

A point of interest in this colorimetric method is that it can be applied to antimony in either state of oxidation; pentavalent antimony is reduced, in presence of excess iodide and acid, to trivalent antimony, which appears to be the valency state necessary for the production of the coloured compound.

Notes

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

THE PRECIPITIN TEST FOR BLOOD.

In connection with the valuable papers by Sir William Willcox, Dr. Roche Lynch and Dr. Hartley (Analyst, 1928, 2, *et seq.*), the following notes on my experience with the precipitin test may prove useful. During the past seventeen years I have frequently given evidence, based on the results of the test, in the Courts of this Colony. Guinea pigs, rabbits and domestic fowls have been used for the preparation of the sera, and Lloyd's method has been found as satisfactory as any. Sera purchased from England, Germany and the United States have also been used, but I can endorse what was said at the meeting concerning their reliability. From practical experience I can say that anti-human serum made from fowls is no weaker than serum made from rabbits or guinea pigs.

It is the experience of this Laboratory that sera will only remain potent in Trinidad for three months, although kept on ice. This seems somewhat incredible, but the fact has been proved a great many times. I cannot suggest an explanation other than the hypothesis that a constant temperature of 0° C. being difficult to maintain in the tropics, fluctuations of temperature occur and give rise to intramolecular changes in the sera which destroy their potency by gradually forming molecules which will not react with the antigens. Possibly the molecules of the anti-bodies gradually change back to original and more stable molecular constitutions occurring in the blood before the biological reaction was excited by introduction of the antigens.

It is advisable to test the reactions of the bloodstain solutions. If they are neutral to litmus they lie within the P_H range laid down for the precipitin reactions, and reactions due to excessive acidity or alkalinity are excluded.

With regard to the application of the antiserum I agree that layering is a very good method, but have obtained more definite zone reactions by allowing the antiserum to roll gently down the side of the tube inclined at 45° through the solution of the stain, and therefore incline to favour this method.

Herbert S. Shrewsbury.

Government Laboratory,
Trinidad, B.W.I.

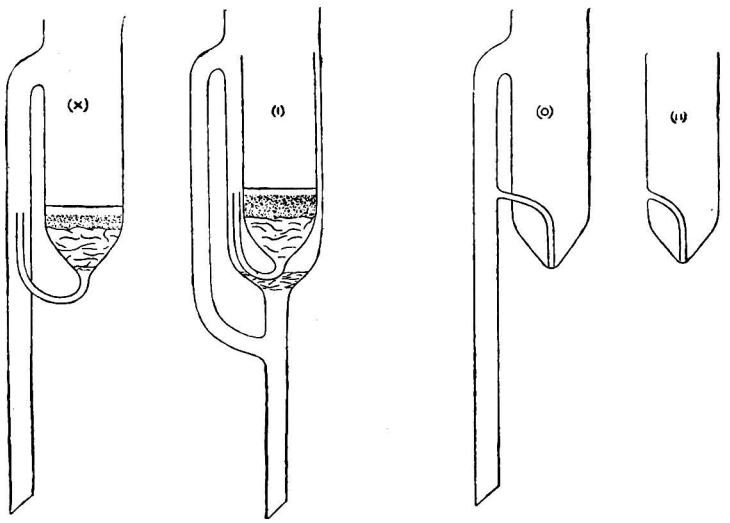
A CONTINUOUS EXTRACTOR.

This apparatus was devised for the rapid extraction of large quantities (50 grms.) of materials from which the oil or other soluble substance can be easily extracted, such as the extraction of phenols from carbolic powders having a siliceous base.

The bottom of the extractor is packed with cotton wool or other suitable filtering medium. It is preferable to use one complete piece of cotton-wool, as, if several layers be used, air is enclosed between them and this may rise during the extraction and disturb the packing. A weighed quantity of material is introduced, and the extractor attached to a reflux condenser. A flask containing the

solvent is fitted to the stem of the extractor. The flask must not contain so much solvent that the extractor could fill up and overflow through the large side tube into the flask. The solvent is distilled into the extractor in the usual way.

Where the extract is heavy, as in the case of the extraction of phenols, etc., or where a considerable depth of a fine powder is being extracted, a certain amount of "head" is at first necessary to drive the extract over and distillation is continued until a rapid flow of solvent is obtained; in addition, air bubbles have a retarding effect, but these gradually disperse.



A large margin of space to allow for the formation of this "head" has been allowed in the design of the apparatus. The 5 grm. size extractor has also been made of such a diameter that the 3 to 5 grm. quantities usually taken for extraction form a shallow layer and do not obstruct the passage of the solvent.

The drawing (X) shows the complete extractor and the drawing (O) shows another type in which the small tube is placed inside.

The apparatus has the following advantages:

(1) Neither thimbles nor filter paper packets are necessary (they can be used if required).

(2) The substance to be extracted is always totally immersed in the solvent.

(3) The "pressure" is directly on the solvent in contact with the material, and the extracting liquid is always in motion. (In the Soxhlet extractor the "pull" acts on the liquid outside the thimble. After each "siphoning over" a little more extract-containing solvent drains from the thimble into the bottom, and the extractor has to be filled again before "siphoning" takes place. This continues until extraction is complete.)

(4) There is a continuous and rapid flow of solvent. The rate of flow is, of course, dependent on the rate of distillation.

(5) If the apparatus be left to "stand overnight," the substance remains totally immersed, and when the extractor is started again the first few ml. of solvent from the condenser cause circulation to continue.

(6) As soon as the solvent is circulating easily, only a few ml. are necessary in the flask to continue the extraction.

(7) The contents of the extractor are visible.

The extractor may be used for the extraction of substances which require no filtering medium, and cakes, etc., in which some form of filter is necessary.

Using asbestos as a filtering medium, cocoa has been successfully extracted without any of the fine powder passing through into the flask.

On the same principle (1) and (11) are glass thimbles which are placed in the container shown. The thimble may rest on cotton-wool, which supplies a further filtering medium, or may rest directly on the glass, when the hot vapour will pass up and round the thimble.

Except for special purposes, when these thimbles might be required, the complete extractor (X) is simpler and more satisfactory in use.

DOUGLAS HENVILLE.

Notes from the Reports of Public Analysts.

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

CITY OF BIRMINGHAM.

REPORT OF THE CITY ANALYST FOR THE FIRST QUARTER, 1928.

OF the 1481 samples submitted during the quarter, 1325 were taken under the Sale of Food and Drugs Acts, 1254 being bought informally (36 adulterated), and 71 formally (7 adulterated).

BAKING POWDER. EGG SUBSTITUTE POWDER.—These articles should contain at least 6 per cent. of available carbonic acid, but one baking powder contained only 4·5 per cent., and an egg substitute powder only 4·6 per cent. The manufacturer of the two powders was cautioned, and undertook to increase the strength.

"PALE SYRUP."—A sample of golden syrup, sold under the name of "pale syrup," contained 20 per cent. of glucose syrup. "Golden syrup" is definitely a sugar product, but "pale syrup" may contain any proportion of adulterant. The wholesale dealer, therefore, protected himself by the use of the term, though the retailer may have been ignorant that it indicated an adulterated product. The retailer was cautioned, and also the wholesale dealer, who was informed that his invoice was unsatisfactory.

SUGAR PIECES.—Eight informal samples contained from 2·4 to 7·0 per cent. of moisture. Another sample was rather high in moisture, containing 6·6 per cent. A firm of sugar refiners was kind enough to inform me that the usual percentage of moisture in this article is 3 to 6 per cent.

ZINC OINTMENT.—Zinc ointment should contain 15 per cent. of oxide of zinc, and the six informal samples contained approximately correct amounts, varying from 13·9 to 16·3 per cent. One sample was rancid, and the vendor was asked to withdraw it from sale.

J. F. LIVERSEEGE.

Legal Notes.

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

CREAM BUNS.

ON May 4th a tradesman was summoned at Birkenhead for selling cream buns not of the nature, substance and quality demanded. Five cream buns, costing 2d. each, were bought at the defendant's shop, and when analysed by the Public Analyst (Mr. H. E. Davies) were found to contain 69 per cent. of pastry and 31 per cent. of filling material, composed of 43·30 per cent. of fat, 34·98 per cent. of sugar, and 21·72 per cent. of water. The fat was margarine, and contained no cream.

The Solicitor for the defence contended that since an order had been made prohibiting preservatives in cream, it was not practicable to use fresh cream. He submitted that the purchaser had not been prejudiced.

Mr. A. C. Shepherd, Deputy Town Clerk, observed that other tradesmen in the town were under no misapprehension in the matter.

The Magistrates dismissed the summonses. They were agreed that what was sold was not prejudicial from the point of view of purity.

PREPARED SALT SOLD AS SALT.

A GROCER was summoned on May 8, at Stradbally, for selling salt which was adulterated, since, according to the certificate of the Public Analyst, it contained carbonates of calcium and magnesium.

The solicitor for the defence contended that at worst the offence was a technical one, the ingredients complained of having been added to keep the salt dry and to enable it to be poured easily from the package.

To this the prosecution objected that if the manufacturers had to add these ingredients, the fact that the salt was a mixture ought to have been notified on the package.

The District Justice pointed out that this was a specially prepared salt which had not been heard of when the Act of 1875 was passed, and that the Act should be brought up to date to cover many articles such as this. He dismissed the charge under the Probation of Offenders Act, the defendant to pay 14s. costs, and he asked the defendant to take the matter up with the manufacturers, since he was liable to prosecution for it every day.

Ceylon.

REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1927.

ACCORDING to the Report of the Government Analyst, Mr. C. T. Symons, 2543 analyses and 920 reports were made during the year, the decrease in the number of analyses, compared with 1926, being due to the smaller number of stamps examined.

MILK.—The number of milk samples analysed was 293, as compared with 259 in 1926. Of these, only 27 per cent. were genuine, the amounts of added water ranging from about 25 to over 70 per cent. (maximum 77 per cent.). There is unlikely to be any improvement in this matter, so long as the penalties imposed on milk dealers are so slight.

CRIMINAL INVESTIGATION.—During the year, 908 articles were examined in connection with 363 cases.

Poisoning Cases.—In only 17 of the 77 cases investigated was poison detected. Of the 22 articles examined in connection with these cases, arsenic was found in 5, nitric acid in 4, strychnine, prussic acid and datura in 2 each, and metallic mercury, kerosene, formaldehyde, *Gloriosa superba*, Jeyes fluid, and an unidentified mydriatic alkaloid in the remainder. Arsenic still remains the popular poison, but possibly, when the provisions of the new Poisons, Opium, and Dangerous Drugs Ordinance are strictly enforced, it will be more difficult for the poisoner to obtain his favourite weapon.

A large number of cases are sent for examination where no one has been poisoned, and the only evidence has been the appearance of the food or its unusual taste. Sometimes this may be due to slight decomposition of food cooked overnight, but in such cases, if the persons concerned are fervent followers of the Ayurvedic Code, they may suspect a derangement of the Dosha Equilibrium of their system, followed by an actual feeling of illness. It would not be unnatural to expect negative results in such cases from the search for an active poison by the methods of western science. One can only hope that Ayurvedic practitioners will come forward with proofs of the evil or benign influence of combinations of seemingly inactive compounds. This point is of particular interest, because the Committee appointed in 1926 to consider the question of Ayurvedic medicine recommended that facilities should be given for research in the Eastern systems of medicine in connection with the Government Analyst's laboratories. So far no action has been taken in this direction.

Prohibited Drugs.—Of the 238 articles examined in 84 cases, ganja was found in 132 and opium in 31, showing an increase of 45 per cent. over the cases in 1926. In four cases the Laboratory was asked to distinguish between Ceylon and Indian opium, presumably to determine whether the drug has been legally purchased from a Government depôt, or whether it had been smuggled in from India.

Police Lectures.—The usual lectures have been delivered to Police Officers in training, and they are now taught as completely as possible to protect any material for scientific examination as circumstantial evidence.

Miscellaneous.—Among the 149 articles examined, in 44 cases were bullets in murder charges, bullet marks on planks, wads, etc. In a case of housebreaking and theft it was necessary to determine whether the dhoby mark on a handkerchief, made with kottan seed juice (*Semecarpus anacardium*), had been placed on the article before washing or after. Actual trial with the same juice showed that

the handkerchief, though used since the last washing, had not been washed since the dhoby marks had been placed on it.

In addition to the numerous forgery cases in connection with the sale of rubber, which are not dealt with officially by this Department, this year has provided a case where it was necessary to prove an attempt to destroy by burning books containing the register of rubber purchases. Two such books, to quote from the report, "showed traces of kerosine oil. Both were partly charred. A much more than B. The charring on A indicates that the first leaves of the book were burnt while the front cover of the book was lifted up. The charring on the front cover of A indicates that this occurred while some other body, of square base, such as B, was resting on A."

It is usually easy to determine whether a currency note is forged or not, but in one case, referred to this Department, it was necessary to determine whether two such notes were forged by one and the same person. The problem was rendered easier by the fact that the notes were not printed. But the Deputy Government Analyst was able to show quite clearly that the papers were different, that different inks had been used, that the forgeries had been produced by different methods, one by tracing and the other by freehand drawing, and that the technical skill of the forgers differed very considerably.

A waft of romance, if a little tainted, was brought into the otherwise somewhat sordid work of the Department, when a red substance, which proved to be an iron ore, red haematite, was sent for analysis, as it was suspected to be an unwholesome drug (love philtre) with the history that it was "alleged to have been given to a woman to be rubbed on betel leaf and given to another woman so as to make the latter not to resist any advances made by this man."

EXCISE WORK.—Of 24 samples of arrack examined, only 6 were passed as sound, the remainder being found to contain an excess of copper.

CUSTOMS WORK: *Milk.*—Legislation has been recently introduced, regulating the import of milk, especially condensed milk, with regard to the dilution figures, bringing the Ceylon regulations into line with those in force in Great Britain. Up to date only three such samples have been analysed, the work being delayed owing to the lack of accommodation and staff in our laboratories.

Department of Scientific and Industrial Research.

FUEL RESEARCH. Technical Paper No. 20.*

THE THOMAS RECORDING GAS CALORIMETER.

IN this instrument (which is fully described in an Appendix and in *Gas. J.*, 23rd Aug., 1922, p. 426) the calorific value of a gas is continuously measured and recorded by the transference of all the heat obtained from the combustion of the gas in air, to a stream of heat-absorbing air, the rise in temperature of which is measured. The streams of air and gas are maintained in a fixed volumetric ratio by separate wet meters, and the gas is burnt in a closed burner round which flows the heat-absorbing air. An independent electrical recording instrument translates heat

* Published by H.M. Stationery Office. Price 9d.

measurements into thermal units. The report describes exhaustive tests of the original calorimeter manufactured by the Cutler Hammer Co., and of the modified forms adopted by the Cambridge Instrument Co. as a result of the earlier tests described. It is concluded that the instrument in its final form, properly installed, adjusted and maintained, should give a reading for a gas of steady calorific value (500–600 B.Th.U.) correct to $\pm$ 1 B.Th.U. compared with a standard Boys calorimeter. A true "cold balance" is obtained 25 to 34 minutes after starting, and 22 to 32 minutes after extinguishing the flame, and the first indication of a change in calorific value is obtained after 4 minutes, though when a different gas is used 14 to 20 minutes are required to show full changes up to 50 B.Th.U. in 600. The periodicity of regular, rapid changes of gas supply at 2·5 minute intervals is shown accurately, and though there is a lag of about 6·5 minutes for the maxima and minima and the amplitude of the variations is greatly reduced, the mean value recorded is correct.

Changes in the speed of the motor (normally 1450 revs./min.), or in the applied voltage, have only small permanent effects, and should not exceed 0·25 per cent., a change which causes a maximum fluctuation of $\pm$ 0·5 B.Th.U., and lasts for 3 minutes. Variations in atmospheric temperature or pressure have negligible effects, but the pressure of the gas supply should not vary more than from 1·5 to 6·5 ins. of water, and changes of level of water in the tank should be avoided. The mechanical parts were found satisfactory, and variations in gas-supply were shown more accurately than by the water-flow or differential expansion calorimeters, though the time-lag was greater, and minor temporary changes recorded by the two first-mentioned were smoothed out and shown as a mean value.

J. G.

Parliamentary Notes.

FOOD AND DRUGS (ADULTERATION) [H. L.]

A Memorandum of a Bill intituled:

An Act to consolidate the Sale of Food and Drugs Acts (*The Lord Gage* [*V. Gage*), was ordered to be printed 23rd May, 1928.

Copies can be obtained from H.M. Stationery Office. Price 9d. net.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

Food and Drugs Analysis.

Composition of the Fatty Acids of Palm Oil. A. Rayner and S. G. Campbell. (*J. Soc. Chem. Ind.*, 1928, **47**, 149T–150T.)—Palm oils from the following sources were examined: Brass, Congo, Hards, Lagos, Lahou, New Calabar, Niger, Red Sherbro, and Sumatra. The iodine values varied from 33·6 per cent. for Congo (with 89·2 per cent. of free fatty acids) to 63·3 per cent. for Lahou (with 39·7 per cent. of free fatty acids). The Sumatra oil had iodine value 56·8 and free fatty acids 3·6, and the titres of the oils varied from 48·5° C. for Congo

to 41·4° for Lahou. The differences were found to be due rather to the proportion of solid acids than to differences in composition of these. The proportion of stearic acid in the solids varied from 12·5 for Red Sherbro to 18·5 for Lahou, and the liquid acids, calculated from the iodine values, contained on the average 20·0 per cent. of linolic and 80·0 per cent. of oleic acid. D. G. H.

The Kreis Test for Rancidity. W. G. Powick. (*Oil and Fat Ind.*, 1928, **5**, 107–108.)—The use of hydrochloric acid containing nitrosyl chloride in the Kreis test for the detection of rancidity in oils and fats may lead to confusing results. The acid should give no positive reaction with phloroglucinol, for nitrosyl chloride appears to react with the double bonds of unsaturated fatty acids, and hence positive Kreis results may be obtained with sweet fats, and negative results with rancid fats. D. G. H.

Chemical Composition of Ergot Oil. W. F. Baughman and G. S. Jamieson. (*Oil and Fat Ind.*, 1928, **5**, 85–89.)—A mixed sample of Russian, Spanish and Austrian ergot contained 34·66 per cent. of oil on a dry basis, and the oil had the following characteristics :—Sp. gr., 25/25° C., 0·9222 ; $n_D^{25°}$ C., 1·4691, saponification value, 196·9 ; iodine value (Hanus), 73·8 ; Reichert-Meissl value, 0·3 ; Polenske value, 0·4 ; acetyl value, 7·3 ; unsaponifiable matter, 1·18 per cent.; acid value, 3·02 ; saturated acids (corrected), 27·2 per cent. (iodine value, 2·6) ; unsaturated acids (corrected), 68·1 (iodine value, 101·2). The unsaturated acids consisted of oleic 87·84, linolic 12·12 per cent.; and the saturated acids, myristic, 1·09 ; palmitic, 77·32 ; stearic, 19·13, and arachidic, 2·46 per cent. Daturic acid or hydroxylated acids were not detected. D. G. H.

Fluorescence of Italian Olive Oils in Ultra-Violet Light. R. Stratta and A. Mangini. (*Giorn. Chim. Ind. Appl.*, 1928, **10**, 205–207.)—Examination of Apulian olive oils shows that, when exposed to ultra-violet (Wood's) light in layers at least 3 mm. in thickness, and with a free surface, pressed virgin oils assume a fluorescence varying in colour from deep lemon-yellow to orange, changing, after an exposure of 30 minutes, to natural Sienna-earth colour. Sansa oils, extracted by solvents and refined, yield a sky-blue fluorescence, unaltered by prolongation of the exposure. Refined lampante (washed and filtered) oils develop a dull, greyish-blue fluorescence. Addition of refined oil, in proportions of 1 per cent. or more, to virgin oil, causes changes in the colour of the fluorescence, which becomes almost white with 10 per cent., pale blue with 30 per cent., and brilliant sky-blue with 60 to 100 per cent. of the refined oil. Examination of a sample of olive oil under Wood's light, either by the eye or by means of a colorimeter, renders it possible to decide if a delivery of oil corresponds with the sample. The fluorescence spectrum of crude olive oil, and particularly of any virgin oil, always exhibits a characteristic and distinct red band, with its mid-point at 669$\mu\mu$; by means of this band, which is lacking with all refined oils, virgin oil may be recognised in the presence of even 90 per cent. of refined oil, and its concentration judged.

T. H. P.

Organic Acids of Tomatoes. A. Borntraeger. (*Z. Unters. Lebensm.*, 1928, **55**, 112–143.)—Oxalic acid does not occur in the pressed juice or aqueous extract or residues of fully grown tomatoes, whether unripe or over-ripe, or in either fresh or properly stored tomato preserves, but it is found to the extent of 0·0016 per cent. in very unripe tomatoes, and may be produced by the action of bacteria or certain moulds (*Aspergillus niger*) on the fruit or the preserve. Pectic substances do not occur in the filtered pressed juice of green tomatoes, or of tomatoes which have ripened and softened, but traces are detected with the semi-ripe and just ripe fruit. In analysing extracts, prepared hot, of tomatoes and their preserves, it is advisable to make sure that pectins are removed. This may be effected by treating 25 c.c. of the juice with 4 c.c. of 5 per cent. sodium hydroxide solution for 24 hours, and then over-neutralising with hydrochloric acid, making up to 50 c.c., and filtering on the following day. Arabinic acid (metapectinic acid) is not removed in this way, as it is soluble in water, but it may be eliminated by precipitation with alcohol.

Malic acid cannot be determined by conversion into oxalic acid. With juices and extracts of over-ripe tomatoes, incineration and titration of the alcoholic precipitate of calcium malate give high results, owing to the entrainment of calcium succinate and, possibly, of calcium arabinate also. Even with heated preserves the presence of arabinic acid may occur, but not that of succinic acid, at any rate with fresh products made from sound fruit. The presence of succinic acid is confirmed by the reaction with ferric chloride. As the ripe tomatoes soften, the proportions of citric and malic acids diminish, and succinic acid, which otherwise is lacking, may then be found. As is always the case when the juices and extracts turn mouldy, the citric acid disappears before the malic acid. When the juices ferment, volatile acids are formed, as well as succinic acid.

The fresh tomato preserves examined contained no oxalic, tartaric, succinic, lactic, or volatile acids, and these do not appear after long storage of the material in glass. The same holds for the solid preserves, except that, when these are kept for a long time under a layer of salt, succinic acid appears to be formed at the expense of the malic acid. A solid preserve prepared without artificial heating lost most of its malic acid in 18 months. (See ANALYST, **1926**, **51**, 151.)

T. H. P.

Honey Diastase. J. Fiehe and W. Kordatzki. (*Z. Unters. Lebensm.*, 1928, **55**, 162–169.)—Koch's method (Zander and Koch, *Der Honig*, 108) and Gothe's method (*Z. Unters. Lebensm.*, 1914, **28**, 286) mark a considerable advance over the qualitative test for diastase given in the " Festsetzungen über Honig," issued by the Reichsgesundheitsamt in 1912.

Koch's method, which measures the diastatic activity under the conditions (acid and salt content) prevailing in the honey itself and hence may give different values for two samples of honey of equal diastase content, is carried out as follows : From a mixture of honey and starch solution in definite proportions, immersed in a water-bath at 40° C., 2 c.c. samples are removed at intervals of 1 minute, treated with dilute hydrochloric acid to arrest diastatic action, and tested with iodine

solution. This procedure is continued until the colour, observed in a colorimeter, corresponds with that obtained by addition of iodine solution alone to the hydrochloric acid. For normal honeys, the time required for the degradation of the starch is 9 to 22 minutes ; for sugar-fed honeys, 31–31 minutes ; for doubtful honeys, 32 to 50 minutes ; and for decomposed honeys, above 50 minutes.

By Gothe's method, measurement is made of the "diastase" number, which represents the number of c.c. of 1 per cent. starch solution degraded by 1 grm. of honey in 1 hour under optimum conditions. A solution of 10 grms. of the honey in water is made neutral to litmus by addition of 0·05 *N* sodium carbonate solution and made up to 100 c.c. A number of mixtures are prepared, each having the total volume 16 c.c., and containing 0·5 c.c. of 0·02 *N* acetic acid and 0·5 c.c. of 0·1 *N* sodium chloride solution. The first mixture contains honey solution, water, and 1 per cent. starch solution (specially prepared and usable only for 2 days after preparation) in the proportions 10:4:1, the corresponding diastatic number being 1 ; for succeeding tubes, the proportions and diastatic numbers are :— 10:2·5:2·5, 2·5 ; 10:0:5, 5 ; 7·7:2·3:5, 6·5 ; 6·0:4:5, 8·3 ; 4·6:5·4:5, 10·9 ; 3·6:6·4:5, 13·9 ; 2·8:7·2:5, 17·9 ; 2·1:7·9:5, 23·8 ; 1·7:8·3:5, 29·4 ; 1·3:8·7:5, 38·5 ; 1·0:9·0:5, 50. The tubes containing the mixtures are kept in a water-bath at 45 to 50° C. for 1 hour, and are then cooled in ice water and treated with 1 drop of 0·1 *N* iodine solution, a purple coloration being taken as the end point. The authors find that, when the honey solution is neutralised by sodium carbonate in presence of litmus, the colour change is not sharp, but that satisfactory results are obtained if 0·05*N* sodium hydroxide and phenolphthalein are used ; the titration is continued until the pink colour persists for some minutes. The starch solution remains good for 5 days, provided that it is kept in an ice-chest and is shaken before use. In most cases the first two of the above mixtures may be omitted, and the acetic acid, sodium chloride, and starch solution may be kept ready mixed, so that 6 c.c. are measured into each tube. Cooling of the tubes with ice water after the diastatic action is unnecessary if the warm water of the bath is quickly siphoned off and replaced by cold water, and the iodine test is applied immediately to the cooled tubes.

Approximate, but not complete, parallelism is found between the results obtained by the two methods, divergences appearing especially with honeys of low diastatic content. For instance, a honey giving the Gothe diastatic number 23·8 required 45 minutes for the starch degradation according to Koch's method. The authors' results indicate that honeys with diastatic numbers below 17·9 are to be regarded as suspicious, and those with values under 10·9 as decomposed or adulterated. T. H. P.

Banisterine, a New Narcotic and Medicament. L. Lewin. (*Chem. Ztg.*, 1928, **52**, 357.)—E. Merck has prepared a new alkaloid, banisterine, $C_{13}H_{12}ON_2$ (m.pt. 256–257° C.), from the plant banisteria, a liana of the order *Malpighiaceae*, found widely in South America, and used by the natives for the preparation of a drink. It is slightly soluble in ether, and crystallises from alcohol or ether in

shining prisms. The hydrochloride (m.pt. 264° C.) produces symptoms of excitement, accompanied by hastened respiration, dilation of the pupils and sometimes madness, when 0·005 grm. is injected into a dog. The alkaloid is identical chemically, but not toxicologically, with harmine. J. G.

Biochemical

Determination of Corn Cockle in Flour by Haemolysis. F. S. Okoloff. (*Z. Unters. Lebensm.*, 1928, **55**, 155–162.)—The following method, based on Kobert's method for determining saponins in medicinal plants, serves for the accurate determination of corn cockle in flour. Twenty-five (or 12·5, if more than 0·6 per cent. of corn cockle is present) grms. of the flour are mixed in a 500 c.c. Erlenmeyer flask with 125 c.c. of physiological sodium chloride solution, and 0·3 c.c of 40 per cent. commercial formalin, the flask being closed with a rubber stopper and tilted to wash down any black particles from the walls. The flask is left in an incubator at 37° C., with occasional gentle stirring, until the next day, when it is vigorously shaken and then left at rest for an hour at room temperature. The supernatant liquid is filtered perfectly bright through a roomy filter. Meanwhile rabbit's blood is obtained by heart puncture with a 10 c.c. Luer's syringe, 5 to 6 c.c. of blood being sufficient for 15 to 20 tests. The blood is placed in a small flask containing glass beads and defibrinated for 10 minutes, then sieved through bandage gauze, diluted sixfold with physiological salt solution, centrifuged, and washed three times. The final suspension of washed erythrocytes is diluted threefold with the salt solution. For a small number of tests, the blood diluted 18-fold may be centrifuged directly.

For the haemolysis test, the first 5 test-tubes are charged with 1 c.c. of the clear flour extract, three with 0·5 c.c., three with 0·25 c.c., and the last three with 0·1 c.c., all volumes being made up to 1 c.c. with the salt solution. Of the erythrocyte suspension, 0·1, 0·2, 0·3, 0·4, and 0·5 c.c. are added to the first five tubes, and 0·3, 0·4, 0·5 c.c. to each of the succeeding groups of three. The tubes are shaken, placed for 1½ hrs. in an incubator at 37°, and then left overnight at room temperature. In order to check the specificity of the haemolysis, a mixture of the same dilution as the last tube showing marked haemolysis is treated, before the erythrocytes are added, with 2 drops of saturated alcohol cholesterol solution, which, under the same treatment as the other tubes, should completely inhibit haemolysis. The haemolytic activity of the flour extract is obtained by multiplying the amount of blood added by the dilution of the extract.

The results may be checked by tests on flour with known proportions of corn cockle. For 0·2 to 0·6 per cent. of corn cockle, results 4·5 per cent. low on the average were obtained, and for 0·6 to 2·5 per cent. the mean divergence was 4·9 per cent. Disturbance of the results due to the use of blood of different rabbits has not been observed, but may be avoided by tests on flours of known corn cockle content. T. H. P.

Phosphorus Compounds of Milk. IV. Presence of Adenine Nucleotide in Milk. H. D. Kay and P. G. Marshall. (*Biochem. J.*, 1928, **22**, 416–418.)—Recently it has been shown by Kay (*Biochem. J.*, 1925, **19**, 433) that there are present in protein-free filtrates from goat's, cow's, or human milk, acid-soluble phosphoric esters which are readily hydrolysable by dilute acids and by extracts of certain tissues, and are readily diffusible through collodion membranes. It has now been shown that adenine nucleotide, associated with some other phosphoric ester with similar precipitation reactions and solubilities, is present in very small quantities in goat's milk. So far, its presence has not been demonstrated in cow's or human milk. Its concentration is of the order of 3 mgrms. of nucleotide per 100 c.c. milk, corresponding with about 0·3 mgrm. phosphorus. The average organic acid-soluble phosphorus of goat's milk is some 13 mgrms. per 100 c.c.; thus, the nucleotide found accounts for only a small fraction of the ester phosphorus, so small that it may be only an adventitious milk constituent, derived from cellular breakdown in the mammary gland. The adenine nucleotide is present, either mixed or in loose combination with an impurity which contains more phosphorus and less or no nitrogen. The nucleotide from pig's blood was similarly prepared, and the substance obtained was identical with the milk compound. Calculated for adenine nucleotide, P=8·93 per cent., N=20·1 per cent. Found for goat's milk compound, P=11·3 per cent., 11·6 per cent; N=16·42 per cent.; 15·38 per cent. Found for pig's blood compound, P=11·3 per cent., N= 16·0 per cent. P. H. P.

Quantitative Pettenkofer Test Applicable to the Determination of Bile Acids in the Blood. M. Aldrich and M. S. Bledsoe. (*J. Biol. Chem.*, 1928, **77**, 519–537.)—The Pettenkofer test (*Ann. Chem. Pharm.*, 1844, **52**, 90) is probably the most sensitive reaction known at present for the bile acids, and as such, although it is not specific, it offers the best basis for a method which can be readily adapted for use with small amounts of blood. A quantitative modification of the Pettenkofer reaction has been devised by which pure bile acids can be determined in amounts of from 0·1 to 0·5 mgrm., with an accuracy of ± 5 per cent.; the method is applicable to bile as well as blood. A series of analyses of commercial preparations of bile salts and of bile from different sources, made to compare this method with that of Schmidt and Dart (*J. Biol. Chem.*, 1920–21, **45**, 415; ANALYST, 1921, **46**, 146) shows that the results obtained by the modified Pettenkofer test agree within the limits of error with those obtained by the gasometric determination of the amino nitrogen liberated by alkaline hydrolysis. The new colorimetric method is easier, more rapid, and can be applied in the analysis of small amounts of material. It was also compared with the colorimetric determination of amino nitrogen, the method of Rosenthal and Lauterbach (*Arch. exp. Path. u. Pharmakol.*, 1924, **101**, 1). The reaction has been applied to an alcoholic extract of 5 c.c. of blood, and a method of extraction which permits of the determination of maximal amounts of Pettenkofer-reacting material is described. Recovery of bile acids added to the blood is made by this method with an average.

loss of about 0·5 mgrm. for each 100 c.c. With few exceptions, the recovery is greater than 90 per cent. When interfering substances are removed as completely as possible, normal blood yields a Pettenkofer value equivalent to from 3 to 6 mgrms. of glycocholic acid for each 100 c.c. of blood. Increased values have been found under certain clinical and experimental conditions. P. H. P.

Colorimetric Determination of Lipoid Phosphorus in Blood. A. R. Harnes. (*J. Biol. Chem.*, 1928, **77**, 405–407.)—A modification of the method of Briggs (*J. Biol. Chem.*, 1922, **53**, 13) for the colorimetric determination of phosphorus is described for the determination of lipoid phosphorus in small amounts of blood. It is time-saving and material-saving, and gives results as accurate as those obtained by other methods now in use. The lipoid phosphorus is converted by hydrolysis with the sulphuric acid present in Briggs's molybdate solution into the inorganic form of orthophosphoric acid, and the blue colour of reduced molybdate is developed at the same time. One c.c. of oxalated blood, serum, or plasma is spread on fat-free filter paper which is cut in strips of $1\frac{1}{4} \times 7$ inches, and then the strips are dried in an electric oven at 50° C. and placed in a Folin sugar tube which contains about 4 c.c. of chloroform. A test-tube, through which water is allowed to siphon, inserted half way into the tube, serves as a condenser. The chloroform is "refluxed" on the water-bath at 75° C. for 3 hours, transferred to a 50 c.c. volumetric flask, diluted with 20 c.c. of distilled water, and 3 c.c. each of molybdate and hydroquinone solutions are added. The flask is placed in the water-bath at 100° C. for 30 minutes, after which time the colour is fully developed, then cooled, and read against the standard. If the chloroform extract is made up to 5 c.c. and an aliquot part taken for the phosphorus determination, cholesterol may be determined on the remaining portion. *Molybdate solution.*—Five per cent. ammonium molybdate in 5 *N* sulphuric acid. In 300 c.c. of water 25 grms. of ammonium molybdate are dissolved, 75 c.c. of concentrated sulphuric acid added, and the whole is diluted with 125 c.c. of water. *Hydroquinone solution.*—Five grms. of hydroquinone and 25 grms. of potassium hydrogen sulphite are dissolved in 500 c.c. of water. *Standard phosphorus solution.*—In 200 c.c. of water 219·3 mgrms. of potassium dihydrogen phosphate are dissolved and made up to a litre. One c.c. of standard solution is equivalent to 0·05 mgrm. of phosphorus.

P. H. P.

New Blood Sugar Method. O. Folin. (*J. Biol. Chem.*, 1928, **77**, 421–430.)—A micro method is described for the determination of sugar in 0·1 c.c. of blood. The author has abandoned the use of copper solutions in this method. The sugar is oxidised with alkaline potassium ferricyanide, and the ferrocyanide produced is measured colorimetrically as Prussian blue. The colour obtained from 0·04 mgrm. of glucose in a 25 c.c. tube is quite as deep as the colour obtained from 0·2 mgrm. of sugar in the Folin and Wu method. Two separate determinations, therefore, can be made on the extract from 0·1 c.c. of normal blood, and several determinations can be made when the extract is obtained from diabetic bloods with high sugar contents. Owing to the difficulties such as evaporation, in obtaining the blood,

very practical 0·1 c.c. " micro blood pipettes " have been made, the filling of which requires neither the force of gravity nor suction. In glass tubes with an internal diameter of 1·0 to 1·7 mm., water will rise by capillary action alone to a vertical height of 1·5 to 2 cm., and 0·1 c.c. will fill such tubes to a length of from 5 to 8 cm. (from the tip). Pipettes made from such tubing can thus be filled automatically to the 0·1 c.c. mark even better than the capillary 0·01 c.c. blood count pipettes are filled, since the speed with which the first named fill is slower, and can be regulated by varying the upward angle at which they are held. These automatic pipettes do not collect air bubbles, and are made to contain (not to deliver) 0·1 c.c. Full experimental details and some results obtained with this method are given. The new method gives unmistakably lower values than the Folin–Wu method. It does not require the use of oxalate or other anti-coagulant, and the Folin–Wu blood sugar tubes are not prescribed. P. H. P.

The Aldehyde Oxidase of the Potato. F. Bernheim. (*Biochem. J.*, 1928, **22**, 344–352.)—It was thought interesting to compare further the properties of potato aldehyde oxidase with those of animal aldehydase, and in the course of the investigation it was found that preparations of the potato enzyme would, under suitable conditions, reduce both nitrate and methylene blue, although Bach (*Biochem. Z.*, 1913, **52**, 412) and Michlin (*Biochem. Z.*, 1927, **185**, 216) were not able to reduce methylene blue with it. It is thus possible to correlate the specificities of the plant and animal aldehydases. A method of preparation of the potato aldehyde oxidase is described, and the properties of the enzyme are studied. The aldehyde oxidase will oxidise all aldehydes, but none of the other hydrogen donators tried. Beside nitrate, methylene blue, Clark's dyes, and quinone are reduced by the enzyme-aldehyde system. The failure of Bach and Michlin to obtain the reduction of methylene blue is explained. Each of their experiments was carried out at such a P_H that reduction would not take place. The P_H-activity curves for the nitrate and methylene blue reductions have been obtained and are given. The action of the enzyme is not dependent on traces of iron. P. H. P.

The Vitamin *B* Content of Malt Extract. A. L. Bacharach and E. Allchorne. (*Biochem. J.*, 1928, **22**, 313–316.)—Experiments have been made on rats to find out whether vitamin *B* (the water-soluble growth-promoting anti-neuritic vitamin complex) is normally present in commercial malt extracts, and, if so, what is the source. A blend of wheat and barley flour, malted and unmalted, was tested, and a malt extract from the flour. The average daily growth per animal per grm. of supplementary ration was almost identical for the unmalted and the malted flour, but considerably greater for the malt extract. Rats on a vitamin *B*-deficient diet were restored to normal growth by 1·3 grms. per day of the malt extract. An " appetite " effect had to be considered since the rats preferred malt extract to the flour, and malted flour to unmalted flour. Those given the flour did not eat all their basal diet. There was no evidence that vitamin *B* was either produced or destroyed during malting. Several brands of commercial malt extract were tested and found to be rich sources of vitamin *B*. The vitamin is derived from the unmalted flour. P. H. P.

***Streptothrix Corallinus* in the Determination of Vitamin B_1. J. Orr-Ewing and V. Reader.** (*Biochem. J.*, 1928, **22**, 440–442.)—A method of obtaining quantitative growth-promoting tests has been developed, which is applicable to the testing of antineuritic concentrates after the charcoal adsorption stage. A method is given for the elimination of erroneous results due to the presence of inhibitory agents such as metals. When testing the more active torulin concentrates (0·1 mgrm. per day pigeon dose), a very considerable difference in growth was noted between flasks which contained 1/20 and 1/40 dose, or again, between those which contained 1/400 and 1/800 dose in 20 c.c. medium. It was then found that, given a fraction, say 1/1000 part, of a torulin concentrate, it was possible to predict the number of doses to be found in the whole. Hence the technique was developed which is capable, with certain limitations, of replacing the pigeon tests in following the fractionation of the vitamin. Different dilutions of test fluid are compared with a series of dilutions of the standard by reading the amount of growth in the flasks after inoculation with *Streptothrix corallinus*, and incubation. Certain anomalous results are obtained with material tested before the charcoal stage.

P. H. P.

Relation of Vitamin *B* to the Growth-Promoting Factor for a *Streptothrix*. R. A. Peters, H. W. Kinnersley, J. Orr-Ewing, and V. Reader. (*Biochem. J.*, 1928, **22**, 445–450.)—An intensive study has been made by parallel tests of two factors, the vitamin *B*, curative for pigeons, and a bacterial growth stimulant for *Streptothrix corallinus*, during the course of numerous fractionations of yeast concentrates. When the pigeon dose is used as a standard, the most active vitamin B_1, preparations (0·027 mgrm.) have the same relative growth-promoting powers as preparations 100 times less pure. Sufficient treatment with alkali always inactivates the curative properties, but the pure extracts after such treatment still retain growth-promoting activity. From this it is concluded that vitamin B_1, and the bacterial growth-promoting factor are not identical. Similarity in constitution is suggested by the fact that during varying types of fractionation (alcoholic, metallic precipitation, etc.) the two properties fractionate in parallel. Since the *Streptothrix* factor is present in the charcoal concentrates, it is not identical with vitamin B_2. Tables showing the parallel *Streptothrix* and pigeon tests are given.

P. H. P.

Bacteriological.

Effect of Alkali Solutions on Bacteria Found in Unwashed Milk Bottles. C. S. Mudge and B. M. Lawler. (*Ind. Eng. Chem.*, 1928, **20**, 378–380.)—At 49° C., a 1 per cent. sodium hydroxide solution destroys all bacteria found in unwashed milk bottle within one minute, whilst a 0·7 per cent. solution of the alkali has the same effect in four minutes. In the case of mixtures of sodium hydroxide and sodium carbonate, the hydroxide appears to be the effective agent.

W. P. S.

Intestinal Bacteria Isolated from Packed Dates. R. F. Hunwicke and G. N. Grinling. (*Lancet*, **214**, 1071–1072.)—Owing to the occurrence of a severe case of colitis in a child who had recently eaten packed dates, various samples of both block dates and packed dates were examined. The block dates all proved free from intestinal bacteria, but six of the seven parcels of dates repacked in fancy boxes contained a bacillus of intestinal type, and the seventh a lactose-fermenting yeast. When dates are repacked in Europe a solution of glucose is sprayed over them. This furnishes a sticky surface to which bacteria would be likely to adhere, and which would presumably form a good pabulum for the growth or preservation of fermentative organisms. It is probable that the dates are handled during packing, and that bacteria are introduced during the process. It seems possible that the intestinal bacteria found indicate faecal contamination, with the concomitant possibility of epidemic dysentery or typhoid fever.

T. H. P.

Toxicological and Forensic.

Carbon Monoxide Poisoning. H. Hartridge. (*Lancet*, 1928, **214**, 1137–1140.)—There is no definitely fatal proportion of carbon monoxide in air, the effect produced being influenced by the length of time during which the air is breathed. Roughly, if the product of the exposure in minutes by the percentage of the oxide in the air is equal to 1, just noticeable symptoms may be observed, these being replaced by severe symptoms if the product amounts to 5. The danger is increased in the case of men performing active work or respiring deeply.

Carbon monoxide occurs in producer gases of various types and in the products of incomplete combustion of gaseous, liquid, and solid fuels. It appears in petrol motor exhausts in amount varying with the degree of completeness of the combustion and with the ratio of fuel to air, and, as is shown in the following table, taken from " Experimental Studies in the Effect of Ethyl Gasoline and its Combustion Products, 1927," it is found in noticeable quantity before this ratio reaches such a value that the free oxygen disappears.

Ratio of air to fuel.	Power developed.	Oxygen. Per Cent.	Carbon Monoxide. Per Cent.
23·9	9·8	7·6	0·0
20·1	15·5	4·8	0·0
18·1	18·1	3·8	0·4
17·2	18·5	2·1	0·1
15·5	19·7	0·6	0·4
14·0	20·0	0·4	2·4
13·5	19·9	0·2	4·0
12·5	19·8	0·1	6·3
11·5	19·8	0·2	8·1
10·9	19·9	0·4	10·6
8·9	20·0	0·6	12·1
8·2	15·1	0·0	15·3

These figures show that the full power of the engine will not be developed

unless there is some carbon monoxide in the exhaust, and that the power will not fall off noticeably until the percentage reaches 15·3. Owing to the expansion of air when heated, a carburettor with jet and air-passages of given sizes will produce a weaker mixture at slow speeds or when the engine is cold, and a carburettor adjusted to give a mixture that fires well and develops nearly full power with a cold engine will give an unnecessarily rich mixture when the engine is hot. Thus, with the average motor-car in adjustment, about 4 to 10 per cent. of carbon monoxide appears in the exhaust, which contains also irrespirable gas, carbon dioxide and, possibly, unburned petrol vapour and therefore produces greater poisonous effects than its carbon monoxide content alone would suggest. (*Cf.* ANALYST, 1928, 349.) T. H. P.

Water Analysis.

Determination of Stable and Unstable Organic Matter in Sewage-Polluted Liquids. W. E. Abbott. (*Ind. Eng. Chem.*, 1928, **20**, 406–407.)—The dissolved oxygen absorbed during the first stage or carbonaceous fermentation of a polluted liquid may be much less than the amount of oxygen absorbed from acid dichromate solution, but in the case of sewages free from suspended solids the first stage oxidation represents the oxidation of most of the soluble carbonaceous matter. With well purified activated-sludge effluents which have been aerated for twenty hours, the first stage oxidation deals with a small fraction only of the organic matter remaining after the purification, whilst in the case of river waters the fraction is larger, but still small, owing to the water having undergone a considerable degree of self-purification. Johnson (ANALYST, 1927, **52**, 130) has stated that the oxygen absorbed from boiling alkaline permanganate solution amounts to about four-fifths of the biological absorption, but the absorption of oxygen from permanganate under these conditions is often considerably less than the quantity absorbed from acid dichromate solution. W. P. S.

Organic Analysis.

New Acrolein Reaction. J. Pritzker. (*Helv. Chim. Acta*, 1928, **11**, 445–448.)—In Powick's modified reaction for acrolein (*cf.* ANALYST, 1923, **48**, 128; 1924, **49**, 188) 2 to 3 drops of acrolein and 1 drop of 3 per cent. hydrogen peroxide are mixed with 5 c.c. of hydrochloric acid (sp. gr. 1·19), the excess of peroxide being removed after 1 minute by a few drops of a 10 per cent. solution of potassium iodide, and the excess of iodine then removed in turn by sodium thiosulphate. The mixture is shaken with 5 c.c. of benzene, the benzene extract washed with water, and then shaken for 1 minute with 5 c.c. of hydrochloric acid (sp. gr. 1·19) and a saturated solution of resorcinol in benzene, when a red-violet colour is produced. A 0·1 per cent. solution of naphthoresorcinol in ether, in place of the resorcinol, gives a green colour. The relation of this and the Kreis reaction to the production of rancidity in fats is discussed, together with the nature of the oxidation product of acrolein responsible for the reaction, and the structure of the compound produced by the condensation of epihydrin aldehyde and phloroglucinol. J. G.

Behaviour of Beeswax towards Trichlorethylene at Ordinary Temperature. G. Buchner. (*Chem. Ztg.*, **32**, 1928, 319.)—Trichlorethylene dissolves beeswax completely when warm, but at ordinary temperatures only dissolves 30 per cent. (ether behaves similarly). The variation in composition between the portions soluble and insoluble in trichlorethylene of one sample were as follows :

	M.pt.	Acid value.	Ester value.	Saponification value.	Ratio of cerotic acid : ester.
Soluble	54·5	3·45	24·36	27·8	1 : 12
Insoluble	67·5	15·6	54·0	69·6	1 : 6·3

Thus the soluble portion contained the greater proportion of the hydrocarbons and only small traces of free wax acids and traces of esters ; the insoluble part contained the greater part of the free wax acids and esters and only small traces of hydrocarbons. The solvent power of trichlorethylene has been tested on a number of waxes, etc., at ordinary temperatures, with the following results :—Easily soluble :—stearic acid, paraffin wax, Japan wax, tallow and pitch ; partly soluble :—beeswax and ghedda-wax ; difficultly soluble :—carnauba wax, montan wax, and ceresin. This property of trichlorethylene may be utilised in analysing mixtures of waxes by allowing 5 c.c. of the solvent to act on 1 grm. of the wax at the ordinary temperature for 24 hours. Under these conditions ordinary beeswax gives a thin slime, beeswax containing 20 per cent. of ghedda wax gives a thick slime, but beeswax containing 20 per cent. of carnauba or montan wax forms a thick slime with the undissolved carnauba or montan wax floating on the surface.

R. F. I.

Solubility of Paraffin Wax in Pure Hydrocarbons. P. Weber and H. L. Dunlap. (*Ind. Eng. Chem.*, 1928, **20**, 383–384.)—In the following table the figures represent grms. of wax dissolved per 100 c.c. of solvent :

Temperature.	Pentane.	Hexane.	Heptane.	Octane.	Isodecane.
0° C.	—	2·77	1·37	0·99	—
5° C.	—	3·69	2·18	1·69	0·94
10° C.	5·11	4·81	3·55	2·90	1·44
15° C.	6·94	6·07	5·06	4·24	2·74
20° C.	9·53	8·31	7·18	5·93	4·98
25° C.	17·16	16·23	14·36	11·66	9·17

The paraffin wax had m.pt. 56° C. and sp. gr. at 20°/4° C., 0·775. W. P. S.

Determination of the Individual Pectic Substances in Nature. D. R. Nanji and A. G. Norman. (*Biochem. J.*, 1928, **22**, 596–604.)—A method for the differential extraction of the individual pectic substances from plant materials, suitable for routine work, is indicated, and the determination of each by an improved method as calcium pectate is described. The principle involved in the determination is that put forward by Carré and Haynes (*Biochem. J.*, 1922, **16**, 60 ; ANALYST, 1922, 263). It consists in the precipitation of the pectin as calcium pectate, after hydrolysis by alkali, and weighing as such. Although rather involved, the method, with due precautions, is accurate. It is proposed eventually

to follow by this method the regional and seasonal changes in some selected types of plant or plants at the same time proceeding with histological investigations on the same material. Results are given in the case of certain leaves, cereal grains and fruits. There are no special outstanding points about the leaves taken. In cereal grains pectic substances are present only to a small extent. The main point of interest in the distribution in the fruits examined is the surprisingly high content of pectates in the rind of the orange and the lemon, and the low content in the pulp of these fruits. The free pectin appears to be distributed through the fruits fairly evenly, the region containing the lowest amount being the pulp. Experiments on the gently-expressed juice show that it contains by no means all of the free pectin, and consequently some of this must be regarded also as forming a part of the cell-wall. P. H. P.

Electrometric Titration of Phenols in Alcoholic Solution. W. D. Treadwell and G. Schwarzenbach. (*Helv. Chim. Acta*, 1928, **11**, 386–405.)—The ionic equilibria of weak organic acids in aqueous and in alcoholic solutions are discussed, and it is shown that numerous phenolic compounds may be titrated with 0·1 *N* sodium ethoxide, in alcoholic solutions, by means of the electrometric titration curves obtained. A platinised platinum and hydrogen electrode is used, and the potassium chloride in the ordinary calomel electrode may be conveniently replaced by an alcoholic solution of lithium chloride. Nitro- and nitroso-groups increase the acid character of a phenol, and in the latter case, as well as for di- and tri-hydroxy anthraquinones, it is necessary to sensitise the electrode by the addition of a drop of hydrochloric acid, the extra amount of alkali required for neutralisation being deducted from the total titration amount. Picric acid and *m*-dinitro-phenol gave unsatisfactory results, owing to depolarisation of the electrode, and in the case of 2.6-dihydroxy anthraquinone only, the two hydroxyl groups were shown as two separate breaks in the titration curve. A table of dissociation constants is given for a number of common phenols and nitro-phenols (titrated in absolute alcohol) and for nitroso-phenols, mono- and poly-hydroxy anthraquinones, and chromazo compounds (titrated in 96 per cent. alcohol), and an attempt is made to correlate the results with the positions of the substituent groups. J. G.

Absolute Essential Oil of Clary. Y. Volmar and A. Germstad. (*J. Pharm. Chim.*, 1928, (8) **7**, 390–395.)—The so-called "absolute" essence of Clary (*Salvia sclarea*), obtained by extraction with volatile solvents, is of a greenish-yellow solid of the consistence of very hard honey. A sample had the following characteristics: Sp. gr., 0·9826; m.pt., 44°–83° C.; solidif. pt., 35°–36° C.; α_D^{18}–4·12°; n_D^{20} (by Féry's refractometer), 1·5038; solubility, 1 c.c. dissolves completely in 0·5 c.c. of 90 per cent. alcohol, in 2·8 c.c. of 70 per cent. alcohol, 1 c.c. of amyl alcohol, 1 c.c. of benzyl alcohol, 0·5 c.c. of methyl alcohol, 0·7 c.c. of chloroform, 1 c.c. of ether, 1 c.c. of ethyl acetate, 12 c.c. of petroleum spirit, 2 c.c. of carbon disulphide. In 1 c.c. of benzene or 2 c.c. of xylene, 1 c.c. of the "essence" dissolves, with formation of a gelatinous mass interlaced with crystals.

It is insoluble in water. The saponification value of the absolute essence was 49·3 ; after acetylation, 81·6 ; acid value, 4·81 ; acetyl value, 36·65 ; dry extract, 73·74 per cent., and volatile substances, 26·21 per cent. A small quantity of acetic acid is present in the essence, and traces of a free unsaturated acid ; 21·8 per cent. of volatile constituents, a small proportion of an ester ($C_7H_{12}O_2$) of a non-saturated acid, 80 per cent. of linalyl acetate, and 20 per cent. of linalol ; a substance having the physical properties of cedrene ; 42·2 per cent. of a crystalline substance, sclareol, and 28 per cent. of a non-crystalline sesquiterpene alcohol, $C_{15}H_{26}O$. The last two substances are not found in the officinal essence, and are characteristic of the absolute essence, of which they form about three-quarters.

D. G. H.

Separation of Phthalic and Homophthalic Acids. H. G. Poole. (*J. Chem. Soc.*, 1928, 1378–1379.)—The mixture is neutralised with sodium hydroxide solution, diluted, and a solution of copper sulphate added in the cold. (If the mixture is boiled a green-blue basic salt is precipitated.) After 12 hours, concentrated hydrochloric acid is added to the filtered precipitate and sufficient boiling water to dissolve it ; 90 per cent. of the homophthalic acid is recovered in a pure state (m.pt. 181° C.) from a mixture with twice the amount of phthalic acid, by crystallisation, since the copper phthalate remains in solution. J. G.

Determination of Volatile Matter in Coke. F. J. Eaton and S. Pexton. (*J. Chem. Soc.*, 1928, 1215–1217.)—The sample is ground to pass a 70-mesh I.M.M. sieve, dried for 1 hour at 125° C. and weighed into a tared platinum crucible which is suspended in an electric furnace by a nichrome wire so that the bottom just touches a thermo-couple. Nitrogen is introduced at the rate of 200 c.c. per minute through a cap sealed by a sand lute, and escapes through the silica tube which carries the thermo-couple. A temperature of 950° ±10° C. should be attained after 1 minute and maintained at 950° ±2° C. for 7 minutes, after which the crucible is re-weighed. The maximum error is 0·05 per cent. and the method compares favourably with the A.S.M.T. or Bone and Silver methods (ANALYST, 1921, **46**, 416), from which it is adapted, since it is rapid, oxidation is avoided, and the temperature of the crucible is accurately shown. J. G.

Direct Determination of Rubber in Soft Vulcanised Rubber. A. R. Kemp, W. S. Bishop and T. U. Lackner. (*Ind. Eng. Chem.*, 1928, **20**, 427–429.)—Two grms. of the sample are extracted successively with acetone and chloroform, and then dried at 70° C. under reduced pressure. This treatment removes resins, free sulphur, oils, waxes, organic accelerators, and mineral rubber. Another portion of the sample is extracted with alcoholic potassium hydroxide solution in order to detect the presence of factice. From 0·7 to 0·1 grm. of the acetone-chloroform extracted sample is then heated at about 160° C. under a reflux condenser with 50 c.c. of tetrachlorethane, the solution is cooled, diluted with 25 c.c. of pure carbon disulphide, 25 c.c. of Wijs solution are added, the flask containing the mixture is placed in ice-water and, after two hours, the excess of iodine is

titrated in the usual way. A control experiment with the reagents, but without the rubber, is made at the same time. Combined sulphur is determined by dissolving 0·5 grm. of the acetone-chloroform extracted sample in tetrachlorethane as described, diluting the solution with carbon tetrachloride to 250 c.c., allowing insoluble matter to settle, evaporating 100 c.c. of the clear liquid, and determining the sulphur in the residue thus obtained. The theoretical iodine value for pure rubber hydrocarbon is 372·8, and the percentage of rubber hydrocarbon in the original sample is found by the equation :

$$\text{Rubber hydrocarbon per cent.} = \frac{\left(\frac{100A}{372{\cdot}8} + 2{\cdot}13B\right)(100 - C)}{100},$$

where A is the iodine value found, B the percentage of combined sulphur in the acetone-chloroform extracted sample, and C the percentage amount of the acetone and chloroform extracts. If the amount of extract obtained by extraction with alcoholic potassium hydroxide solution exceeds 1 per cent., the amount is added the value C in the above equation. W. P. S.

The Reaction between Albumin and Various Metaphosphates. D. Balarew. (*Z. anal. Chem.*, **73**, 1928, 411.)—Four different metaphosphates were prepared : I. Tammann's trimetaphosphate, II. Tammann's tetrametaphosphate, III. that produced by heating sodium ammonium hydrogen phosphate to a dull red heat, and IV. that prepared by dissolving phosphorus pentoxide in water. To 2 c.c. of albumin solution were added 1 c.c. of the metaphosphate solution under examination, together with 2 c.c. of $N/5$ acetic acid. The lowest concentration of the added metaphosphate solution at which the reaction showed clearly was found to be :—I, $N/40$; II, $N/480$; III, $N/2500$; IV, $N/5600$. These observations suggest a method for distinguishing between various pure metaphosphates.

R. F. I.

Chemistry of Jaffe's Reaction for Creatinine. IV. Compound of Creatinine, Picric Acid and Sodium Hydroxide. I. Greenwald. (*J. Biol. Chem.*, 1928, **77**, 539–546.)—When a fairly concentrated solution of creatinine, sodium picrate and sodium hydroxide is acidified with hydrochloric acid, a red tautomer of creatinine picrate is precipitated. With a mixture of barbituric acid solution, sodium picrate and sodium hydroxide the addition of hydrochloric acid does not yield a red precipitate, but the addition of several volumes of alcohol does. The precipitate appears to be a compound of 2 molecules of picric acid, 3 of barbituric acid, 9 of sodium hydroxide, and 1 or 2 of water. When alcohol was added to a mixture or creatinine, sodium picrate and sodium hydroxide, a red precipitate resulted, which could be dissolved in water and reprecipitated without having its composition much altered. The aqueous solution contained a mixture of sodium hydroxide, sodium picrate, creatinine, the new compound and probably at least one other. The substance is an orange, hygroscopic powder which darkens when heated, but does not melt, even at 265° C. When heated at

100° C., or even at 80° C., *in vacuo*, it loses a small quantity of water, and decomposition occurs, since it darkens and gives less picric acid after solution and acidification. The substance is readily soluble in water, forming an orange-red solution. The colour is not nearly deep enough to account for the red colour of Jaffe's reaction. It is slightly intensified by the addition of more sodium picrate and more sodium hydroxide. The full colour value of the creatinine can be obtained only if the substance is first dissolved in dilute acetic acid. Therefore this compound is not the substance responsible for Jaffe's reaction. That may be a similar compound which contains more sodium picrate and more sodium hydroxide. The results of analyses correspond closely with those calculated for a compound which consists of 2 molecules of creatinine, 1 of picric acid, 3 of sodium hydroxide, and 3 of water, but the sodium content is always slightly greater than that calculated. When a solution of the new substance is treated with basic lead acetate solution, a red precipitate is obtained which when filtered out, washed, and dried over sulphuric acid, *in vacuo*, forms a red powder. This appears to be a compound of 2 molecules of creatinine, 1 of picric acid, 3 of lead hydroxide, and 3 of water. The base is present in even greater excess. Both compounds lose nitrogen upon treatment with concentrated acids. No attempt has been made to formulate the manner in which the creatinine, the sodium (or lead) picrate, the sodium (or lead) hydroxide, and the water are combined. It is believed that the tenacity with which the water is held, the destruction of picric acid upon heating, the loss of nitrogen upon treatment with concentrated acids, and the excess of base over that calculated are all of significance. P. H. P.

Inorganic Analysis.

Analytical Chemistry of Beryllium. (*a*) **L. Moser and M. Niessner.** (*Monatsh. für Chem.*, 1927, **48**, 113–121.) (*b*) **L. Moser and J. Singer.** (*Ibid.*, 1927, 673–687.)—(*a*) The published methods for the *separation from aluminium* are criticised. Fischer's tetraoxyanthraquinone reaction (ANALYST, 1928, **53**, 303) was found to be highly sensitive and was used in this investigation. According to the authors, none of the processes advanced for the actual separation of the two elements is really satisfactory (*cf.* ANALYST, 1921, **46**, 359, 437; 1922, **47**, 50). They propose a separation method based on the precipitation of aluminium as the light-brown, voluminous tannin adsorption compound in acetate solution, in which beryllium remains dissolved as a complex acetate. The hot, feebly acid sulphate solution (500 c.c. for less, 600 to 800 c.c. for more, than 0·1 grm. Al_2O_3) is treated with the hot reagent (a cold-saturated solution of ammonium acetate containing 3 grms. of pure tannin per 100 c.c., filtered if necessary) while the liquid is stirred. After 2 minutes' boiling, the precipitate is allowed to settle and complete precipitation tested for with a few drops of the reagent. After cooling, the precipitate is collected on a filter, washed with warm 5 per cent. ammonium acetate solution, dried, ignited in platinum, and evaporated 2 to 3 times with nitric acid. After strong ignition it is weighed as Al_2O_3. If this exceeds 0·06 grm., the voluminous precipitate must be collected by suction in a glass crucible, washed as

before, and dissolved in the covered crucible in nitric acid (1:3). The solution is received in a tall beaker, boiled, and treated with fuming nitric acid until the tannin is completely oxidised, the brown liquid turning colourless ; the solution is precipitated with ammonia in the usual manner. The filtrate from the aluminium precipitate is likewise oxidised with nitric acid, the beryllia precipitated with ammonia, washed with dilute ammonium nitrate, ignited over a blast burner, and weighed. The results quoted are excellent.

(*b*) The only recognised method for the *determination of beryllium* is precipitation of the hydroxide with ammonia and ignition of the precipitate to BeO. a process possessing the same disadvantages as the corresponding procedure for alumina. The solubility of beryllium hydroxide was found to equal 0·002 grm. BeO per litre for water, 0·0045 for aqueous one per cent. ammonia, and 0·003 for (1 per cent. NH_4Cl+0·1 per cent. NH_4OH). Three new gravimetric methods were worked out. (1) *Hydrolysis with ammonium nitrite* : the feebly acid solution (0·1 grm. BeO per 100 c.c.) is cautiously neutralised with sodium carbonate ; any opalescence is removed with a drop of acid. The solution is heated to 70° C., a current of air is passed through it, and 50 c.c. of 6 per cent. ammonium nitrite solution and 20 c.c. methyl alcohol added per 0·1 grm. BeO. The solution gets cloudy in a few minutes ; precipitation is complete after ½ hour's gentle boiling. Another 10 c.c. of methyl alcohol is added ; the solution is filtered after 10 minutes, the precipitate washed with hot water, dried, ignited strongly, and weighed as BeO. The procedure aims at the rapid removal of acid-forming oxides of nitrogen, in the form of methyl nitrite. Beryllium oxide being hygroscopic, the weighing of the crucible in a tared weighing-bottle is recommended. (2) *Precipitation with tannin:* The weakly acid solution (300 to 400 c.c.) free from other metals except alkalis, is boiled with 20 to 30 grms. of ammonium nitrate, and treated with a 10 per cent. solution of tannin (10 times as much tannin as beryllia) followed by ammonia, drop by drop, until precipitation is complete. The bulky precipitate is collected and washed with hot water. If alkali salts are present, the precipitate is dissolved on the filter in a little sulphuric or hydrochloric acid ; the solution is neutralised with ammonia and the precipitation repeated. The dried precipitate is ignited in platinum or silica, treated with a little nitric acid, strongly ignited, and weighed as BeO. The method is especially suitable for minute quantities. (3) *Pyrophosphate method:* To the weakly acid sulphate or nitrate solution are added diammonium phosphate (5 grms.), ammonium nitrate (20 grms.), and 30 c.c. of a cold-saturated ammonium acetate solution. The liquid is boiled and cleared with nitric acid, added drop by drop. The boiling liquid is then titrated with ammonia (2·5 per cent.; 5 to 6 drops per minute). When the finely-crystalline beryllium ammonium phosphate is completely precipitated, the ammonia may be added more quickly until the liquid smells of it. After cooling, a little cold water is added, and enough ammonia to impart a rose tint to added phenolphthalein. When clear, the liquid is poured through a porous porcelain crucible ; the precipitate is washed with hot 5 per cent. ammonium nitrate solution, and ignited in an electric muffle to constant weight. Factors, Be:$Be_2P_2O_7$, 0·0947 ; BeO: $Be_2P_2O_7$, 0·2610.

The *separation of beryllium* by tannin from a number of metals besides aluminium (*v. supra*) is practicable and satisfactory. *From iron*: The neutral solution, containing iron as ferric salt, is treated with ammonium acetate (30 to 40 grms.), ammonium nitrate (20 to 25 grms.), water to a volume of 400 to 500 c.c., and 1·5 c.c. of 80 per cent. acetic acid per 100 c.c. The solution is boiled, stirred, and precipitated completely with 10 per cent. tannin solution. An addition of a few drops of 3 per cent. hydrogen peroxide before precipitation prevents slight reduction to ferrous salt. The precipitate is collected (the filtrate should be yellow-brown, not mauve), washed, dissolved in a few c.c. of hot dilute sulphuric acid, and the filter washed to neutral reaction. The resulting solution is nearly neutralised with ammonia, partial precipitation taking place, and the precipitation repeated as before. The precipitate is left to settle, collected, washed free from sulphate with hot, dilute ammonium nitrate solution, dried, and ignited in porcelain, with subsequent ignition to Fe_2O_3 of constant weight after evaporation with nitric acid. The combined beryllium filtrates are precipitated with excess of ammonia. *From chromium:* The procedure is the same as for iron; the solution should contain 2 per cent. of free acetic acid. Double precipitation is prescribed. *From titanium:* The titanium complex being insoluble at greater acid concentration, a single precipitation suffices. The cold solution is treated with ammonia to incipient cloudiness, ammonium acetate (10 grms.) and nitrate (20 grms.), and 20 to 25 c.c. of acetic acid; it is boiled, stirred, and the titania precipitated with about 10 times its weight of tannin. After short boiling, the red precipitate is collected, washed with 10 per cent. acetic acid containing a little ammonium nitrate, and converted into TiO_2 by the technique applied to the other precipitates. The beryllium is precipitated in the filtrate as tannin complex by neutralisation with ammonia. *From zirconium:* The white tannin precipitate is insoluble in acetic, and even in hydrochloric (1:20) acid. The procedure is the same as for titanium; if the solution has not been neutralised to cloudiness, the precipitation from the boiling liquid is more gradual. A single precipitation is adequate. *From thorium:* The white thorium precipitate is insoluble in 2 to 2·5 per cent. acetic acid. Double precipitation is required, the procedure being the same as for iron. *From tungsten:* The neutral or alkaline (*i.e.* beryllate) solution is diluted to 300 to 500 c.c., treated with ammonium nitrate (30 to 50 grms.), 10 c.c. of sulphuric acid (1:2), and, while boiling, with 10 parts of tannin (calculated on WO_3) in 10 per cent. solution. After 5 minutes' boiling, another 10 grms. of ammonium nitrate are added, boiling being continued for a short time with a view to rendering the precipitate denser. It is washed with sulphuric acid (1:10) containing ammonium nitrate. The filtrate is left for some hours on the water-bath, when a further small quantity of the tungsten complex often deposits (*cf.* ANALYST, 1927, **52**, 505); this is added to the bulk, which is ignited, etc., as before, and weighed as WO_3. The beryllium in the filtrate is precipitated with ammonia. *From vanadium:* The neutral solution of alkaline vanadate and beryllium salt (400 to 500 c.c.) is treated with 20 grms. of ammonium acetate and 30 of nitrate, and 2·5 c.c. of 80 per cent. acetic acid per 100 c.c., boiled, and precipitated with 10 per

cent. tannin solution (10 parts of tannin to one of V_2O_5). After short boiling, the voluminous deep-blue precipitate is filtered hot and washed with 10 per cent. ammonium acetate solution. After ignition and nitric acid-treatment, it is heated till it fuses, and weighed as V_2O_5. The tannin precipitate is insoluble in acetic, but soluble in mineral acids; the filtrate has a green tint, but vanadium cannot be detected therein by known reactions. *From molybdenum:* The separation by tannin is not feasible, the primarily-formed precipitate re-dissolving. On the other hand, beryllium may be precipitated by the above nitrite method, a few drops of ammonia being added after the hydrolysis to prevent the separation of a little molybdic acid during the filtration. The precipitate is washed with very dilute ammonia water containing a little ammonium nitrate. W. R. S.

Ceric Sulphate as a Volumetric Oxidising Agent. I. Preparation and Standardisation of Solutions. Determination of Calcium. H. H. Willard and P. Young. (*J. Amer. Chem. Soc.*, 1928, **50**, 1322–1334.)—A 0·1 *N* solution of ceric sulphate is prepared by dilution of a solution in sulphuric acid (sp. gr. 1·3) of the oxide obtained by ignition of cerium oxalate at 625° C., any insoluble material being filtered from the solution after 1 hour at 80° C. The strength remains constant over a long period, and pure material is unnecessary, since in the presence of other rare earths the oxide is more readily dissolved and the titration is not affected. The reagent is a more active oxidising agent than potassium permanganate in strongly acid solution, it is more stable towards heat, and has only one possible valency change, *i.e.* from $Ce^{\cdots\cdot}$ to $Ce^{\cdots}$. It is standardised against a solution of sodium oxalate in the presence of hot sulphuric, hydrochloric or perchloric acid (maximum amounts 2·5 c.c., 20 c.c., and 30 c.c. of a 73 per cent. solution, in 100 c.c. of liquid, respectively), but nitric acid, which attacks the oxalate, and phosphoric or hydrofluoric acids, which precipitate the cerium, should be absent. The end-point is determined electrometrically with a silver chloride and platinum electrode, or from the change from colourless to yellow, but if the titration is done at room temperature with 10 c.c. of a 0·005 *M* solution of iodine chloride (prepared from 250 c.c. of hydrochloric acid and a solution of 0·279 grm. of potassium iodide with 0·178 grm. of potassium iodate in 250 c.c. of water), a sharp change is obtained when 2 drops of a 0·1 per cent. solution of methylene blue are added within 0·3 c.c. of the end-point. Calcium precipitated as oxalate has been determined by titration, and in the determination of ferrous iron the reverse titration may be made in the presence of nitric acid (*cf.* following abstract and Furman, ANALYST, 1928, **53**, 302). J. G.

Ceric Sulphate as a Volumetric Oxidising Agent. II. Determination of Iron. H. H. Willard and P. Young. (*J. Amer. Chem. Soc.*, 1928, **50**, 1334–1338.)—Iron in ores may be titrated accurately with a standard solution of ceric sulphate (*cf.* preceding abstract) after it has been reduced by stannous chloride and hydrochloric acid, or by aluminium or zinc and sulphuric acid. The end-point may be determined electrometrically in the presence of 10 c.c. of a saturated solution of mercuric chloride, or colorimetrically, with 0·8 c.c. of a 0·1 per cent solution of diphenylamine as internal indicator, in the presence of 15 c.c. of phosphoric acid (sp. gr. 1·37). Arsenious and phosphoric acids do not interfere in this

case (*cf. loc. cit.*), and the consistent negative error of about 1 part per 1000 disappears when the titration is carried out in an atmosphere of carbon dioxide, being attributed to oxidation. It is preferable, however, to standardise the ceric sulphate solution against sodium oxalate (*loc. cit.*), and to apply a small correction factor, or else to use iron of known purity. J. G.

Microchemical Determination of Phosphoric Acid as Strychnine Phosphomolybdate. C. Antoniani and R. B. Jona. (*Giorn. Chim. Ind. Appl.*, 1928, **10**, 203–205.)—An investigation into Embden's method for determining small quantities of phosphoric acid (*Z. physiol. Chem.*, 1921, **113**, 138–145) is described. Two solutions are used : (1) Ammonium molybdate is dissolved in three times its weight of hot water, and the solution filtered if necessary. One volume of this solution is added slowly, by means of a pipette, to three volumes of nitric acid (2 vols. of sp. gr. 1·40+1 vol. of water), with constant stirring. (2) Fifteen grms. of strychnine nitrate are dissolved in 1 litre of water. Just before use, one volume of (2) is added slowly, and with stirring, to three volumes of (1) ; the mixture should be clear. The strychno-molybdic reagent is stirred into the cold phosphate solution, the liquid being left for 30 to 40 minutes, and then filtered through a Gooch crucible, the precipitate being washed first with the reagent diluted to five times its volume with water and then with water alone until all acidity is removed. Multiplication of the weight of precipitate, dried for two hours at 100° C., by 0·0354, gives H_3PO_4, or by 0·0257 gives P_2O_5.

More constant results are obtained if the precipitate is washed with 10 per cent. nitric acid solution instead of with the diluted reagent, and the method is applicable to quantities of P_2O_5, ranging from 1 to 6·3 mgrms. The time of sedimentation of the precipitate is without effect, provided that it is at least 30 to 40 minutes, and the precipitation is not impaired by excess of the reagent, but 6 parts of strychnine per 1 part of P_2O_5 represent optimum conditions. Excess, either of nitric acid beyond that of the reagent, or of other free acid, is harmful. Salts of organic acids, extraneous inorganic salts, and silicic acid are without influence on the results, but arsenic acid behaves similarly to phosphoric acid. Good results are obtainable with nitro-hydrochloric acid extracts or soil or mineral superphosphate, and with sulphuric acid solutions of Thomas slag, the acid solutions being neutralised with sodium hydroxide solution, and then re-acidified with nitric acid to dissolve the separated hydroxides, prior to precipitation with the strychno-molybdic reagent. T. H. P.

Reviews.

FOOD INFECTIONS AND FOOD INTOXICATIONS. By S. R. DAMON. Pp. viii+260, with 18 plates and 13 figures. London: Baillière, Tindall & Cox. 1928. Price 18s. net.

This work, which from its title might appear to be an exhaustive treatise on all forms of food poisoning, has been deliberately restricted by the author to include only those diseases in which "transmission of the infectious or toxic substance takes place, as a rule, *only* through the medium of food." Even when making allowance for this restriction, which places a definite limit on the general utility

of the book, it is difficult to understand why enteric fever should have been entirely omitted, and amoebic dysentery dismissed in one short paragraph, as not coming entirely within the scope of the book.

The book is divided into three main sections, dealing with infections, intoxications, and zoo-parasitic infections, respectively. Of these, the first section is the least good. The bacterial infections are all treated far less fully than the intoxications and parasitic infections, and the paragraphs devoted to the bacteriology of each disease are such that previous knowledge of the subject, or reference to a bacteriological text book, would be necessary for an adequate comprehension of the subject. Many statements are made regarding the transmission of bacterial infections by meat and other foods which are usually eaten after cooking, and one could wish that the author had seen fit to include some data as to the heat resistance of the various bacteria dealt with, and the temperatures reached in the interior of pieces of meat of given size during the ordinary processes of cooking.

The last two sections are dealt with much more fully, and are consequently likely to be more useful. It is rather surprising to find solanin referred to, on page 154, as the active principle of deadly nightshade, but, apart from this, the whole section on the Intoxications is good, and the chemistry of the subject has been dealt with in a comprehensive way. The section on parasitic diseases contains some useful illustrations and tables, but it has been slightly affected by the author's self-imposed restriction.

Apart from one or two places where the punctuation is rather strange, due apparently to the compositor's fondness for commas, the general style of the book is quite good. It should prove a useful book, but it would have been far more useful if it had been made slightly more comprehensive. G. ROCHE LYNCH.
R. M. FRY.

THE MECHANISM OF HOMOGENEOUS ORGANIC REACTIONS FROM THE PHYSICAL-CHEMICAL STANDPOINT. By F. O. RICE, Associate Professor of Chemistry in the Johns Hopkins University. Pp. 217. American Chemical Society Monograph Series. The Chemical Catalogue Company. Inc. New York. 1828. Price $5.00.

In the Chandler Lecture of 1928, Prof. Gomberg summarises our knowledge of organic chemistry as follows:—"In the study of phenomena three questions present themselves in succession. What happens? How does it happen? Why does it happen? In organic chemistry we know the answer to the first question in a number of cases; but our replies to the second are by no means plentiful." Prof. Rice has attempted to collect the answers to the second question, and if his collection of answers will disappoint the average organic chemist, the fault lies not in Prof. Rice's book, but in organic chemistry itself. To appreciate Prof. Rice's work in full it must be read in this spirit; only then does one realise the amount of work and thought involved in it. It is a wonderful collection of facts, full of suggestions, and it should be in the possession of every thinking organic chemist.

M. NIERENSTEIN.

LABORATORY MANUAL OF COLLOID CHEMISTRY. By H. N. HOLMES. Second edition. Pp. xviii. + 228, with 70 figures. Med. 8vo. London: Chapman & Hall. 1928. Price 15s. net.

The first edition was published in 1922 and reviewed in THE ANALYST, 1923, **48**, 49. It contained 127 pages, whereas the new re-written edition has been enlarged to 228 pages. The extended and revised matter deals with sedimentation, electrodialysis and ultra-filtration, surface tension, gels, viscosity and plasticity, and soils. Greater attention has been devoted to adsorption, "the heart of the subject," whilst entirely new chapters have been added on froths and films, proteins, and disperse systems in gases (aerosols). The author's statement, however, that "so much theory has been interspersed between the experiments that the manual is now almost a text as well" is too ambitious.

Although a very wide range of experiments is presented, the book suffers severely from its abundance of abrupt statements. To be of value to a practical student, a sound knowledge of the theoretical side is really essential, and this, apart from the fact that the author directs the student to read various references bearing on the experiments undertaken.

There is evidence of quite careless proof-reading. On p. 10, figure 4 is discussed, but the figure itself lacks the lettering mentioned. In the description of colloid mills (p. 44) the author is at pains to discuss the principle of two rotors, and then illustrates and describes a mill with one rotor and one stator. On p. 57, "emulsoids" is an error for "suspensoids"; "valves" for "values" (p. 105; "thin" for "this" (p. 97); "symph" for "symp" (p. 209); "cardoid" for "cardioid" (p. 211). Again, there is Ph on p. 106, and pH subsequently (p. 125).

In the matter of references no uniformity is apparently attempted. Occasionally the reference is missing altogether (Ferguson, p. 67), or incomplete (Green, p. 68). The references are given indiscriminately in the text or as footnotes (p. 79). In the index, the references under Gibbs (W. E.) include one to Willard Gibbs (p. 90), and omits one (p. 79).

It is unfortunate that the reader will inevitably gain the impression that the book has been hastily compiled, especially as the printing, illustrations and binding are excellent. To the student who has worked through a smaller manual, such as that by Hatschek, and who has a good theoretical knowledge of his subject, the experiments described by Professor Holmes are worth working through. Many have immediate value for the chemist confronted by colloid problems in his technical career. There is no other laboratory manual on colloid chemistry which covers so wide a field as this one, and the price is not excessive. WILLIAM CLAYTON.

TEXTILE MICROSCOPY. By L. G. LAWRIE. Pp. 144, with 44 illustrations and 3 plates. London: E. Benn, Ltd. 1928. Price 25s.

This volume is intended to serve as a guide to the general microscopical technique necessary for the examination of textile fibres and provides instruction suitable for the student of the subject who has little or no knowledge of microscopy. Such a volume cannot be expected to contain much original matter, nor the results of recent research, and from this point of view the title is somewhat misleading, being far too comprehensive. Considerably more than one-half of the book is

devoted to the use of the microscope and its numerous accessories, the remaining 45 pages of text providing descriptions of various reagents, mounting media, stains, etc., and general details for the preparation and mounting of textile fibres as micro sections.

On the whole, the volume is a reliable guide, but considerable importance appears to be attached to the identification of different fibres by various iodine reagents, although experience has shown that the results are greatly modified by the previous chemical and mechanical treatment of the textile under examination. In this connection it is noticeable that no reference is made to some hydrocelluloses and other substances which resemble starch in yielding a blue colour on the addition of iodine in potassium iodide solution. Some confusion in the mind of the reader will be produced by the antagonistic statements regarding the use of the draw-tube on pp. 17 and 21, and no indication is given of how the exact tube-length for cover-glass correction is ascertained, although this is of extreme importance when the best definition is desired. Considerable inaccuracy will result if the method given on p. 75 for calibration of the fine adjustment head is adopted, since no correction is made for the refractive index of the glass slip. The above appear to be the only serious defects in the volume, but others of minor importance occur. In Fig. 2, depicting the path of light rays through the microscope, the rays appear to be focussed on the lower surface of the condenser and within the objective, instead of on the object (Ob), as described in the text. An object illuminated by dark ground illumination is seen principally by refracted light, and not "by light reflected from its surface," as stated on p. 36.

In spite of these defects, however, the volume contains much accurate information, and is well adapted for its intended use. It may appear that recent more specialised and frequently adopted methods are neglected, but sufficient is included to give the student a sound basic knowledge of the microscope and microscopical methods. Chapter VI, dealing with the "Reproduction of Observations," by freehand drawing, tracing and by the camera, contains a well-balanced discussion on the merits of the three methods, and throughout the text is lucid and legible. The illustrations both in the text and on the plates are excellent, and the comprehensive index is accurate, but for a volume of this size and nature the price appears excessive. T. J. WARD.

ICE CREAM. By G. D. TURNBOW and L. A. RAFFETTO. Pp. ix.+407. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. Price 20s. net.

The *raison d'ètre* for this volume is given in the Preface "to supply the students in the Dairy Industry with a complete and modern text-book; and to give the practical man in the commercial plant a ready reference work." There is also a statement that "While some of the subject-matter may appear technical, it is difficult to cover many of the newer phases of ice cream manufacturing without dealing with the subject in this manner." It is good to bear these two extracts in mind when reading this book, otherwise one is liable to become too critical of what is a type of potpourri, and one is likely to think of old English expressions, such as "Jack of all Trades" and "Neither fish, flesh nor good red-herring." The

book is very similar to others which seek to deal in a comprehensive manner with the scientific principles, the bacteriology, the dietetics, the process of manufacture, the engineering, the artistry and the sales organisation of an industry. Certainly in the chapter on bacteriology there is a note that it is only to be taken as supplementary to a course of study—and one wishes the same had been said about the physical chemistry section.

The book is a curious blend of obvious facts, of technical points and of references to research work. There is also a certain carelessness of expression which is to be deplored, *e.g.* on page 164 one reads "such reducing agents as sodium bicarbonate," on page 100 "dextrose" is quoted as "being an invert sugar," on page 101 occurs the expression "Several invert sugars . . ." Unfortunately, however, there are some quite serious mis-statements, *e.g.* on page 87 occurs "a definite relation exists between the fat and solids-not-fat in milk," which is so obviously incorrect that one can only assume the authors meant something else. Again on page 113 the phrase "a liquid without viscosity" is surely a serious error.

The meaning in many cases is obscure. What is meant by (page 106) ". . . a certain amount of water is adsorbed in the mix . . ."? or by (page 104), "The problem of stabilising viscosity to produce an ice cream that is palatable and nutritious offers a large field for research"?

Statements of the obvious are made. For example, on page 129, after quoting figures from a table of results of research work which is so elementary that it need never have been carried out, the statement is made ". . . it is safe to conclude that the gelatin regardless of its P_H changes to that of the ice cream mix." Further, page 129, a table of viscosities of mixes with various butter-fat contents is given, and follows the comment ". . . . it seems that butter-fat exerts an influence upon the viscosity of the mix."

One hardly likes to criticise the chapter on Viscosity and Surface Tension, as it gives the impression, probably through the wish to make the subject understandable by the public for whom the book is prepared, that the authors are under misapprehension as to the subject. For example, on page 114, occurs "In the whipping of cream the oil-in-water emulsion is changed to an oil-in-air emulsion," and on page 133, "Increasing the dispersed phases makes the continuous phase, separating the fat globules, thicker, and raises the surface tension."

The chapter on the analysis of ice cream and dairy products is, on the whole, satisfactory, although not entirely adequate to the scientific control of ice cream manufacture; thus the question of the determination of sugars is not dealt with at all, and the method given for the jell-strength of gelatin is in its preliminary precaution very elaborate in relation to the actual determination itself.

One is particularly pleased to see the value of extreme care in the sanitation of plant and factory emphasised, and it would be good if all manufacturers of ice cream would take the advice to heart.

The chapters devoted to the actual manufacturing of the various ingredients used in ice cream are of considerable value, and from this point of view the book is to be commended.

The book has few typographical mistakes, and (one is not called upon to criticise form) is decidedly readable.

Finally, one can sum up by saying: For the technical man who will skip the scientific matter, the book is good; for the student, not too concerned with the scientific bases, the book is passable; for the scientist with a knowledge of the subject, the book is boring and annoying in many details. L. H. LAMPITT.

ANNUAL REPORTS OF THE PROGRESS OF APPLIED CHEMISTRY. Vol. XII, 1927. Published by the Society of Chemical Industry. Pp. 743. Price to members 7s. 6d., to non-members 12s. 6d.

The Annual Reports of Applied Chemistry published under the auspices of the Society of Chemical Industry are so well compiled and so full of information that no laboratory can afford to be without them. The chemist who has neither the time nor the facilities for perusing the large amount of chemical literature being published at the present time may be almost certain that reference will be given to any work of importance, either in these volumes or in the Annual Reports of the Chemical Society, with such details as may be required to fit it into a complete outline of chemical progress.

The section on Explosives has been omitted this year, but the volume is of approximately the same size as the last edition. The analyst will find much of interest in most sections, whether dealing with foods, leather, soil or even glass, the only parts which do not introduce points of analytical interest being the metallurgical sections.

The desirability of standardising methods of analysis is mentioned on several pages, although the difficulties connected with procedure are fully recognised. In this connection tribute is paid to the work carried out by the Milk Products Sub-Committee of this Society, and also to the work of Kent-Jones and Herd on the extraction of gluten from flour, the results of which were published in THE ANALYST. Reference is made to the investigation of milk solids carried out at the Midland Agricultural College, when it was shown that, of fifteen herds, only four gave no samples below the standard for solids-not-fat.

It is pointed out that there is no hope of obtaining reliable chemical methods for the determination of vitamins in the near future; even the arsenic or antimony chloride tests may not always be reliable, owing to the presence of carotin or xanthophyll.

In connection with food preservation it is mentioned that fresh fruit may be preserved in an aqueous solution containing 0·08–0·1 per cent. of sulphur dioxide, and jam from such fruit is stated to be superior to that made from pulp. The ideal method of preservation, however, is to omit sulphur dioxide, and Matzka has patented electrolytic methods for dealing with fruit juice and egg products.

In the section on the fermentation industries an interesting reference is made to the use of "vaccines" for the protection of wines against secondary fermentation, and in the section on oils, fats and waxes, mention is made of a Spanish Royal Decree which defines Castile soap and gives analytical constants to which the fatty acids must conform.

These reports show an abundance of steady progress and are, in themselves, indicative of the spirit of team work, to which reference is made above.

T. MCLACHLAN.