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The Determination of the Milk Proteins.

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Pedler Research Scholar of the Institute of Chemistry, 1928–1930.

(*Read at the Meeting, November* 5, 1930.)

II. THE IDENTITY OF THE CASEIN PRECIPITATE.

Introduction.—Recent extensive experiments which have been carried out by Linderstrom-Lang[1] have raised the question of whether casein is one substance or several. By heating casein with various solvents (60 per cent. alcohol at about 60° C. with or without $N/1000$ HCl, and also dilute HCl-NaCl solution) he has been able to separate it into fractions differing in various respects, notably in their phosphorus content. The interpretation to be placed upon these experiments depends upon whether the effect of the solvent is truly and only physical, or whether it is accompanied by some slight chemical action between the protein and the solvent. It is quite to be expected that in a substance like casein, with a molecular weight in the vicinity of 200,000,[2a] or even more,[3] there may be quite large different groups that are held together in the molecule by comparatively weak linkages, so that they are capable of being separated by mild treatment. Only if the solvent applied to casein acts in a purely physical manner can it be correct to say that casein is not homo-molecular. Linderstrom-Lang admits that "It would seem as if no especial clearness has been brought by the results obtained, which instead of simplifying the chemistry of casein apparently complicate it"; but he also summarises his results thus: "Casein is a hetero-molecular substance, a more or less homogeneous mixture of several sorts of

colloidal molecules, components which *on account of mutual interaction* follow along through the processes to which casein is, in general, subjected, and thereby *form a co-precipitation system.*" (I have italicised two phrases.) The "mutual interaction" may quite possibly be of the nature of residual valencies or bonds, which, it has been suggested, play an important part in linking together portions of the large molecules of proteins. The large number of –CO.NH– groups which are supposed to be present in proteins could very easily be the sites of such residual bonds, which might be readily broken by comparatively mild chemical treatment. Subsequent experiments may confirm the idea that casein is hetero-molecular, but the experiments so far carried out seem to me to come short of proving it. In the absence of proof, the important thing to remember, for the present purpose, is that casein certainly does form a very definite co-precipitation system.

The foregoing discussion draws attention to the desirability of producing evidence that the material precipitated from milk by the proposed analytical method is really casein and nothing but casein. The problem thus raised of the composition and characterisation of a genuine protein substance is not a simple one, a fact which was emphasised not many years ago by one of the foremost modern protein chemists, S. P. L. Sörensen.[4]

FUNDAMENTAL CONSIDERATIONS.—One physical property of proteins that is of especial value here is their isoelectric point. In a review of the "Physical Chemistry of the Proteins," Cohn[2b] wrote: "The concept of the isoelectric point has a two-fold origin. Born of colloidal chemistry, it has acquired a theoretical significance and a mathematical definition in terms of electrolytic dissociation"; and "The minimum solubility should coincide with the isoelectric point on the simplest assumptions that can be made."

Casein is a protein which is typically insoluble at its isoelectric point, and it is to be separated in milk from two other proteins which are typically soluble, even at their isoelectric points, in salt solutions such as exist in milk. Cohn later (p. 402) says of casein, "The precipitation of this protein at this point is remarkable, and its purification depended upon its relative insolubility under these conditions long before the theory of the isoelectric point had been developed." The analytical proposal, first made by Waterman, to precipitate the casein from milk at a *p*H as close as possible to its isoelectric point, has thus an obvious fundamental basis, and one might even go so far as to define casein as the material which is precipitated from milk at this particular *p*H (4·6) by means of acetic acid and sodium acetate. The necessity for specifying the buffer to be used is obvious from the experiments of Michaelis.[5]

Before discussing methods of investigating the casein precipitate, some consideration may, with advantage, be devoted to the precise nature of the problem. The improved analytical method which I have evolved gives figures which are usually about 1–2 per cent. higher than those obtained by the use of acetic acid alone. The additional material which is thus precipitated must be either casein or one of the other milk proteins. If it is casein, then the protein precipitated at

*p*H 4·6, and at *p*H 4·2 must have the same chemical properties. If the higher values obtained at *p*H 4·6 are due to contamination with, say, lactalbumin, then methods, which are incapable of showing differences due to as little as 1–2 per cent. of the contamination, cannot be used. For this reason several possible methods must be ruled out.

Experimental.

Investigation of the phosphorus-nitrogen ratio of the precipitate at first sight appears attractive, but it is complicated by the fact that very great difficulty has always been found in removing from a casein precipitate the last traces of inorganic calcium and phosphorus which are combined with it in the milk. When casein is precipitated at different H-ion concentrations it is not to be expected that the inorganic impurities will be removed to the same extent, and so a factor will be introduced which will interfere with the value of phosphorus determinations. When some analyses were carried out, this expectation was confirmed.

The method of dissolving the casein and reprecipitating it was suggested as a possible means of discovering whether the precipitate was contaminated with soluble protein material. When this was tried by dissolving the casein in dilute sodium hydroxide solution, lower amounts of re-precipitated protein were certainly obtained, but unfortunately this evidence cannot be relied upon. The reason for these lower values is that the action of dilute alkali has been shown by Carpenter[6] to split off soluble material from the casein. On account of this and other important changes[2c] which are produced in this protein by alkali, it can be quite definitely asserted that after casein has been subjected to even faintly alkaline *p*H values it is no longer the same material as it was before.

A number of difficulties stand in the way of using the specific rotation of alkaline solutions of the casein. Racemisation is liable to occur and alter the values. Some experiments were carried out to see if the method used by Woodman[7] and Wright[8] to identify various proteins by means of their racemisation curves might be used to examine the casein precipitated at different *p*H values. The method was abandoned because of the fact that exceedingly opalescent solutions of casein were obtained, so that the accurate readings required could not be made. The opalescence was, no doubt, due to the presence in the solution of traces of calcium phosphate. To obtain reliable readings it was found essential to have the temperature of the solution in the polarimeter tube very carefully controlled.

Formol Titration Method.—Another method of obtaining a curve which shows the progressive decomposition of the protein by alkali solutions, in a manner similar to the racemisation curve, is that proposed by Abderhalden and Kroner.[9] This consists in measuring the increase of amino-nitrogen by means of the formol titration. For the purpose of the experiment casein precipitated from skim milk at two *p*H values was dissolved in sodium hydroxide solution and made up to a definite volume, so that the strength of alkali in each case was normal. After pipetting portions for Kjeldahl determinations of the amounts of casein dissolved,

the flasks were placed in a thermostat at 37° C., and portions of 10 ml. were removed at intervals for formol titration. For this purpose, the casein solution was neutralised in each case by adding carefully from a pipette the same definite quantity of normal hydrochloric acid, drop by drop, so that precipitation of the casein was avoided. The formol titration was carried out by a modification of the method of Northrop.[10] The solution of formalin (5 ml. containing thymol blue) was added to the neutralised casein solution and the mixture titrated colorimetrically to *p*H 8·8 with *N*/20 sodium hydroxide solution. The figure thus obtained was taken as the basis for the results which appear in the following table:

TABLE I.

Casein precipitated at *p*H 4·14 0·4403 grm. *N* per 100 ml. milk.		Casein precipitated at *p*H 4·61 0·4518 grm. *N* per 100 ml. milk.	
Time from start. Hours.	Amino-*N* in per cent. of total *N*.	Time from start. Hours.	Amino-*N* in per cent. of total *N*.
0	10·35	0	10·2
½	13·6	¼	11·9
1	13·5	0⅕	14·3
1½	14·7	1¼	14·9
2	15·2	1¾	15·25
3	16·0	2⅖	16·2
4⅓	18·35	3⅓	16·5
6	20·0	5	18·4
8	21·0	7	20·0
20¼	26·2	19	25·3
27¾	28·1	27	28·1

When these figures are plotted on a graph the points from both caseins lie close to a single curve. Since Abderhalden and Kroner have shown that different proteins give distinctly different curves, the two samples of casein (which were precipitated from the same skim milk) appear to be identical. The slight deviations of the points from the curve are due to errors of the method. A normal soda solution is neutralised by a normal acid solution before proceeding to titrate with *N*/20 sodium hydroxide solution. An error of 1/200 ml. in the pipetting of either of the two first solutions would give rise to an error of 0·1 ml. in the final titration, or about 0·3 per cent. of amino-nitrogen. Much better results were obtained by neutralising always with the same definite pipetted quantity of acid instead of trying to adjust colorimetrically to *p*H 7·0. The results would probably have been still better if the titrations had been carried out electrometrically.

ALKALINE HYPOBROMITE OXIDATION METHOD.—Goldschmidt and Steigerwald[11] have described a method which enabled them to distinguish clearly between different proteins—gelatin, casein and albumin. The method depends on the fact that, owing to structural differences, the proteins react differently with alkali hypobromite and, as in the racemisation and formol titration methods, distinctly different curves are obtained. Of the three methods the hypobromite is much the easiest to use.

The hypobromite solution was prepared by dissolving 2·66 ml. of bromine in 250 ml. of (approx.) $N/2$ sodium hydroxide solution. To avoid the possibility of this solution reacting with the milk fat, skim milk was used to provide the casein. The casein nitrogen content of the milk was determined by means of acetic acid alone at pH 4·23, and found to be 0·405 grm. of N per 100 ml.; the improved method at pH 4·65 gave 0·410 grm. of N per 100 ml. Portions of 100 ml. of the same milk were precipitated at these two pH values and, after being filtered and washed in the usual way, the casein obtained was used for the experiment. A part of each sample was used to prepare two solutions in such a way that each contained exactly the same amount of casein nitrogen, the solution of which was effected by the addition of 25 ml. of $N/9$ sodium hydroxide solution. The volume was made up to 200 ml., which contained 0·152 grm. of N.

TABLE II.

0·152 grm. casein N and 25 ml. $N/9$ NaOH per 200 ml.				0·247 grm. casein N and 20 ml. $N/9$ NaOH per 200 ml.			
Casein pptd. at pH 4·23.		Casein pptd. at pH 4·65.		Casein pptd. at pH 4·23.		Casein pptd. at pH 4·65.	
Time from start	$N/10$ NaOBr used per grm. N.	Time from start	$N/10$ NaOBr used per grm. N.	Time from start	$N/10$ NaOBr used per grm. N.	Time from start	$N/10$ NaOBr. used per grm. N.
Minutes.	Ml.	Minutes.	Ml.	Minutes.	Ml.	Minutes.	Ml.
2½	53·6	3	62·8	2	57·8	2	61·3
9	90·3	9	97·1	9½	93·5	8½	93·2
18	121·4	20	133	19	121·4	22½	131·3
41½	160·1	53	185·6	37½	154·4	39	160
57½	183·8	78½	213·2	59	183·4	61	187·2
130	245·8	125	246	114	227	119	229
270½	298	272½	304	250	282	264	284
352	322	384	330	354	307	359	307·6

In preliminary trials the reaction was found to proceed very slowly at 0° C., the temperature used by the original workers, so that for the experiment a thermostat at 19° C. was used.

Two 200 ml. dry flasks, each containing a dry 10 ml. pipette, had previously been placed in the thermostat. After using each pipette to determine accurately the strength of the sodium hypobromite solution by pipetting it into excess of acidified potassium iodide solution with $N/10$ thiosulphate, the same pipette was used to transfer 40 ml. of the hypobromite solution into the flask. Ten ml. of water and 25 ml. of $N/9$ sodium hydroxide solution were also added, so that the addition of 25 ml. of the casein solutions made a total volume of 100 ml. At intervals 10 ml. were pipetted from each flask into potassium iodide solution acidified with ample hydrochloric acid, and the unused hypobromite thus estimated. In Table II appear the results obtained from the two casein solutions, together with other figures obtained in exactly the same way from solutions of the same samples of casein, but of different strength—0·247 grm. of casein N dissolved

in 20 ml. $N/9$ of sodium hydroxide solution and made up to 200 ml. When these figures are plotted on a graph two curves are obtained corresponding to the different concentrations of protein and alkali in the solutions used. This and other experiments which I have carried out show that it is not possible to make an accurate comparison between two samples of casein unless the concentrations of protein, alkali and hypobromite are the same in the two reaction mixtures being compared.

This condition is fulfilled in each of the two separate parts of this experiment, but not for all four reaction mixtures, hence the two curves. In the first part of the experiment the values from the two caseins (precipitated at *p*H 4·23 and 4·65) fall on one curve, and in the second part, where the solutions differed in strength from those used in the first part, the values from both caseins fall along another curve. From this it may be concluded that the same protein material is precipitated at both H-ion concentrations.

SUMMARY.—1. Upon the fundamental basis of the conception of the isoelectric point, it has been suggested that casein be defined as the material which is precipitated from cows' milk at *p*H 4·6, by acetic acid buffered by sodium acetate.

2. Two methods have been used to show that casein precipitated in this way is chemically identical with the material precipitated at *p*H 4·2 by acetic acid alone.

(*a*) When the two caseins are digested with normal caustic soda at 37° C., the production of amino-groups, as determined by the increase of the formol titration, takes place at the same rate.

(*b*) The rate of oxidation of the two caseins by sodium hypobromite under comparable conditions is the same for both.

This paper represents part of the work carried out during the author's tenure of the Pedler Research Scholarship of the Institute of Chemistry, the assistance of which and the interest of the members of the Pedler Fund Committee is gratefully acknowledged. I wish to add my appreciation of the facilities made available to me at the National Institute for Research in Dairying, and especially of the valuable advice of Capt. J. Golding, Head of the Chemical Department.

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(Part III, "Proposed Modified Method for Casein" will be published in the March issue.)

The Hydroxylamine Method for the Determination of Ketones.

Carvone in Caraway and Dill Oils.

By C. T. BENNETT, B.Sc., F.I.C., and T. TUSTING COCKING, F.I.C.

The method described by Bennett and Salamon (Analyst, 1927, **52**, 693) has been applied by us to several aldehyde- and ketone-containing oils with a view to its adoption as a general method. Our experiments show that a uniform method is not applicable in all cases, and that the method requires modification according to the particular ketone present.

In the case of aldehyde-containing oils, the modified method recommended by the Essential Oil Sub-Committee for the determination of citral in oil of lemon (Analyst, 1930, **55**, 109) was found to be of almost universal application, provided that a little benzene was added as a solvent for the non-aldehydic portion of the oil.

This modified method was found to be quite unsuitable for the determination of ketones; the reaction took place very slowly, and did not proceed to completion, even after many hours' contact with the reagent.

The reagent in this case was $N/2$ hydroxylamine hydrochloride solution in 60 per cent. alcohol; a small excess only was used, and the reaction was carried out in the cold, with continuous shaking.

In order to hasten the reaction, the use of a stronger alcohol (80 per cent.), as in the method used by Bennett and Salamon, was tried as a solvent; in alcohol of this strength the oil was soluble, and distinctly better results were obtained, but the reaction was still too slow, taking about 24 hours for completion. Methyl orange, the indicator recommended in the case of oil of lemon, where 60 per cent. alcohol is the solvent, is unsuitable in stronger alcohol, and dimethyl yellow (dimethyl-amino-azo-benzene) was used instead. This has the same colour change and works over the same pH range as methyl orange.

The experiments with the 80 per cent. alcoholic solutions were repeated, the determinations being made at a temperature of about 70° C., and a vast improvement resulted. The reaction was complete in about 60 minutes, and the end-point was fairly distinct, but it was found somewhat difficult to get concordant results with different operators.

The following results were obtained on several ketone-containing oils by varying the conditions:

THE DETERMINATION OF KETONES BY HYDROXYLAMINE.

DILL OIL.

By sodium sulphite method,
57 per cent. by volume = 60·3 per cent. by weight of carvone
„ cold hydroxylamine method, 30 minutes = 45·5 „ „ „ „ „ „
60 minutes = 52·8 „ „ „ „ „ „
18 hours = 57·2 „ „ „ „ „ „
24 hours = 58·7 „ „ „ „ „ „
„ hydroxylamine method,
heated to 70° for 30 minutes = 58·2 „ „ „ „ „ „

CARAWAY OIL.

By sodium sulphite method,
57 per cent. by volume = 60·3 per cent. by weight of carvone
„ cold hydroxylamine method, 30 minutes = 44·6 „ „ „ „ „ „
60 minutes = 51·8 „ „ „ „ „ „
18 hours = 55·8 „ „ „ „ „ „
24 hours = 56·8 „ „ „ „ „ „
„ hydroxylamine method,
heated to 70° C. for 30 minutes = 56·8 „ „ „ „ „ „

PENNYROYAL.

By sodium sulphite method, 84 per cent. by volume (4 hours) = 83 per cent. by weight
„ cold hydroxylamine method after 2 days = 82·4 „ „ „ „
„ hydroxylamine method boiling for 1 hour (under reflux) = 87·1 „ „ „ „
„ hydroxylamine method, heated to 70° C. for 90 minutes = 86·5 „ „ „ „

PIPERITONE.

1. By sodium sulphite method (1 hour in water-bath) = 97 per cent. by volume
2. By hydroxylamine method, 70° C. 1 hour = 75·4 „ „ „ „
1½ hours = 91·9 „ „ „ „
2 hours = 97·7 „ „ „ „

After numerous experiments it was found that more uniformity was obtained when a still stronger alcohol (90 per cent.) and a stronger reagent were used, and when a larger excess of the reagent was present. Eventually the conditions for the determination of carvone in the oils of caraway and dill were standardised, and concordant results, agreeing well with those obtained by neutral sulphite absorption, were obtained.

The method finally adopted is as follows: About 1·5 grms. of the oil are weighed into each of two stoppered tubes, and to each tube are added 10 c.c. of approximately *N* hydroxylamine hydrochloride reagent, and the tubes are placed in a beaker of water on a boiling water-bath, the temperature rising to about 75° C. The colour of the solutions changes from yellow to red owing to liberated hydrochloric acid, and at 5 minute intervals the tubes are removed from the water-bath,

and neutralised with *N* alcoholic potash contained in two separate burettes. As the reaction proceeds it becomes slower, and towards the end the indicator does not become red, but only a golden orange, and the exact end-point is a little difficult to judge without practice. When it is thought that the end has been reached, the readings of the two burettes should be taken, and then to one of the tubes an additional 0·5 c.c. of the alcoholic potash should be added. This titration is now definitely over-run and the full colour of the indicator is developed. If this colour is a paler yellow than that of the other tube, it is evident that this has not been titrated to the full yellow colour, and the titration is continued until it is exactly the same colour as that in the tube with the excess of alkali. The calculation of the result is based on this latter titration. The reaction is complete in about 35 to 40 minutes.

During the titration a certain amount of potassium chloride is precipitated, but this does not interfere with the result nor make the end-point more difficult to see.

The reagent is prepared by dissolving 6·95 grms. of pure hydroxylamine hydrochloride in 95 c.c. of 90 per cent. alcohol, adding 0·4 c.c. of a 0·2 per cent. solution of dimethyl yellow and sufficient *N* alcoholic potash to bring the reaction of the reagent to the *full* yellow colour of the indicator.

This adjustment is best carried out by dividing the solution into two portions and adding alcoholic potash to each in turn until the last drop produces no change in colour; about 0·8 c.c. is required. The addition of the alkali produces a turbidity due to precipitation of potassium chloride, but, on shaking, this slowly redissolves. As the reaction of the reagent, and of the solution at the end of the test, is more alkaline (or, rather, less acid) than pure hydroxylamine hydrochloride, it is necessary to introduce a small factor as a correction.

This is accomplished by multiplying the theoretical figure by 1·008. 1 c.c. of *N* potassium hydroxide solution is therefore equivalent to $0{\cdot}1501 \times 1{\cdot}008$ grm.$=$ 0·1513 grms. of carvone.

Results of Determinations of Carvone in Caraway Oil.

By neutral sulphite and acetic acid 54·5 per cent. (v/v) = 57·8 per cent. (w/w).

By *N*/2 hydroxylamine reagent in 90 per cent. alcohol.

2·5 c.c. excess reagent used, 2 hours, temperature about 75° C., 57·1 per cent.
,, ,, ,, ,, 58·4 ,, ,,
,, ,, ,, ,, 58·4 ,, ,,

By the method finally adopted, using 10 c.c. of *N* hydroxylamine reagent in 90 per cent. alcohol.

Equivalent to approximately 100 per cent. excess, 50 minutes 58·9 per cent.
58·5 ,, ,,
,, ,, ,, ,, 40 minutes 58·7 ,, ,,
57·6 ,, ,,
,, ,, ,, ,, 29 minutes 58·2 ,, ,,

Further determinations carried out by different observers 58·2 per cent.
58·4 ,, ,,
58·4 ,, ,,
58·5 ,, ,,
58·7 ,, ,,
58·9 ,, ,,

The accuracy of the results was confirmed by determinations carried out on pure carvone. Using 10 c.c. of the reagent and about 0·8 grm. of the carvone, the results obtained were 99·3, 99·8, 99·9, and 100·3 per cent.

On a mixture of the above pure carvone and a pure terpene, the method gave results within $\pm$0·2 per cent. of the calculated figure.

The advantages of this method over the neutral sulphite method are several:—(1) Only a small quantity of the oil is required. (2) Duplicate determinations are carried out at the same time. (3) The result can be obtained in less than an hour.

The method has been applied to the determination of ketones in other oils; menthone in oil of peppermint, and pulegone in oil of pennyroyal. In both these cases the end-point was found to be difficult to see, owing to the darkening of the oil itself, when heated with the reagent, obscuring the colour-change of the indicator. Instead of a pure yellow colour being obtained at the end, the colour was golden orange, and the result was uncertain to the extent of several per cent. It is hoped that further research will result in improvements and modifications of this useful method whereby it may be applied to all ketone-containing oils.

The experimental work in connection with the above has been carried out in the laboratories of The British Drug Houses, Ltd., and Wright, Layman & Umney, Ltd., and we are indebted to our Directors for permission to publish our results.

The Lead Reduction Method for the Volumetric Determination of Tin and the Interference with it by Copper and Antimony.*

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

(*Read at the Meeting.*)

This paper contains the results of an investigation of the determination of tin by reduction from the stannic condition by means of lead and direct titration with iodine in presence of the lead, a process which was first introduced by A. R. Powell

* Communication from the Research Department, Woolwich.

in 1918. It is shown that by keeping the solution during the reduction and titration in an atmosphere of carbon dioxide, for which purpose an apparatus already described by B. S. Evans was used, quantitative results can be obtained when the amount of tin is calculated from the absolute amount of iodine used. This is the case even with small amounts of tin down to 0·5 mgrm., which is the practical lower limit when 0·01 *N* iodine is used, no modification of the standard process used for the higher amounts being required beyond the use of the more dilute iodine solution as the titrating liquid. The investigation was extended to cover the effects on this method for tin, of copper and antimony, which have been generally supposed to have no appreciable influence. The effect of copper, however, is profound, the results for tin becoming markedly lower in proportion as the copper is increased, and this is due to the precipitation of tin in the form, it is believed, of a compound with copper. The effect of antimony is less marked, but distinct.

REDUCING AND OXIDISING AGENTS.—Innumerable methods have been published based on the reduction of stannic tin by metals followed by an oxidising titration; almost all of the common metals have been advocated as reducing agents by one author or another, and the same is true of iodine, ferric chloride, etc., as oxidising agents. Several authors have recently drawn attention to an inherent drawback to the majority of these methods (Evans, ANALYST, 1927, **52**, 590; Willey, *J. Soc. Chem. Ind.*, 1924, **43**, 70T; Boller, *Diss.*, Zurich, 1915, quoted by Sandved, ANALYST, 1927, **52**, 2), namely, that they do not admit of using the theoretical factor for calculating tin from the amount of oxidising agent (generally iodine) used, but rely on special factors found by reducing and titrating known amounts of tin under similar conditions. These authors share the view, which is supported by experimental evidence, that one of the most important conditions for the complete reduction of tin is that air shall be rigorously excluded from the test solution during the reduction. Inaccuracy due to the tin in the sample and in the standard not being reduced to the same extent would be at a minimum when these methods are used by skilled workers who can carry out determinations under strictly reproducible conditions, but is apt to be pronounced when determinations are only carried out occasionally, as, for example, in certain "umpire" analyses.

LEAD AS REDUCING AGENT.—Metallic lead would seem to offer advantages over practically every other metallic reducing agent suggested for tin, by reason of the great purity of the commercial metal, thus avoiding "blanks,"* very often associated with metals like iron and nickel, and also because it does not introduce any coloured products into the solution to affect the delicacy of the end-point; neither does it need to be withdrawn from the solution before the titration with iodine, the end-point of which is sharp and reasonably stable (Powell, *J. Soc. Chem. Ind.*, 1918, **37**, 285T). Furthermore, lead is free from the erratic behaviour in reducing properties met with in the case of zinc, which is apparently due to the free

* The lead used in the present work gave a "blank" of 0·3 c.c. of 0·01 *N* iodine, which is of significance only in the determination of the smaller quantities of tin.

reducing surface becoming protected by a layer of spongy tin (*cf.* Sandved, ANALYST, **1927, 52**, 2, and authors he cites).

It was, therefore, decided to use Powell's lead reduction in a modified method in which air could be rigorously excluded during the reduction and titration. As has been mentioned, several authors have suggested apparatus for this purpose, but no apparatus seemed so simple or suitable as that described by Evans (ANALYST, **1927, 52**, 570). It consists of a conical flask of 750 c.c. capacity having a rather wide neck into which fits a rubber stopper carrying the following attachments: (*a*) Leading tube, from a Kipp's apparatus delivering carbon dioxide, passing down to about $\frac{1}{2}$-inch above the surface of the liquid which is to be reduced in the flask (it is preferable that the lower part of this tube should be bent towards the wall of the flask, so that it is well clear from the drops of iodine during the titration); (*b*) a small tapped funnel with its stem bent twice, so that the bulb of the funnel clears the top of the rubber stopper (to allow of the jet of a burette being inserted in the third hole); (*c*) a removable glass plug fitting into the third hole in the stopper.

METHOD.—The following method was adopted for the determination of tin: The solution to be analysed, which should contain the equivalent of 50 c.c. of concentrated hydrochloric acid, is transferred to the 750 c.c. flask, diluted to approximately 250 c.c., and a strip of stout lead foil, $12'' \times 1''$, added.* The stopper carrying the attachments is placed in position. The tap of the funnel being open, the liquid is boiled gently for $1\frac{1}{2}$ hours (at such a rate that the volume is not reduced to less than 100 c.c. during this time), a stream of carbon dioxide (which has bubbled through water and is free from air) being passed into the flask meanwhile. It is advisable that the flask should be protected from draughts during the boiling, to avoid the possibility of air being drawn into the flask. Quite a slow stream of carbon dioxide is all that is necessary, particularly as a certain volume of liquid collects in the tap funnel and helps to prevent back diffusion of air.

At the end of the specified time the flask is removed from the heat, the current of carbon dioxide being simultaneously turned full on. The tap of the funnel is now closed, and the flask removed to a cooling bath and allowed to remain under pressure of the gas from the Kipp's apparatus until quite cold. Sufficient starch indicator is poured into the funnel. The glass plug is withdrawn from the stopper and the starch solution is run into the flask, the tap of the funnel being closed before the funnel is quite empty. The jet of a burette containing iodine is now inserted into the hole formerly occupied by the glass plug (the jet must make a tight joint with the stopper, and must be long enough to project below it). The interior of the flask is now under pressure from the Kipp's apparatus, so that before iodine can be run into the flask the pressure must be released by turning off the carbon dioxide supply and opening the tap of the funnel for a moment. The titration is now proceeded with up to the starch blue end-point. For amounts of tin below 10 to 15 mgrms., 0·01 *N* iodine solution is used. One c.c. of 0·1 *N* iodine = 0·00594 grm. of tin; 1 c.c. of 0·01 *N* iodine = 0·000594 grm. of tin.

* Powell used granulated lead.

TESTS OF THE PROCESS.—For the larger quantities of tin, known weights of metal were dissolved in warm hydrochloric acid (50 c.c. of sp. gr. 1·18), with sufficient bromine to impart a yellow colour to the final solution, which was diluted to 250 c.c. before reduction. Smaller quantities of tin were taken in the form of measured amounts of a standard solution, which were added to hydrochloric acid and water, making the conditions the same as for the larger quantities of tin. The process was carried out as described and the following results were obtained:

TABLE I.

Tin taken. Grm.	Iodine required. c.c.		Tin found. Grm.
0·2025	34·00	0·1 *N*	0·2020
0·1022	17·15	,,	0·1019
0·0521	8·80	,,	0·0523
0·0220	3·70	,,	0·0220
0·0103	1·75	,,	0·0104
0·0100	16·8	0·01 *N*	0·0100
0·0050	8·1	,,	0·0048
0·0030	4·95	,,	0·0029
0·0020	3·2	,,	0·0019
0·0010	1·65	,,	0·00096
0·0005	0·85	,,	0·00051

(A blank of 0·3 c.c. of 0·01 *N* iodine was deducted from the total titration amount in the last six of the above experiments.)

The iodine solution used for the titration was standardised with arsenious oxide.

The time taken for the reduction is somewhat longer than is usually specified for reduction by lead; but the time cannot be shortened in this method with safety. For example, with a 40-minute period of reduction (timed after some bromine which the liquid contained had become reduced) a titration registered 0·0093 grm. of tin, instead of 0·0123 grm. which the solution actually contained. It is possible that reduction would be more rapid in a solution of greater acid concentration than that used in the present method; a more strongly acid liquid would, however, be prone to attack the rubber stopper, and a further disadvantage would be the need for diluting the reduced solution, before titrating, with oxygen-free water, since it has been noticed that the end-point is the less stable the higher the acidity of the solution, indicating a certain interaction between the lead strip and iodine in presence of much acid.

The lead reduction method for tin is generally regarded as being very little subject to interference by, at any rate, those metals commonly met with in non-ferrous analysis. Indeed, I have not seen any statements in the literature to contradict the notion that the method is suitable for the direct determination of tin in non-ferrous metals containing appreciable amounts of copper or antimony.

Prior to the experimental work described in this paper, the practice in this laboratory was to separate copper before determining tin, mainly on account of a belief that copper which would collect on the lead during the reduction process may reduce the efficiency of the reduction at the lead surface.

THE INTERFERENCE OF COPPER.—A noticeable effect occurs when copper is precipitated on lead from a boiling solution of hydrochloric acid containing stannic chloride. In the absence of tin in the solution, copper precipitates on the lead as a red metallic film which separates as brownish spongy masses when the amount of copper is large. In the presence of tin, there may be a momentary coating of the lead by the red film, but this rapidly turns black and becomes detached from the lead in a black flocculent form when appreciable amounts of copper and tin are present in the solution.

A series of determinations of tin was carried out by the method just described upon solutions containing known amounts of tin and copper, and the following results were obtained:

TABLE II.

	Taken.		Titration.		Found.	
	Tin.	Copper.		Iodine.	Tin.	Tin precipitated (by diff.).
	Grm.	Grm.	c.c.		Grm.	Grm.
(1)	0·2000	Nil	34·00	0·0990 *N*	0·1999	—
(2)	0·2000	0·01	33·15	,,	0·1949	0·0053
(3)	0·2000	0·05	29·10	,,	0·1711	0·0289
(4)	0·2000	0·10	24·90	,,	0·1464	0·0536
(5)	0·2000	0·20	15·10	,,	0·0888	0·1112
(6)	0·2000	0·40	1·65	,,	0·0097	0·1903
(7)	Nil	0·20	less than 0·05	,,	—	—
(8)	0·0103	Nil	17·6	0·0099 *N*	0·0103	—
(9)	0·0103	0·0010	16·9	,,	0·0099	0·0004
(10)	0·0103	0·0050	12·7	,,	0·0075	0·0028
(11)	0·0103	0·010	8·2	,,	0·0048	0·0055

A unit weight of copper causes the loss, roughly, of half a unit weight of tin. The effect of copper in the lead reduction method for tin is, therefore, to cause the results for tin to be low in direct proportion to the amount of copper present.

In experiments (3) to (6) the major portion of the deposit flaked off the lead and became suspended in the solution in a flocculent form. After the titration, the suspended matter was filtered off and washed with water. The precipitates from experiments (3), (4) and (6) were tested qualitatively by dissolving them in concentrated nitric acid; blue solutions were produced containing heavy white precipitates which were identified as a tin hydroxide. The proportion of copper to tin in the precipitate from experiment (5) was determined as follows: It was washed from the filter with water, nitric and sulphuric acids were added, and the liquid evaporated until fumes of sulphuric acid appeared; on treatment of the residue with cold water a clear solution was produced, which remained clear and

free from deposit after being kept several hours, showing that it contained no appreciable amount of lead; the solution was electrolysed for copper, and the tin was precipitated from the copper-free solution as sulphide, which was ignited and weighed as stannic oxide. Found: Copper, 0·1700 grm. (63·63 per cent.); tin, 0·0972 grm. (36·37 per cent.). (These weights are, of course, less than those appearing for Expt. (5) in the table, owing to a part of the metals remaining adherent to the lead strip.) The deposits obtained with the smaller amounts of tin and copper (Expts. (8) to (11)) likewise showed the presence of tin when tested by dissolving the whole lead strip, carrying the deposit, in nitric acid (a clean lead strip which had not been used for a reduction showed a trace of tin in this test which was, however, qualitatively less than the amounts shown in the above tests).

It is unlikely that the precipitated metal reacted to any extent with iodine during the titration, since reasonably stable end-points were obtained, or, in view of the result of Expt. (7), that any copper in the cuprous condition remained in the solution after reduction. It is, therefore, sufficiently certain that the figures given in column 4 of Table II, which are the differences between the tin taken and that calculated from the iodine consumed in the titration, represent tin precipitated with the copper under the influence of the lead used in the reduction.

These results, plotted as a graph, show that the relationship between the amount of copper present in the original solution and the amount of tin precipitated in Expts. (1) to (5) is very nearly linear. From an extrapolation of this graph, 0·2 grm. of tin would require 0·3525 grm. of copper for its complete precipitation, thus corresponding to a precipitate containing 63·8 per cent. of copper and 36·2 per cent. of tin. These figures are closely similar to the results given above of the analysis of a specimen of one of the precipitates, and seem to suggest that the tin is precipitated with the copper as a *compound*, $Cu_{10}Sn_3$, which has the calculated composition: copper, 64·1 per cent.; tin, 35·9 per cent. While there is little doubt that the consistency of the results obtained indicates compound formation between the copper and the tin, it is unsafe to draw too definite a conclusion, from the evidence available, as to the chemical individuality of the substance precipitated.

The Interference of Antimony.—When a solution of antimony in dilute hydrochloric acid is boiled with lead, the antimony, as is well known, deposits on the lead in a non-adherent form. Experiments were carried out to ascertain whether the precipitation during the reduction of tin by lead would exercise any influence on the accuracy of the determination. Solutions containing varying amounts of tin dissolved in 50 c.c. of concentrated hydrochloric acid and oxidised with bromine, with antimony added in the form of a solution of antimonious chloride, were diluted to 200–250 c.c. The reduction with lead and titration with iodine were carried out exactly as in the standard method described for the determination of tin (Expts. (*a*), Table III); the precipitated antimony suspended in the solution, however, rendered the detection of the end-point of the titration

difficult. After the titration, the solution in the flask was filtered to remove the metallic precipitate, and the clear filtrate, together with the liquid used in washing the precipitate, was re-reduced, using a fresh strip of lead, and titrated as usual (Expts. (*b*)). The bulk of the antimony separated during the first reduction process and only a small quantity appeared on the lead during the second reduction. The following results were obtained:

TABLE III.

	Taken.		Titration. Iodine.		Found.
	Tin. Grm.	Antimony. Grm.	c.c.		Tin. Grm.
(1) (*a*)	0·1057	0·5	18·7	0·1 *N*	0·1111
(*b*)			17·3	,,	0·1028
(2) (*a*)	0·2004	0·5	34·7	,,	0·2061
(*b*)			32·65	,,	0·1939

The amounts of tin found in Expts. (*b*) show that co-precipitation of tin had taken place during the reduction process in Expts. (*a*). In spite of this, the results of Expts. (*a*) are seen to be high. This can only be explained by the assumption that antimony in the fine suspension reacted to some extent with iodine during the titration process. In this connection it may be noted that I have obtained distinctly high results in the determination of tin, using antimony powder which had been very finely ground in an agate mortar, and which remained largely suspended in the liquid during the titration with iodine (the end-point was not distinct); the reduction and titration were carried out in the same manner as in the method described using lead.*

It is evident from Expts. (*b*) that a considerable amount of tin is removed from the solution by the precipitate of antimony. More conclusive evidence of this effect than is provided by these titration experiments has been obtained by actually determining the co-precipitated tin in the antimony precipitate. For this purpose the precipitate of antimony obtained by carrying out the usual lead reduction process on solutions containing tin and antimony was collected by filtration (most of what remained adherent to the lead strip was recovered by spreading the strip diagonally upon a glazed tile, rubbing off the deposit with a rubber-tipped rod, and rinsing it on to the filter), and well washed with hot dilute hydrochloric acid. The deposit was dissolved by treating the filter with dilute hydrochloric acid and bromine. The clear solution, to which was added 1 grm. of citric acid, was boiled to remove excess of bromine, neutralised with ammonia and 2 per cent. (by volume) of hydrochloric acid added. To the cold solution (volume 200 c.c.) cupferron was added, when a white precipitate was produced, showing the presence of tin, since interfering metals were absent and quinquevalent antimony gives no precipitate with cupferron under these conditions. The precipitate was filtered

* Incidentally, when somewhat coarser antimony (over half of which passed through a 90-mesh sieve) was used in the reduction distinctly low results were obtained.

off, and washed with a dilute solution of cupferron; the filter was ashed, ignited, and the residual tin oxide weighed. The following results were obtained:

TABLE IV.

Taken.		Found.	
Tin. Grm.	Antimony. Grm.	Weight of tin oxide. Grm.	Tin recovered. Grm.
Nil	0·2	(0·0004)	—
0·100	0·2	0·0070	0·0056
0·200	0·2	0·0122	0·0096

Wide variations from the theoretical results are, therefore, obtained in the determination of tin by the lead reduction process in presence of appreciable amounts of antimony. It is clear that this is due to errors caused by (*a*) reaction of iodine, during the titration, with the precipitated antimony, (*b*) co-precipitation of some tin with the antimony. These errors, while acting in opposite directions, do not necessarily compensate one another.

THE EFFECT OF ANTIMONY AND COPPER TOGETHER.—Metallic copper is known to form antimonides readily, the compound Cu_2Sb having been found to be the chief constituent of antimony Reinsch films (Evans, ANALYST, **1923**, **53**, 1). The extent to which tin is carried down with either copper or antimony in the lead reduction process might be expected to be less if copper and antimony were present together than if they were present separately, as in the foregoing experiments, owing to the possibility of copper combining with the antimony in preference to tin. This was found to be the case in an experiment in which copper (0·51 grm.) and antimony (0·5 grm.) were added (as chlorides), in approximately the proportions in Cu_2Sb, to a solution containing stannic tin (0·2530 grm.); after reduction by the usual process, 0·2414 grm. tin was found by titration. This result is low, but is very much higher than would have been obtained in absence of the antimony.

Many commercial alloys in which tin is likely to be determined by the lead reduction process, such as tin base bearing metals, contain several units per cent. of antimony and copper, and the antimony is usually present in greater proportion than the copper. The interference of these metals with the tin determination in these cases is not likely to be very noticeable, although it may account for some of the difficulties met with in obtaining a satisfactory analysis of these alloys.

It may be concluded from the above work that where copper and antimony are present it is, in general, necessary to effect a preliminary separation of the tin if results are to be obtained of the accuracy of which the lead reduction method, in its present form, is capable.

The Electrolytic Separation of Lead and Antimony, and its Application to the Determination of Lead in Tartar Emetic.

By ELLA M. COLLIN, B.Sc., A.I.C., and
HENRY J. S. SAND, D.Sc., Ph.D., F.I.C.

During the course of some experimental work on the analysis of lead bullion by internal electrolysis (E. M. Collin, Analyst, 1930, 314) it was found that antimony may be deposited quantitatively from a solution containing the metal in the antimonious state, when only the limited potential supplied by a lead anode is available. On the other hand, antimonic antimony is not deposited at all under these conditions. It was previously known that, under conditions in which the deposition potential is not limited, antimony deposited from a solution containing antimonious chloride is less compact than that obtained from the antimonic compound (Lassieur, *Electroanalyse Rapide*, Paris, 1927, p. 87), and that the metal in the pentavalent state is not deposited at all from fluoride solutions in the presence of potassium dichromate (*ibid.*, p. 95). These facts form the starting point of the present investigation.

The deposition potentials of trivalent antimony, of pentavalent antimony, and of lead in acid tartrate solutions were compared, the following being made up:

I. In Tartaric Acid.

A. 0·25 grm. of tartar emetic, 5 grms. of tartaric acid, 3 grms. of sodium tartrate, and about 200 c.c. of water.

B. 0·25 grm. of potassium antimonate, 5 grms. of tartaric acid, 3 grms. of sodium tartrate, and about 200 c.c. of water.

C. 0·25 grm. of lead nitrate, 5 grms. of tartaric acid, 3 grms. of sodium tartrate and about 200 c.c. of water.

The deposition potentials were measured against a tenth normal quinhydrone electrode. In A, antimony was deposited when the potential was raised to 0·9 volt, in B, no antimony was deposited even when the potential was raised to 1·1 volt, and in C, the deposition potential was 0·98 volt.

II. In Nitric and Tartaric Acid Solutions containing Chlorides.

D. 10 c.c. of concentrated nitric acid, 1 grm. of tartaric acid, 2 c.c. of 2 *N* hydrochloric acid, 0·5 grm. of hydroxylamine hydrochloride, 0·25 grm. of tartar emetic, and about 200 c.c. water.

E. As D, but 0·25 grm. of potassium antimonate instead of the tartar emetic.

F. 5 c.c. concentrated nitric acid, 2 grms. of tartaric acid, 2 c.c. of 2 *N* hydrochloric acid, 1 grm. of hydroxylamine hydrochloride, and 0·25 grm. of lead nitrate.

In D, antimony was deposited at a potential of 0·75 volt, in E no antimony was deposited when the potential was raised to 1·1 volt, and in F, the deposition potential was 0·92 volt.

These experiments furnish a sufficient explanation of what has been stated concerning the deposition of antimony in the internal electrolysis apparatus.

The deposition of antimony from alkaline solutions was next tried, and it was found that, whereas antimony in a spongy form may be deposited from an antimonite solution, none is precipitated even by high current densities when an antimonate solution is employed. Lead, on the other hand, may readily be deposited as metal in a satisfactory form from alkaline solutions (Sand, *J. Chem. Soc.*, 1907, **91**, 397).

On these observations a method of separating lead from antimony was based, the principle of which is the following:—The antimony is oxidised to the antimonic state by iodine in the presence of bicarbonate. Tartaric acid is then added, and the solution made strongly alkaline with potassium hydroxide. It is then analysed by means of a current which may be derived either from an external source or from internal electrolysis.

Ordinary Electrolysis.—Tests were first made in which an externally generated current of about 3 amperes was employed, the electrodes previously described being used (Sand, Analyst, 1929, **54**, 275). Five grms. of "lead-free" tartar emetic were dissolved in water, 3 grms. of potassium bicarbonate added, and the antimony oxidised with a solution of iodine in potassium iodide, after which about 4 grms. of potassium hydroxide and 2 to 3 grms. of tartaric acid were added. The solution, after dilution to a volume of about 300 to 350 c.c., was electrolysed cold. The platinum cathode was previously coated with copper and the anode rotated. There was a small deposit of lead on the cathode, the presence of which was confirmed by a chemical test. The sample of tartar emetic was tested chemically and found to contain lead. A standard solution of lead acetate was made, and known amounts added to a solution of tartar emetic prepared as described above and electrolysed. A deduction was made from the weight of the deposit to allow for the lead in the tartar emetic unless this had been removed previously by electrolysis. The following results were obtained:

Lead added. Grm.	Lead found. Grm.
0·0033	0·0031
0·0024	0·0022
0·0016	0·0017
0·0011	0·0011
0·0023	0·0022
0·0016	0·0016

INTERNAL ELECTROLYSIS.—The solutions for electrolysis were prepared as described above and electrolysed in the internal electrolysis apparatus (Sand, ANALYST, 1930, 309), using zinc anodes and a 10 per cent. solution of potassium cyanide in the anode compartments. Zinc nitrate as the anolyte did not give a sufficient e.m.f. to effect the deposition of the lead. Results obtained were as follows:

Lead added. Grm.	Lead found. Grm.
0·0034	0·0033
0·0022	0·0019
0·0028	0·0028
0·0016	0·0016
0·0011	0·0011
0·0033	0·0033
0·0033	0·0031
0·0033	0·0032

The time of electrolysis was in each case 15 to 20 minutes.

DETERMINATION OF LEAD IN TARTAR EMETIC.—In the following experiments samples of tartar emetic were analysed for lead both by ordinary and internal electrolysis, and the results were checked by a chemical method, namely, the precipitation of the lead as sulphide and weighing it as lead sulphate.

	Ordinary Electrolysis.		Internal Electrolysis.		Chemical.	
No.	Amt. taken Grams.	Lead Per Cent.	Amt. taken Grams.	Lead Per Cent.	Amt. taken Grams.	Lead Per Cent.
1	2	0·096	3	0·076	2	0·096
2	3	0·080	2	0·075	2	0·092
	3	0·083				
3	3	0·09	3	0·09	2	0·09
	2	0·11	2	0·085	*2	0·097
	2	0·10	2	0·075		
4	*2	0·021	5	0·020	5	0·028
					*2	0·026
					*5	0·021
5	10	0·007	10	0·009	10	0·005
	*10	0·0083			*5	0·0076

The results we have obtained are satisfactory, both when a current derived from an external source and when one obtained by internal electrolysis was employed. We have no hesitation in recommending the first-named method; before, however, definitely advocating the use of the second for analysis, we should like to have a further opportunity of investigating the effect on the copper cathode, of cyanide, which may find its way into the catholyte from the anode compartment.

In addition to the gravimetric method, three of the samples were tested by a colorimetric method, in which the lead is precipitated as chromate and determined

* Colorimetric determination.

with diphenylcarbazide as indicator (B. Jones, ANALYST, 1930, **55**, 318). The sample was dissolved in water, the solution made alkaline with potassium hydroxide, and the lead precipitated as sulphide, the subsequent procedure being as described in the paper quoted. In addition, the deposits obtained electrolytically from the two samples of low lead content were dissolved and tested by the same colorimetric method. The copper used for plating the cathode did not interfere.

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The Testing of Admiralty Disinfectant Fluid.*

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AND
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INTRODUCTORY.—The Admiralty Disinfectant Fluid is of the type commonly known as a "coal-tar disinfectant," and in modern practice the raw material is obtained almost exclusively from coke-oven, producer-gas, and blast furnace plants. The active ingredients derived from the tar distillate fraction are phenoloids; phenol itself is, or should be, absent. The germicidal action of the fluid is due to the homologues of phenol, the cresols, xylenols, and still higher homologues.[1]

These compounds, with increasing molecular weight, exhibit greater germicidal power and decreased toxicity. The higher homologues are relatively insoluble in water, and, therefore, to render them available for use as disinfectants, they have to be emulsified. Soaps are largely employed for this purpose, but are inadmissible for use in the Admiralty Disinfectant Fluid, which is required to be miscible with sea-water in all proportions. The germicidal power of such a disinfectant is related not only to its chemical composition, but directly also to its physical condition, as expressed by the fineness and stability of the emulsion.

SAMPLING.—An original unopened container is forwarded for examination, unless the capacity is greater than one gallon, in which case a sample of about 500 c.c. is withdrawn after thorough mixing.

PHYSICAL CONDITION.—The entire sample is transferred to a narrow-mouthed stoppered bottle of capacity slightly in excess of the volume received for examination. It is mixed by pouring the whole, five times backwards and forwards, from the bottle into the container, with the aid of a funnel 15 cm. in diameter, and finally into the bottle.

* Published by permission of the Director of the Medical Department, Royal Navy.

Quantities of 250 c.c. and 100 c.c. are placed in stoppered cylinders of 1 litre capacity. Each is made up to 1 litre with artificial sea-water, and thoroughly mixed. The artificial sea-water is prepared by dissolving 27 grms. of sodium chloride (A.R.) and 5 grms. of magnesium sulphate (A.R.) in sterile distilled water, and making up to 1 litre. The cylinders are allowed to stand in the dark for 6 hours, at the end of which time visual examination is made of these dilutions (25 and 10 per cent.); and after 24 hours, the 1 per cent. dilution, prepared for estimation of "phenol coefficient" (*vide infra*), is similarly examined to determine whether the sample is *miscible with sea-water in all proportions.*

The stoppered bottle, with the remainder of the sample, is allowed to stand in the dark for 7 days. It is then inspected to ascertain whether it is *entirely free from sediment and does not separate out or precipitate, also whether it has any objectionable or disagreeable smell.*

If the sample has been received, as is usual, in its original container, the latter is examined for evidence of *corrosive action on metals.*

GERMICIDAL VALUE.—It must be recognised that the germicidal value expressed as "phenol coefficient" has a very limited interpretation in actual surgical and sanitary practice. The test consists in comparing the lethal power of the product under consideration with that of pure phenol. In most methods, whether the original[2] or the improved Rideal-Walker,[3] the U.S.A. Hygienic Laboratory,[4] or the Chick-Martin,[5] the basis of comparison is the inhibitory effect on a certain standard test organism, namely *Bacillus typhosus.*

The behaviour of a germicide with *B. typhosus* is no criterion of its action against other more common pathogenic organisms. Before it could be laid down that such and such dilutions should be used for, say, disinfection of drains, utensils, clothing, hands, it would be necessary to test the product against the actual bacteria usually found in these situations. Antiseptics for use on the skin and mucous membrane should be examined in the presence of pyogenic organisms such as *Staph. aureus* and *Strep. haemolyticus.* If a disinfectant is recommended for general use, the manufacturers should be able to guarantee that the dilutions specified will kill all the non-sporing organisms which cause diseases of an epidemiological nature.[6] Again, the optimum temperatures for pathogenic organisms differ from what is laid down in the laboratory test. The organic matter with which they may be associated is a very variable quantity; the time of exposure and the opportunities for penetration may not coincide with the artificial conditions of phenol coefficient technique. For these reasons it has been stated that a phenol coefficient test is unfair and has no value in practical medicine. These objections are valid only if an estimation of exact germicidal effect is desired. This information was never expected to be obtained from such a process as the Rideal-Walker or its modifications, the aim being confined to a numerical comparison of disinfectants with a standard.[7] Its applications are limited, but it is a definite means of ascertaining the comparative value of disinfectant fluids; it gives also at least a rough indication of their practical value.

The determination of the phenol coefficient by any method is entirely empirical, and the strictest attention to every detail is essential to secure concordant

results. Unless every step is carefully standardised, different operators may report entirely different values for the same sample.

Two disinfectant fluids, which may be designated A and B, were divided into two series of identical samples, and were sent to thirteen laboratories recognised as undertaking the examination of such fluids. The phenol coefficient of these samples, as determined by the Rideal–Walker technique, was reported by the various laboratories as follows:[8]

Laboratory	1.	2.	3.	4.	5.	6.	7.
Sample A.	8·95	5·5	5·5	6·0	6·0	6·25	7·0
Sample B.	11·8	6·0	14·4	9·0	12·0	9·0	14·0

Laboratory	8.	9.	10.	11.	12.	13.
Sample A.	10·0	10·5	10·0	11·0	11·5	11·2
Sample B.	11·0	12·7	14·0	17·0	16·4	16·4

The most cursory examination reveals the enormous differences in these figures, which are quoted to show the necessity for the extreme, and perhaps tiresome, detail with which the Admiralty method is described in the subsequent pages.

In outline, the method consists in preparing a 1 per cent. dilution of the fluid with artificial sea-water, allowing this to stand for 24 hours; and then determining its action in presence of organic matter, against a particular strain of *B. typhosus*, as compared with pure phenol.

Some of the causes of the great variation in the results of phenol coefficient determinations are common to all methods, and are referred to in discussing certain details of the Admiralty method under the headings:

THE TEST ORGANISM AND ITS PRELIMINARY SUBCULTURE.—The same species of organism must be used in all series of tests if any uniformity of results is to be looked for, and also, for comparative work, the ideal is to have a culture of standard strength and resistance. Discrepancies up to 300 per cent. may occur if different species are taken, and there may be variation even between strains of the same species. *B. typhosus*, adopted originally by Rideal and Walker, is still recognised as the best choice. It is more constant in behaviour than most organisms, produces an easily recognised turbidity in broth as evidence of growth, and can be readily counted or agglutinated in the endeavour to arrive at a standard strength. The use of a set type such as *B. typhosus*, Rawling's (National Collection of Type Cultures) further diminishes the opportunity for variation. This is a remarkably constant strain, and has retained its virulence, antigenic power, and agglutinability for years. *B. coli*, recommended in the *Lancet* method[9] on account of its non-pathogenicity, is an organism whose strains vary considerably in resistance.

In order that the culture shall be of optimum vitality on the day of the test it is important to have a recent growth in broth—24 hours at 37° C. The resistance, even when subculturing from the same stock, may vary from day to day[10]; forty-eight hour cultures generally are more resistant. The number of

times the organisms are subcultured before use will considerably affect the result. A coefficient may vary according to this number, and tend to be higher the younger the generation.[7] Conditions can, therefore, be further standardised by subculturing daily in a set medium for a set period, say 7 days, starting from a stock agar of a certain age, *e.g.* one month, that has been stored at room temperature.

In this laboratory the present routine for preparing the test cultures is as follows:—The organism is *B. typhosus*, Rawling's strain (N.C.T.C.). Monthly subcultures are grown on nutrient agar made with the same broth basis as is specified for the test. Dry slopes are chosen, as moist media favour the change from smooth to rough growth. Stocks are maintained at room temperature. It is assured that the strain reacts constantly to high titre serum and retains smoothness and motility. Subcultures in broth (prepared as detailed) are started afresh every week. Taking a month old stock agar and plating out overnight at 37° C., a smooth colony of 1 mm. diameter is lifted and sown whole into the first broth. Re-inoculations are made every 24 hours with the standard 4 mm. loop (*vide infra*). Tubes are of uniform size and contain 5 c.c. of broth. Suitable strength is assured by matching between stock tubes (1000 millions and 2000 millions per c.c.), preserved from previous satisfactory tests, sterilised, counted and sealed. If turbidity is insufficient, a heavier inoculum is prepared ready for the next 24 hours. The series is maintained at 37° C., and the day before the test, transfers are made to four or more such broths, according to the amount of culture required. Satisfactory readings are obtained with from 3 days to a fortnight's subculturing, after which time there is a tendency for the rough phase to supervene. For the sake of uniformity the routine period is made 7 days. The object is to stabilise the resistance of the test organism. On plating out for a new series of broths a pure smooth colony is chosen—moist, translucent, domed, free from radiation or granular appearance, and giving uniform turbidity in the fluid medium. The organism is then at the height of its virulence and antigenic power; its strength is at a definite level—the optimum. It can likewise be accredited with possessing some definite degree of susceptibility to antiseptics. On the other hand, there are countless gradations of the partially rough variants, with corresponding uncertainty in their reactions down to the wholly rough type—dry, crenated, granular, opaque, precipitating in broth, auto-agglutinable and weak in antigenic power. Similarly, their resistance to antiseptics must vary. If the series is always initiated from a smooth strain of a definite age; is subcultured in standard broths over a definite period; and gives a standard opacity, there should be more likelihood of standard resistance in the test culture.

PRESENCE OF ORGANIC MATTER AND ITS NATURE.—In order that the conditions under which the disinfectant is tested for phenol-coefficient shall simulate what occurs in practice, organic matter is added. From time to time various authorities have favoured admixture of blood serum, dead cultures, urine, faeces, peptone, gelatin, egg albumin, pus, mucin, casein, and starch. It is important to prove the efficiency of the sample in the presence of some such material; but it is essential that it shall be sterile and of known definite composition. The difficulty of ensuring sterility of blood serum as such, makes its routine use hardly practical. The presence of urine, faeces or pus would, perhaps, represent natural conditions best, but their organic content is widely inconstant. Where every endeavour is being made to reduce variable factors something more reliable is required. As

it is impossible to obtain morbid secretions and excretions of uniform character and composition, combinations of certain types and strengths of standard material are used. Thus the interaction between the organic matter and the disinfectant is more under control.

In the Hygienic Laboratory (U.S.A.) method,[2] 3 per cent. of organic matter, in the form of a peptone-gelatin combination, is mixed with the disinfectant dilutions. The materials are carefully standardised and satisfactory results are claimed. Carbohydrate plus protein makes a still more natural combination. Adapting from the work of Sommerville and Walker,[11] particulate matter, which will introduce the influence of adsorption, is incorporated in the Admiralty technique in the form of a suspension of rice starch. This substance can be ground very fine. Compared with the other commonly occurring starches the rice granule is one of the smallest (3–7μ); also it is more uniform in size.[12] Protein, in the form of gelatin, is introduced, but only a small percentage can be added, on account of its physical condition at the temperature of the test.

The gelatin-starch mixture is prepared in the following manner: Five grms. of gelatin are dissolved in one litre of artificial sea-water; 0·5 grm. of specially prepared rice starch is added to 100 c.c. of the stock gelatin solution immediately before the test. The rice starch is prepared by drying it at 100° C., then passing it through a 60-mesh to the inch sieve into a Petri dish, and finally sterilising it in the hot air oven at 130° C. for 4 hours. This starch is kept in the Petri dish in the cold oven until required. The method of sterilising starch by treatment with alcohol or chloroform in alcohol and then drying in an oven has not proved satisfactory.

Experiments show that it is unnecessary to add the organic matter to the carbolic dilutions. These are aqueous solutions; therefore, the same strength of phenol is present in every ultimate portion, and admixture of particulate matter will not noticeably lessen the amount of germicide which comes in contact with the bacteria. Addition of such a substance to disinfectant emulsions affects the germicidal value to a much greater extent. Here the physical state being that of an emulsion, all the active ingredient is in the form of microscopic globules moving in the inert watery diluent. Adsorption of the disinfectant globules by the organic particles will reduce the number available to act on the bacteria.

Diluent.—As the uses to which disinfectant fluid is put in the Royal Navy are generally associated with the presence of sea-water, a similar medium is prescribed in the Admiralty test. It is used as the diluent for both disinfectant and organic matter. The composition of the artificial sea-water has been given on p. 94.

The Disinfectant Emulsion.—The main factors governing the bactericidal powers of an emulsified disinfectant of the type under discussion are (*a*) the nature and amount of tar acids, (*b*) the perfection of the emulsion. These products are physico-chemical in their action,[13] and have marked adsorptive properties, whereby the antiseptic constituent concentrates round the bacterial protoplasm. The

degree of homogeneity has a considerable effect upon the activity. An irregular emulsion tends to separate, and will give different values with different portions of the one sample. This de-emulsification is accelerated in the presence of an electrolyte such as sea-water. The amount of shaking employed in preparing the test dilutions is a marked source of variation. A product heavily charged with tar acids, in order to enhance its potency, may yield an unstable emulsion. If sufficiently vigorous shaking is given, the value is artificially improved; but if the dilution stands for some hours before the test is made, quite a different result is obtained.[8] Therefore, even in the minor matter of shaking, there should be a standard method. A good emulsion, with normal shaking, will retain a uniform homogeneity and coefficient value.

THE PHENOL.—Carbolic acid lends itself admirably as the coefficient denominator. It is obtainable pure, and remains stable if stored under proper conditions. Leroux[14] has shown that the best test for determination of purity of phenol is by means of the melting point, which should be 40·85° C.*; the presence of 0·2 per cent. of water lowers the melting point to 40·10° C. Cresols cause a reduction in this temperature of half a degree centigrade per cent. of admixture.[15] The Admiralty method prescribes Calvert's No. 1 carbolic, but it is proposed to alter this to "pure carbolic acid having a crystallising point of 40·5° C."

The accuracy of the phenol coefficient test is fundamentally dependent upon the reliability of standard solutions prepared from the phenol. The usual practice is to keep a stock 5 per cent. solution by weight, from which other dilutions are freshly made for each test as required. The routine followed in this laboratory is to keep stock quantities of all the test dilutions. The dry crystals are weighed in a stoppered weighing bottle, dissolved in sterile distilled water, and made up to the required volume. In order to obtain suitably close dilutions, and to have them arranged in mathematical order, figures are chosen which give a geometrical progression of 4 per cent. on either side of 1 in 100, *i.e.* 1 in 88·90, 92·45, 96·15, 100, 104, 108·16, 112·48, etc. Each successive denominator shows an increment of 1/25. Five hundred c.c. quantities are made up and stored in amber-coloured bottles with the original stoppers, in a cool, dark cupboard. Such dilutions remain unchanged for at least a year. Five c.c. portions of the required strengths are taken for each test.

PROPORTION OF CULTURE TO DISINFECTANT.—The employment of various sized drops, spoonfuls, and other measures in different modifications of the phenol coefficient test must mean variation in the number of organisms introduced into the tubes. Discordant results are inevitable if some workers use, say, "0·2 c.c.," and others "5 drops" of varying size[4,8]. If tests are to be comparative, the dilution tubes must always be inseminated with a definite quantity of culture accurately measured by graduated pipettes. The larger the dose of culture, the greater is the effect of mass resistance, and the stronger will be the dilution of

* Stratton and Partington (*Phil. Mag.*, 1922, **43**, 411) found the melting point of pure phenol to be 41·5° C.

emulsified disinfectant required to kill in standard time. Its coefficient will, therefore, be lower.

In the U.S.A. Hygienic Laboratory method[4] a 0·1 c.c. dose is used, measured by pipettes graduated in tenths, while 0·5 c.c. quantities are recommended by Rideal and Walker,[3] to be distributed by means of a dropping pipette calibrated to deliver this amount. Even with the most careful measurement, the inoculum may vary in a series of tests unless the culture is always of the same strength. In this discussion an account is given, under the heading "Test Organism," of the steps taken in an endeavour to arrive at a standard resistance. The resulting culture is somewhat susceptible, but at the dosage employed (0·25 c.c.) it works very satisfactorily, also within the limited range of dilutions chosen. The volume is delivered by means of a Cole's automatic pipette.

Amount of Inoculum to Broths.—As the operation must be completed within a scheduled time, the platinum-loop method is used to transfer portions of the mixture of culture and disinfectant to the final broths. The quantity thus carried over is small enough to ensure a dilution in the broth which will prevent any further inhibitory action on surviving bacteria. If these quantities vary, a positive growth may occur after exposure to slightly stronger solutions of disinfectant and a lower coefficient will be obtained. Hence the recommendation for a standard-sized loop in standard gauge wire, the transfer to be carried out in standard manner by immersing the loop at a definite angle to a definite depth. Here the U.S.A. Hygienic Laboratory technique is adopted as most reliable, except that, as far as possible, the same platinum loop is used throughout the test, instead of having a number to choose from. This lessens the chance of variation in size of inoculum. Platinum wire, gauge, 0·015 mm. B.W.G. No. 2, is employed. A perfectly circular loop, 4 mm. inside diameter, is fashioned on round-nosed pliers leaving a shank, 3·5 cm. long, to fit into the holder. A wooden end prevents the rod from getting too hot for the hand with the repeated sterilisations necessary during the test. The loop is set accurately at an angle of 45° with the shank. The loopfuls are taken from the dilution tubes without moving these from their upright position in the water-bath. The rod is held vertical, and the wire is immersed to the same depth and lifted with the same quick motion for each tube. Similar stereotyped movements are used when inoculating the broths.

Media.—The constitution of the subculture broth and its *p*H value are most important considerations. The medium must be at optimum reaction for the test organism; otherwise growth may be inhibited after transplanting. For example, if a transplantation is made from a test dilution which is just under the killing strength of the disinfectant, the inhibitory action of the medium may be sufficient to prevent growth and give a false result.[4]

Broth made from fresh meat encourages growth better than meat extract broth, and may give a positive finding from a stronger dilution tube. The brand of peptone and the percentage of this ingredient also affect the result. Broth with a higher content of peptone gives higher coefficients.

Commercial peptones of different brands vary greatly in composition, and this lack of uniformity causes variations in nutrient value of broths. The co-efficient returned for identical samples of a disinfectant may vary as much as 100 per cent. in different laboratories from this one factor. Most authorities recommend that the broth should be standardised to *p*H 7·6. At this optimum value constant findings are obtained, whereas at higher or lower reactions the coefficient varies indefinitely.[16] Even into the preparation of broth a personal element may enter and affect results; workers may differ in their readings of a colour reaction in titration.

A successful technique will be one where these opportunities for variation are reduced to a minimum; the broth must be prepared according to one standard method, in which the same approved peptone is always used and the medium is brought to the optimum *p*H for the organism concerned. In this laboratory the method of preparing the broth is that described by McIntosh and Smart[17]; Armour's peptone is used.

TIME GIVEN FOR THE TEST.—Where transplantation from the seeded tubes is repeated every 2½ minutes (2½ minutes, 5, 7½, ... 15 minutes—Rideal-Walker), a killing level may be found at more than one of these time periods. Either could be used for the coefficient reading, and the figures might be different. Also, the actual time taken for killing would not be indicated by the number reported. By having one set time for contact, the same for all dilutions, instead of a series of 2½ minutes' intervals, the technique is simplified, and more samples can be tested at once. The finding indicates that disinfection has been obtained after a known interval of action. Rideal later recommended such a variation of the test.[18] Some cultures, being more resistant, are less affected in the first few minutes of contact with a germicide. Others are more susceptible, and suffer most in the early stage of the test, after which the action of the antiseptic gradually decreases. Transplanting at a period about midway between the Rideal–Walker limits offers a satisfactory standard. Using such a method of time allowance, different workers are more likely to get similar results. The test thus modified is more suitable for commercial use and for inclusion in a contract specification.[7]

TEMPERATURE OF EXPERIMENT.—In general, the higher the temperature the greater the velocity of disinfection and the stronger the apparent germicidal power.[19] For example, phenol kills *B. typhosus* in 2½ minutes when the dilution is 1/80 and the temperature 15° C.; at a temperature of 30° C. a dilution of 1/120 has the same effect. Series of tests carried out at dissimilar temperatures cannot be accurately compared. To minimise error, the technique must be so adapted as to ensure the smallest possible range. For ordinary use the disinfectants under consideration are generally required to work at about room temperature, and a test carried out at this level is, therefore, practical, and is suitable for most climates. Rideal and Walker allow a range 15 to 18° C., whilst the U.S.A. Hygienic Laboratory specifies 20° C. The Admiralty schedule prescribes 18° to 20° C., and in this laboratory the water-bath is kept as nearly as possible at 19° C.

METHOD.—*Apparatus required.*—Suitable wooden racks for preliminary handling of dilution tubes.

The following glassware, sterilised in the hot air oven for ½ hour at 150° C.:—A wide-mouthed specimen jar (*e.g.* 3″×2″) to contain culture; glass cap and funnel with cotton-wool filter. Requisite number of dilution tubes (3″×1″) cotton-wool plugged. Test tubes (6″×⅝″) to hold convenient portions of 1 per cent. sample. One c.c. graduated pipettes for disinfectant dilution. Ten c.c. pipettes, marked at 4·4 and 8·8, for measuring organic matter. Cole's automatic pipette in a suitable wide test tube.

Water Bath.—A water-bath with electric heating is used. It is fairly shallow, to facilitate the manipulation of the short tubes employed, and of a size capable of taking three metal racks of 24 tubes apiece. Thus the dilutions for four samples and a control are accommodated, in addition to receptacles for culture and 1 per cent. samples, *e.g.* five rows of seven dilutions. As a rule, fewer dilution tubes suffice, it being known within what limits the critical change from "killing" to "growing" must occur.

Preliminary.—Twenty-four hours before the test is to be made the sample dilution is prepared. A stoppered graduated 1000 c.c. cylinder, of not greater diameter than 7 cm., is filled to about the 980 c.c. mark with sterile artificial sea-water. A pipette graduated to hold 10 c.c.* is filled above the mark, wiped clean outside, and run down to the mark. With the nose of the pipette in the sea-water it is allowed to discharge, and thereafter the pipette is rinsed out three times in the clear portion of the liquid. The whole is made up to 1000 c.c. with artificial sea-water. The cylinder is stoppered, and the contained liquid is thoroughly mixed by inverting, with a corkscrew motion, 100 times. It is now allowed to stand undisturbed at room temperature and away from light for twenty-four hours. Any separation is noted before using for coefficient determination.

At the same time as the sample dilution is prepared, 5 c.c. quantities of the phenol dilutions are pipetted, with a standard pipette, from the stock bottles, into appropriately marked tubes.

Other matters which receive attention the day before the test are:—The test culture (*q.v.*). The last twenty-four hour subculture is put up in a number of broths (4 or 5) sufficient for inoculating the total number of dilution tubes.

The provision of the requisite number of final broth tubes appropriately marked to tally with the individual dilution tubes. All broth is prepared according to the method referred to under "Test Organism" (*q.v.*).

The 0·5 per cent. gelatin in artificial sea-water and the starch for admixture prepared as already described.

THE TEST.—The first care is to bring the bath to a temperature of 19° C. Thenceforward throughout the manipulation this temperature is maintained by adjustment of the thermostat.

The following articles are then placed in the bath in order that they may attain the standard temperature:—The row of phenol dilutions transferred to one of the racks; test tubes for ready use portions of sample dilution—pipetted from the middle of the 1000 c.c. cylinder; flask of gelatin-starch mixture; jar containing filtered culture (the broths having been poured into the funnel and the apparatus covered with its sterile cap).

* Those made for us have a sand-blasted band above and below the mark, so as to be readily distinguishable from the ordinary type.

The short test tubes for disinfectant dilutions are set out in their wooden racks, and each receives 4·4 c.c. of the organic suspension; care is taken to pipette from the body of the mixture and to avoid the heavier material at the bottom. Each tube is transferred at once to its appropriate rack in the water-bath.

A minimum coefficient of 8 being demanded by the contract specification, the strength of the phenol and of disinfectant sample can be so arranged that this reading will occur within the limits of the tubes—*e.g.* of phenol, a sequence of dilutions above and below 1 in 100, and of sample a sequence above and below 1 in 800. Were it desired to determine the coefficient of a fluid, the value of which was unknown, it would be necessary to make preliminary tests in coarser dilutions. The fluids considered here, however, are contract specimens; therefore, the end-point should occur within a limited number of tubes.

A suitable block of dilutions is prepared from the following table, *e.g.* 1/1200 to 1/589:

Starch and gelatin.	1 per cent. sample.	Total mixture.	Final dilutions.
4·4	0·1	4·5	1 in 4500
4·4	0·15	4·55	3033
4·4	0·2	4·6	2300
4·4	0·25	4·65	1860
4·4	0·3	4·7	1567
4·4	0·35	4·75	1357
4·4	0·4	4·8	1200
4·4	0·45	4·85	1078
4·4	0·52	4·92	946
4·4	0·6	5·0	833
4·4	0·7	5·1	729
4·4	0·8	5·2	650
4·4	0·9	5·3	589
4·4	1·0	5·4	540

The final strengths (column 4) are in rough geometrical progression, corresponding each time to three successive dilutions of 4 per cent.*

As each final dilution is completed the tube is re-plugged, shaken, and replaced in the water-bath.

The water-bath is now ready with its racks of dilution tubes of both samples and standard phenols.

The exact time is now noted, and at regular intervals (a stop-watch is used), say, 15 or 20 seconds, 0·25 c.c. of filtered culture is pipetted into each tube in succession. These tubes must now be shaken again so that the organisms will be evenly distributed. It is convenient for the assistant to lift the tubes out of the rack one at a time, remove the wool plug, and hold it resting upright on the carbolised towel ready for the culture. Each tube is then shaken and returned to its position in the rack. Allowing 15 seconds per tube, up to three dozen tubes can be treated, well within the prescribed ten minutes' action period.

* Here all the tubes carry an excess of organic matter, and it might be argued that the weaker dilutions of sample have to act against an unfair load. This objection has been met by mixing the sample dilution with organic suspension in bulk; and distributing in arithmetical series by means of burettes; but this process has proved too lengthy for routine work. The method described above has been found satisfactory in practice. Comparison of results shows no appreciable difference in coefficient findings.

At exactly 10 minutes from the time first noted, and at the same number of seconds' interval as before, an inoculum is taken from each tube in the same order and planted into broth. The assistant hands each broth tube to the operator and removes the corresponding dilution tube from the water-bath as soon as the inoculum has been taken. For this purpose the platinum needle, with its standard 4 m.m. loop set at 45°, is plunged vertically into the solution to a definite depth, and then lifted with a quick motion—uniform throughout, in order always to produce a loopful of the same size. The broth tube has been taken in the left hand and the plug removed with the right little finger. The tube mouth is flamed, and the loop is carefully inserted so as not to foul the sides. After 15 seconds the loop is plunged into the broth, withdrawn, and flamed. The re-plugged tube is then handed to the assistant in exchange for the next, and the manœuvre is repeated similarly for the whole series.

On completion, the broth tubes, now replaced in order in their wooden racks, are incubated at 37° C. for 48 hours. An end-point between killing (clear) and growing (turbid) should occur somewhere in each row, corresponding to a definite dilution of disinfectant.

$$\text{The ratio:—}\ \frac{\text{Sample (dilution for first clear tube)}}{\text{Phenol (dilution for first clear tube)}} = \text{phenol coefficient.}$$

The coefficient is shown by a table compiled from the dilution figures, reading down from the highest sample tube and across from the highest phenol tube that corresponds to the end-point in the broth subcultures. For example:

Dilutions of carbolic—1 in:—	Dilutions of sample—1 in:—								
	•	589	650	729	833	946	1078	1200	1356	etc.
		Give coefficients:—								
	88·90	6·6	7·3	8·2	9·4	10·6	12·1	13·5	15·3	
	92·45	6·4	7·0	7·9	9·0	10·2	11·7	13·0	14·7	
	96·15	6·1	6·8	7·6	8·7	9·8	11·2	12·5	14·1	
	100	5·9	6·5	7·3	8·3	9·5	10·8	12·0	13·6	and so on.
	104	5·7	6·2	7·0	8·0	9·1	10·4	11·5	13·1	
	108·16	5·4	6·0	6·7	7·7	8·7	10·0	11·1	12·5	
	112·48	5·2	5·8	6·5	7·4	8·4	9·6	10·7	12·0	
	116·98	5·0	5·6	6·2	7·1	8·1	9·2	10·3	11·6	
	121·66	4·8	5·3	6·0	6·9	7·8	8·9	9·9	11·2	
	126·53	4·7	5·1	5·8	6·6	7·3	8·5	9·5	10·7	
	etc.									

The fluids kill up to, and including, the furthest clear tube from the "killing end" of the rack.

For range of coefficient low to high, read one down and one across from dilution corresponding to clear tube.

Thus: If carbolic kills in 116·98 and does not kill in 121·66, and sample kills in 729 but not in 833, the reading lies between 6·0 and 7·1.

The limits of coefficient by this method are:

$$\text{Upper limit} = \frac{\text{lowest dilution where disinfectant failed to kill}}{\text{highest dilution where phenol killed}}.$$

$$\text{Lower limit} = \frac{\text{highest dilution where disinfectant killed}}{\text{lowest dilution where phenol failed}}.$$

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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.

SOME NEW COLOUR REACTIONS WITH CERTAIN OILS AND FATS.

A NUMBER of aromatic hydroxy-compounds, *e.g.* phenol, resorcinol, menthol, phloroglucinol, salicylic acid, etc., give well-marked colour reactions with certain oils and fats, in the presence of a slight excess of bromine, in chloroformic solution. Resorcinol is a convenient reagent, being slightly soluble in chloroform, so that a saturated solution is easily prepared. The test is carried out as follows:

About 3 drops of the oil, or melted fat, are added to the resorcinol solution, followed by 5 drops of fairly strongly brominated hydrochloric acid. On shaking, a colour gradually forms with the reacting oil.

Cod-liver oil gives a pink coloration, which gradually turns to a deep red on standing. Of the wide range of oils and fats tested, butter-fat, whale oil, seal oil, oleo-oil, soya bean oil and rape oil give varying shades of pink. Linseed oil gives a deep green, and palm oil a light green coloration. Oils giving no reaction include olive oil, coconut oil and almond oil.

It seems probable that these phenolic reagents react with those oils which are said to contain vitamin *A*, and which also react with Rosenheim and Drummond's

antimony or arsenic trichloride reagent. Cod-liver oil gives by far the most pronounced coloration, butter fat coming next, though it is not nearly so reactive as cod-liver oil.

The insoluble fatty acids separated from cod-liver oil give no definite colour. The unsaponifiable and alcohol-soluble portions, however, after careful separation of free oil, give colour reactions with the phenolic reagents.

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INVESTIGATION OF THE OCCURRENCE OF *B. ABORTUS* (BANG) IN THE MILK OF ENGLISH HERDS (COUNTY OF SOMERSET).

The milk (100 c.c.) from each herd was centrifuged and the deposit injected subcutaneously into a guinea-pig. After about four weeks the guinea-pig was killed and its spleen removed with precautions to ensure sterility. Cultivations were made from the interior of the spleen on glucose, neutral-red, vitamin agar in an atmosphere of about 10 per cent. of carbon dioxide and 90 per cent. of air at 37° C.

The glucose and neutral-red show at once whether any growth that may be obtained is due to contamination with *B. coli*. Usually no growth was obtained.

The number of herds so tested was 379 (confined to the County of Somerset), and *B. abortus* was demonstrated in the milk of 4. It was identified by its failure to grow, except at reduced oxygen tension, and by agglutination with standard anti-serum provided by the Medical Research Council. No further steps were taken to track down individual cows. These experiments were carried out concurrently with the examination of the milk for tubercle bacilli, on the suggestion of Dr. W. G. Savage.

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Official Appointment.

The Minister of Health has confirmed the following appointment:

Mr. Sydney Emsley, B.Sc., F.I.C., as Public Analyst for the County of Isle of Wight (December 5, 1930).

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.

LINIMENT OF TURPENTINE.

On November 18, a Bethnal Green druggist was summoned at Old Street Police Court for having sold liniment of turpentine which was deficient in rectified oil of turpentine to the extent of 38 per cent.

Mr. Glyn Jones, for the defence, said that people who asked for liniment of turpentine often mean white liniment, which was not prepared in accordance with the British Pharmacopoeia.

The Public Analyst (Mr. A. E. Parkes), replying to the Magistrate (Mr. Clarke Hall), agreed that there was a considerable sale for preparations not so strong as the B.P. liniment.

The Magistrate said that, as many people wanted the white liniment, and not the B.P. preparation, he thought that the case would be met by payment of three guineas costs.

SYRUP OF FIGS.

A TRADESMAN was summoned at Eglinton (Donegal) for selling syrup of figs which contained a foreign ingredient, namely, 0·01 per cent. of salicylic acid. The certificate of the Public Analyst was produced.

The solicitor for the defence laid stress upon the point that syrup of figs was not a food, and that, therefore, the prosecution must fail. The manufacturers described it as compound syrup of figs, which meant that it contained various ingredients. The salicylic acid was added as an antiseptic.

The Chairman of the Bench said that the Magistrates, having considered the legal definitions, were not prepared to hold that syrup of figs was a food, and the case would, therefore, be dismissed.

It was intimated that a further prosecution for selling an adulterated drug would probably be instituted.

Ceylon.

REPORT OF THE AGRICULTURAL ANALYST FOR THE YEAR 1929.

IN addition to advisory work on all matters pertaining to soils, manures and fertilisers, 361 samples from experiment stations or private persons were examined in the laboratories, which are under the direction of Mr. A. W. R. Joachim, B.Sc., A.I.C. Special investigations were also undertaken in connection with soil erosion, green manures, sugar cane products, coconut products, drugs, insecticides, waters, etc.

CITRONELLA OIL INVESTIGATIONS.—An investigation to determine the relationship between the chemical and physical characteristics of local citronella oils, the Schimmel's test on which Ceylon oils are generally sold, and market price showed that there was no connection between physical characteristics and market price, but that oils with the higher geraniol content fetched, on the whole, higher prices. Schimmel's test was found unreliable as a means of gauging the quality and purity of citronella oils, and the sale of those oils on the basis of the test alone is unsatisfactory. The purchase of the oil, on the basis of geraniol content, in addition to Schimmel's test, is recommended.

RELATION OF SOIL REACTION TO VEGETATION.—A preliminary study of the relation of soil reaction (pH value) to grass-land vegetation in Nalanda-Dambulla district was made in co-operation with the Systematic Botanist, and interesting results have already been obtained. Leguminous species and certain grasses occurred with greatest frequency on soils with pH about 7, while other grasses

occurred only in areas with *p*H of 6 and less. The greatest number and frequency of species occurred where the soil was about neutral, *i.e.* with *p*H 7, and the least where the soil was most acidic in reaction. The *p*H determinations were made by means of the Bijlmann hydrogen electrode.

Water Pollution Research Board.

REPORT FOR THE YEAR ENDED 30TH JUNE, 1930, WITH REPORT OF THE DIRECTOR OF WATER POLLUTION RESEARCH.*

A LARGE part of the Third Annual Report, which is on similar lines to that of last year (ANALYST, 1930, **55**, 510), is concerned with the *Survey of the River Tees* as a typical river. The biological and chemical surveys of the tidal and non-tidal reaches have been extended for another year, but the hydrographical survey has been practically concluded, and a separate report is being prepared dealing with the measurements recorded. The flood appears to be at its greatest strength at a depth below one fathom, and the ebb is strongest on the surface. The volume of water moving down river in the top layer is greater than the volume moving up in the lower layers. Mixing of water in the different layers does occur, but any matter heavier than water, as it sinks, will tend to be carried up river. Biological and chemical work on the tidal reaches has indicated a stretch of polluted water containing less than the normal amount of dissolved oxygen, the centre of which is between Stockton and Newport at high water, and near the Transporter Bridge at Middlesbro' at low water. The small number of living forms in the central portion of the estuary and their complete absence in the bottom mud between Stockton and Newport may be due to this deoxygenated and polluted water, or to the fact that this is also the area of greatest variability in salinity. The decomposition of the organic matter of the sewage effluents discharged into the river is at its maximum in the hot summer, and this markedly affects the dissolved oxygen. The study of the algae and their distribution and seasonal variation is being continued, and the zoological work has shown the presence of about 70 different species.

BEET SUGAR EFFLUENT.—This work was continued, and it is concluded that percolating filters can be operated satisfactorily through a beet sugar campaign for the purification of pulp press liquor suitably diluted with river water or with effluent from the filters. A satisfactory rate of filtration (effecting an 80 to 90 per cent. purification) is about 100 gallons per cubic yard of filtering material per day for a pulp press liquor diluted to give a mixture with a five days' oxygen absorption value of 60 to 70 parts per 100,000. The laboratory experiments at Rothamsted have led to the conclusion that the partial conversion of sucrose into acid products assists the biological filtration, and the oxidation of the acid products is encouraged by the addition of nitrogen as albumin or as ammonium salt.

BASE-EXCHANGE OR ZEOLITE PROCESS OF WATER SOFTENING.—The investigation of the process has been continued and some results already published (Martin, *Chem. and Ind.*, 1930, **49**, 389T). The influence on the process of variation in the temperature of the water, and in the concentration of dissolved calcium, magnesium, and sodium salts is now being investigated. The base exchange process makes no material difference in the bacterial population of the water.

* Obtainable at H.M. Stationery Office, Adastral House, Kingsway, W.C.2. Price 9d. net.

OTHER INVESTIGATIONS.—An investigation into the treatment of *Corrosive and Plumbo-solvent waters* is being arranged, and the work now being done on *Colloids in connection with Sewage Disposal* is to include an examination of colloids in waste beet sugar factory waters.

CO-ORDINATION OF RESEARCH.—The Research Sub-Committee of the Institution of Gas Engineers has issued its 4th Report, which includes the various suggested methods for removal of phenols from liquor effluents from gas works, and an account of observations on the biological purification of sewage at Cheltenham containing definite quantities of spent gas liquor.

D. G. H.

Ministry of Agriculture and Fisheries.

STATUTORY RULES AND ORDERS, 1930, No. 370.*

AGRICULTURAL PRODUCE (GRADING AND MARKING), ENGLAND.

THE AGRICULTURAL PRODUCE (GRADING AND MARKING) (CANNED FRUITS, PEAS AND BEANS) REGULATIONS, 1930, DATED JUNE 4, 1930, MADE BY THE MINISTER OF AGRICULTURE AND FISHERIES AS TO GRADE DESIGNATIONS AND GRADE DESIGNATION MARKS FOR CANNED FRUITS, CANNED PEAS AND CANNED BEANS.

In exercise of the powers conferred on him by the Agricultural Produce (Grading and Marking) Act, 1928, the Minister of Agriculture and Fisheries hereby makes the following regulations:—

1. Grade designations to indicate the quality of plums, strawberries, loganberries, raspberries, blackcurrants, gooseberries and apples produced and canned in England and Wales shall be as follows:—

SELECT DESSERT PLUMS
SELECT STRAWBERRIES
SELECT LOGANBERRIES
SELECT RASPBERRIES
SELECT BLACKCURRANTS
SELECT DESSERT GOOSEBERRIES
SELECT APPLES

and the quality indicated by such grade designations shall be deemed to be as described in column 2 of the First Schedule hereto.

2. A grade designation to indicate the quality of peas produced and canned in England and Wales shall be as follows:—

SELECT PEAS

and the quality indicated by such grade designation shall be deemed to be as described in column 2 of the Second Schedule hereto.

3. A grade designation to indicate the quality of beans produced and canned in England and Wales shall be as follows:—

SELECT BEANS

and the quality indicated by such grade designation shall be deemed to be as described in column 2 of the Third Schedule hereto.

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

4. A grade designation mark shall be one of the grade designations specified in regulations 1, 2 and 3 above, associated with the words "*Empire Buying Begins at Home*" and with the following mark, namely, a map of England and Wales, in silhouette, with the words, "Produce of England and Wales" inscribed in a circle placed centrally in the map within which circle is a design representing the Union Jack and which is more particularly described in the Fourth Schedule hereto.

5. These regulations shall come into operation on the 4th June, 1930.

6. These regulations may be cited as the Agricultural Produce (Grading and Marking) (Canned Fruits, Peas and Beans) Regulations, 1930.

In Witness whereof the Official Seal of the Minister of Agriculture and Fisheries is hereunto affixed this 4th day of June, 1930.

(L.S.) CHARLES J. H. THOMAS.

SCHEDULE I.

FRUIT PRODUCED AND CANNED IN ENGLAND AND WALES: GRADE DESIGNATIONS AND DEFINITIONS OF QUALITY.

Grade Designation. 1.	Definition of Quality. 2.
Select Dessert Plums.	The fruit shall be firm, ripe, free from blemishes and reasonably uniform in size; it shall be canned in a syrup containing not less than 40 per cent. by weight of sugar (saccharine and glucose free) when packed; no preservatives and/or artificial colouring agents shall be present other than those permitted under the Public Health (Preservatives, etc., in Food) Regulations in force for the time being.
Select Strawberries. Select Loganberries. Select Raspberries. Select Blackcurrants. Select Dessert Gooseberries.	The fruit shall be firm, ripe, free from blemishes and reasonably uniform in size; it shall be canned in a syrup containing not less than 45 per cent. by weight of sugar (saccharine and glucose free) when packed; no preservatives and/or artificial colouring agents shall be present other than those permitted under the Public Health (Preservatives, etc., in Food) Regulations in force for the time being.
Select Apples.	The fruit shall be a recognised cooking variety, cored, peeled, and packed solid in slices of uniform size and colour; no preservatives and/or artificial colouring agents shall be present other than those permitted under the Public Health (Preservatives, etc., in Food) Regulations in force for the time being.

SCHEDULE II.

PEAS PRODUCED AND CANNED IN ENGLAND AND WALES: GRADE DESIGNATION AND DEFINITION OF QUALITY.

Grade Designation. 1.	Definition of Quality. 2.
Select Peas.	The peas shall be undried, freshly gathered at the time of canning, of uniform variety, size and colour; no preservatives and/or artificial colouring agents shall be present other than those permitted under the Public Health (Preservatives, etc., in Food) Regulations in force for the time being.

SCHEDULE III.

BEANS PRODUCED AND CANNED IN ENGLAND AND WALES: GRADE DESIGNATION AND DEFINITION OF QUALITY.

Grade Designation. 1.	Definition of Quality. 2.
Select Beans.	The beans shall be freshly gathered at the time of canning, of uniform variety, size and colour; no preservatives and/or artificial colouring agents shall be present other than those permitted under the Public Health (Preservatives, etc., in Food) Regulations in force for the time being.

SCHEDULE IV.

GRADE DESIGNATION MARK.

The Mark shall be a grade designation mark when used in association with a grade designation and with the words "Empire Buying Begins at Home."

Ministry of Health.

SALE OF FOOD AND DRUGS ACT.

EXTRACTS FROM THE ANNUAL REPORT FOR 1929—1930, AND ABSTRACT OF REPORTS OF PUBLIC ANALYSTS FOR THE YEAR 1929.*

OF a total of 133,584 samples analysed (an increase of 4550 over 1928, *cf.* ANALYST, 1929, **55**, 44), 7260, or 5·4 per cent., were reported against, and of these, 511 were contraventions of the Public Health Preservatives Regulations. Boric acid was present in foods, and sausages and other meat products were the main offending articles.

MILK.—Of 68,115 samples of milk, 5293 (7·8 per cent.) were reported against, a decrease over the previous year (8·2 per cent.). If "appeal to cow" samples are excluded, the percentage would be 7·5. One milk was 43·3 per cent. deficient in fat, and a fine of £50 was imposed; in another instance a fine of £25 was imposed for a deficiency of 10·3 per cent. Annatto was present in 10 samples, 22 samples contained dirt, and formaldehyde or boric acid was present in 5 cases. Added water was reported in 13 cases in separated or skimmed milk. Of 226 samples of dried milk, only 3 were reported against, and 41 of 1294 samples of condensed milk were defective, mostly in fat.

CREAM.—Thirty-seven of 51 adulterated creams, from a total of 2368 samples examined, contained boron preservative. Two "fresh" creams were artificial or reconstituted; 10 tinned creams were deficient in milk fat or solids, and 95 per cent. of the fat of a restaurant cream was not derived from milk.

BUTTER AND MARGARINE.—Only 149 of 10,965 samples of butter were reported as adulterated, and, of these, 81 contained excess water, 47 consisted wholly or in part of foreign fats, 19 contained boron preservative, 1 cane sugar, and 1 traces

* Obtainable at H.M. Stationery Office, Adastral House, Kingsway, W.C.2. Price 3d. net.

of sand. Of 60 of 3951 margarines, 21 contained excess water, 3 boric acid, 1 had been attacked by fungoid growth, and one contained 85 per cent. of butter fat.

LARD.—Seven of 3152 lards, 20 of 553 drippings, and 12 of 212 suets were adulterated. Two lards contained free fatty acids, and 5 were not derived wholly from pig's fat; the drippings mostly contained excess of water and free fatty acids, but one consisted wholly of vegetable fat, and excess of rice flour was present in suets.

CHEESE.—Of 1321 cheeses, 27 were adulterated. Cream cheese was ordinary whole-milk cheese; skimmed milk cheese was sold as Cheshire; 2 Gorgonzolas had a covering, equivalent to 10 per cent. of the cheese, consisting of 21 per cent. of tallow and 79 per cent. of barytes coloured with oxide of iron. No tin-wrapped cheese examined was free from contamination by tin.

BREAD AND FLOUR.—Three samples of flour (of 543) and 14 of self-raising flour (of 723) were reported against; 2 flours contained arsenious oxide and 1 ammonium sulphate, and one sample of self-raising and one of "diabetic" flour were ordinary flours.

OTHER FOODS.—Thirty-one of 1324 samples of *jams and marmalade* were reported against, 21 for containing excess sulphur dioxide, 2 for glucose, 6 for apple pulp or fruit juices other than indicated on the label, and one sample was decomposed. The sale of artificial for malt vinegar and deficiency in acetic acid accounted for 142 of 1856 *vinegar samples* examined. Of 2444 samples of *spirits*, 275 were reported against as more than 35 degrees under proof. Fifteen of 548 beers contained lead or excess of sulphur dioxide. In 2 cases, 199 and 366 parts respectively, of sulphur dioxide per million were present. Other articles of food examined included 1131 *cocoas* (3 contained extraneous mineral matter); 1692 *coffees* (8 contained chicory); 961 *sugars*; 2940 samples of *sausages, polonies*, etc., of which 265 contained sulphur dioxide not indicated on the label; 10 were deficient in meat and some contained boric acid. One sample of *non-alcoholic wine* consisted of sugar and water acidified and coloured with an aniline dye. Copper sulphate was present in 9 samples of food, and tin in 19 of tinned fruits, fish, etc.

DRUGS.—The 5544 samples of drugs represented 109 different kinds, and 254 were reported as adulterated. These included several camphorated oils deficient in camphor, and one made from mineral instead of olive oil; potassium iodide; distilled water which was ordinary water; cod-liver oil tablets devoid of vitamin *A*; 31 samples of sweet spirit of nitre deficient in ethyl nitrite, and short weight packets of 13 Seidlitz powders.

D. G. H.

Department of Scientific and Industrial Research.

FUEL RESEARCH. Technical Paper, No. 27.

MEASUREMENT OF A RAPIDLY FLUCTUATING FLOW OF GAS.*

THE apparatus described measures and records the volume of gas made during each part or cycle of the normal water-gas process, or during the whole operation,

* By J. G. King and B. H. Williams. Obtainable at His Majesty's Stationery Office, Adastral House, Kingsway, London, W.C.2. Price 6d. net.

and in conjunction with an orifice-plate is recommended for recording variable gas-flow in industrial processes. It is to be used for future investigations of this nature at the Fuel Research Station.

In the present instance the average rate of gas-flow was about 300 cb. ft. per minute, and the process was subject to periodic interruptions for the purpose of removing clinker from the generator. The period of the test was further divided into 14 periods, at the beginning of which coke was charged into the generator, and finally, sub-divided into 6 cycles comprising 1 minute's blow and 4 minutes' gas-making.

GAS-FLOW.—Preliminary experiments were made with an orifice-plate and Pitot tube. The latter was not accurate enough for the small differences and rapid variations in pressure involved, and the former was used, employing Hodgson's formula, the simplified form of which was in the present instance $Q=170\sqrt{P/d}$, where Q is the gas-flow in cb. ft. per minute at 760 mm. and 15° C., P the pressure-drop across the orifice in inches of water, and d the density of the gas at the orifice in lbs. per cb. ft.

P was found as a function of time by means of a sensitive inclined gauge tube, 2·5 mm. wide, containing commercial aniline, and giving a movement of 4 inches for a pressure-change equivalent to 0·5 inch of water. A photographic record was obtained by arranging that the meniscus should move along the tube between a beam of light, and a slit in a light-tight box, behind which was fixed a perforated copper strip and sensitive photographic paper moving round a roller in a direction at right angles to the slit. The perforations, which were 0·3 mm. in diameter and 1 mm. apart, produced a series of parallel straight lines on the chart, which were interrupted by a movement of the opaque gauge-liquid between the paper and the light. The range of the instrument was altered by changing the angle of slope of the tube, and from the speed of movement of the paper and the relation between the pressure-difference at the orifice plate and the movement of the gauge, Q could be calculated. Typical diagrams are shown and the corresponding calculations given.

Calibrations of the gauge against known static pressures showed that the motion of the liquid was proportional to the static pressure for 0 to 0·5 inch of water, errors due to change in shape of meniscus and shrinkage of the photographic paper being negligible. Comparison with values obtained by means of a calibrated gas-holder showed differences varying from +1·77 to −1·89 per cent. for 10 individual cycles, the total difference for the whole experiment being −0·13 per cent.

AIR-FLOW.—It has hitherto been difficult to measure the flow of air in such experiments, owing to the large and fluctuating volumes concerned, so that the volume used during the "blow" period of the water-gas process is usually obtained by calculation from the carbon balance, and is thus based on a difference figure. The extent of the error involved is 5 to 10 per cent. of the thermal value of the coke with which the generator is charged.

Orifice plate and Pitot tube methods applied to exact measurements of air supplied during the blow period are, therefore, described. The agreement is of the order of 1 per cent., but it is concluded that the former method, though not necessarily more accurate, is more practicable in such circumstances. Thus, the Pitot tube must be fixed in one position in the air-main, and a relation found to connect the readings with the pressure distribution along the remainder of the main. The orifice-plate responds rapidly to changes in rate of flow, and the pressure-differences across it are much bigger than those set up in the Pitot tube.

J. G.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

Food and Drugs Analysis.

Detection of Heated Milk. K. Eble and H. Pfeiffer. (*Z. Unters. Lebensm.*, 1930, **60**, 311–314.)—Variations of the benzidine test are described which, it is claimed, give less ambiguous results than the original method. Five c.c. of milk are shaken with 0·5 c.c. of a 4 per cent. solution of benzidine in 96 per cent. alcohol, 1 drop of 1 per cent. (by weight) hydrogen peroxide added after 1 minute, and the mixture again shaken. Raw milk gives a stable blue-green colour, slightly heated milk (*e.g.* 30 minutes at 63° C.) a similar but fading colour, whilst strongly-heated milk is colourless. After 1 minute, 10 c.c. of a saturated solution of magnesium sulphate are added, and the mixture shaken, when raw milk becomes grey-green and turns bluish-green in 10 minutes, whilst heated milk is grey-green and turns to greyish-blue. After a further 10 minutes, dark blue curds separate from slightly-heated milk, whilst in strongly heated milk the curds are white. Alternatively, 5 c.c. of milk, 10 c.c. of magnesium sulphate solution and 1 drop of peroxide may be mixed, and the benzidine added after 1 minute, when raw milk appears bright blue and milk heated at 63° C. bright green, both colours becoming deeper on standing. If magnesium sulphate is replaced by sodium sulphate, the respective colours are chocolate to violet-brown and green-brown. Heated milk having an abnormally high leucocyte content or lactic acid acidity, or heated for a short period below 63° C., tends to give the colour reactions of raw milks, and it is advisable before making the test to add 0·25 *N* potassium hydroxide solution till the acidity is 6°.* These exceptions are due to the peroxidase associated with the proteins or leucocytes of the milk which normally passes into solution when the milk is heated, and so loses its activity, but may be re-activated by lactic acid, etc., and inactivated by addition of amyl alcohol. In doubtful cases a mixture of 10 c.c. of milk, 10 c.c. of a saturated solution of magnesium sulphate and 1 c.c. of 20 per cent. acetic acid is shaken and centrifuged for 6 minutes at 120 revs. per minute. Slightly-heated milk gives a clear serum, raw milk a definitely opalescent serum, and mixed milk containing 20 per cent. or more of raw milk a faint turbidity. J. G.

Phosphatide Contents of Varieties of Bread. B. Rewald. (*Z. Unters. Lebensm.*, 1930, **60**, 315–318.)—The phosphatide content was determined by the author's method (*Biochem. Z.*, 1928, **198**, 103; **202**, 394), the sample being extracted in succession with a large excess of acetone and a mixture of benzene and alcohol. The extracts were evaporated separately, the residues extracted with dry ether, and the phosphorus determined in each case in the residue after evaporation of the ether. The sum of these two figures was taken as the total phosphatide content, the following values (percentages on the dry sample) being found:—Rye bread-crumb 0·174 (crust 0·174), white bread (crust+crumb) 0·287, pumpernickel

* One degree = 0·01 per cent. of acidity as lactic acid.

(crust+crumb) 0·073, black bread-crumb 0·183 (crust 0·194), Graham bread-crumb 0·267 (crust 0·302). The respective phosphorus contents of the residues after extraction were 0·20, 0·19, 0·24, 0·32, 0·34, 0·35, 0·30, and 0·29 per cent. With the exception of pumpernickel, therefore, the different breads examined contain approximately the same amounts of phosphatide, whilst the phosphorus contents of the residues are approximately equal in all cases. The fact that almost equal quantities are found in crust and crumb indicates that temperature is without influence on the phosphatide content, and thus confirms the conclusions resulting from a study of phosphatides of hard-boiled eggs, though, in the case of bread, the phosphatide isolated is a typical plant product. J. G.

Effect of Sodium Bisulphite on the Polarising Power of Sugars. Y. Tomoda and T. Taguchi. (*J. Soc. Chem. Ind. Japan*, 1930, **33**, 434B.)—A table is given showing the effect of sodium bisulphite on eleven sugars, the polarising powers of five of which are markedly affected (*d*-glucose, *l*-xylose, *l*-arabinose, *d*-galactose, and lactose), whilst the polarising powers of the other six are hardly affected at all (maltose, *d*-mannose, sucrose, raffinose, dextrin, *d*-fructose). For instance, the polarising power of glucose is decreased from +15·4° V. to −0·3° V., but that of fructose only from −26·8 to −26·5. This property of sodium bisulphite can be utilised in the analysis of mixtures of sugars containing glucose. Thirty grms. of sodium bisulphite are effective for 100 c.c. of the sugar solution containing 2·5 to 24 per cent. of glucose. If a saccharimeter reading be taken with a 200 mm. tube of a mixed solution of glucose and fructose, in the absence and in the presence of sodium bisulphite, the amounts of glucose and fructose can be calculated from the formula:

$$x = 0{\cdot}3225 \times (P - P')$$
$$y = 0{\cdot}1859 \times (-P')$$

where x = grms. of glucose per 100 c.c.
y = grms. of fructose per 100 c.c.
P = saccharimeter reading °V. at 20° C. in 200 mm. tube
P′ = ditto in the presence of 30 grms. of sodium bisulphite.

The composition of invert sugar can be found in a similar manner after neutralisation with sodium bicarbonate.

In the case of mixtures of glucose and maltose a modified formulae has to be used, since the polarising power of maltose is decreased to a somewhat greater extent (+37·2° V. to +30·9° V.) than that of fructose. Where x and y represent the grms. per 100 c.c. of glucose and maltose, respectively—

$$x = 0{\cdot}3225 \times \left(P - \frac{p}{p'} P'\right)$$
$$y = 0{\cdot}1247 \times \left(\frac{p}{p'} P'\right)$$

where P and P′ represent saccharimeter readings of the mixed solution in the absence and in the presence of sodium bisulphite; p and p', saccharimeter readings

of a maltose solution at a certain concentration in the presence and absence respectively of sodium bisulphite.

The method may also be applied to the analysis of honey and hydrolysed products of starch, etc. R. F. I.

Detection of *Iso*propyl Alcohol in Brandy, Spirits, Tinctures, Cosmetics and Liniments by means of Piperonal. G. Reif. (*Z. Unters. Lebensm.*, 1930, **191**, 243–254.)—The author's method (*id.*, 1929, **57**, 277; ANALYST, 1928, **53**, 497) has been tested with 124 prepared or purchased liniments (the compositions and uses of which are described), 25 samples of brandy, 40 of cosmetics, and a number of spirits and tinctures representing many different types, and the following general procedure found satisfactory:—The sample (10 c.c.) is distilled on the water-bath into a small cylinder immersed in ice-water till no more alcohol is evolved, 0·3 c.c. of distillate shaken with 0·7 c.c. of a mixture of 80 c.c. of absolute alcohol and 20 c.c. of water, and a solution of 0·1 grm. of hydroxylamine hydrochloride in 3 c.c. of water added. The mixture is shaken for 3 minutes at the ordinary temperature, 0·4 grm. of absorbent charcoal (*carbo medicinalis*) added, the mixture shaken and filtered through a small dry paper into a 100 c.c. round-bottomed flask. Five c.c. of a freshly-prepared solution of 0·5 grm. of piperonal in 100 c.c. of absolute alcohol are added, followed by the slow addition, to prevent boiling, of 20 c.c. of sulphuric acid (sp. gr. 1·84). The mixture is shaken, placed on the water-bath for 5 minutes in a large test-tube, when a red colour indicates the presence of *iso*-propyl alcohol, and a green-brown colour its absence. If 30 c.c. of 30 per cent. acetic acid are then added, a rose to red colour, stable for 30 minutes, is a positive reaction (brown in the presence of small amounts of the alcohol), whilst a colourless solution or weak red tinge stable for a minute and turning yellow-grey or colourless shows the alcohol to be absent. The sensitiveness is, in general, 1 to 2 per cent., but 0·1 per cent. for liniments. With these formaldehyde, if present, must be destroyed before distillation by heating the sample with 5 c.c. of sodium hydroxide solution (sp. gr. 1·125) under a reflux condenser for 1 hour. Less than 20 per cent. of acetone has no influence on the reaction. J. G.

Composition and Determination of Water in Tomatoes and Preserved Tomatoes. A. Leonhard. (*Z. Unters. Lebensm.*, 1930, **60**, 185–195.)—The various methods of determination of the dry solids in tomato-products, and their disadvantages, are discussed. The author prefers to obtain the percentage by difference from the water-content determined on 5 to 10 grms. of the mixed sample weighed in an aluminium boat and distilled with 150 c.c. of xylene in a 300 c.c. Erlenmeyer flask on a sand-bath. A delivery-tube, bent at 45° and 350 mm. long, leads into a vertical 150 c.c. graduated receiver, completely immersed in running water, and closed except for a glass tube drawn-out at one end to 2 mm. in diameter and inserted through a rubber stopper. The bottom of the receiver should contain 1 c.c. of 1:1 potassium hydroxide solution, and 150 c.c. should distil over in about

20 minutes. Any condensate is then removed from the delivery tube by application of a small flame, the contents of the receiver shaken gently, and the volume of the separated aqueous layer read after 2 hours, the 1 c.c. of alkali originally present being deducted. Any volatile oils are retained by the xylene. The results were compared for 40 samples of Italian and German origin with those calculated from the sp. gr. of the extract obtained by filtering a warm mixture of 20 grms. of sample and 100 c.c. of water, and washing the residue with small quantities of hot water up to a total filtrate of 200 c.c. This solution was also used for determinations of sugar, acidity, soluble acids, etc., the full analytical results being tabulated. In general, the dry solids obtained by the above method were lower than those obtained from the sp. gr., especially for samples of the more liquid type. The following table gives the percentage compositions of some typical tomato-products:—

Description.	Artificial colour.	Water (direct).	Dry residue (calc.).	Sp. gr. (1:10, filtered).	Dry residue (calc.) from sp. gr.).	Invert sugar.	Sucrose.	Acidity (c.c. *N*KOH).	Ash.	Alkalinity of ash (c.c. *N*KOH).	Chlorine.
Thin liquid purée (Swiss)	+	89·42	10·58	1·0082	10·6	3·72	0·65	11·0	2·39	—	0·87
Thin liquid sauce (German)	+	84·10	15·90	1·0070	18·2	7·64	0·79	11·0	2·60	—	0·90
Thin liquid purée (Swiss)	0	85·45	14·55	1·0133	17·5	6·57	1·62	9·2	3·33	10·5	1·56
Pulp (German)	0	83·85	16·15	1·0069	17·8	7·63	0	13·5	3·20	16·5	1·14
Solid pulp (Italian)	0	67·39	32·61	—	—	13·90	0	31·8	8·43	33·0	3·35
Extract (Italian)	—	64·10	35·90	1·0153	39·5	17·58	1·47	31·0	6·59	34·5	2·20
Almost solid purée (Italian)	0	74·38	25·62	1·0105	27·1	15·94	0·87	22·5	2·48	23·2	0·20
Solid pulp (Italian)	+(?)	71·55	28·45	1·0110	28·9	11·75	0	18·5	4·89	32·0	1·27
Solid pulp (Italian)	+	67·50	32·50	1·0140	36·2	13·66	0	32·0	7·40	33·2	2·78
Solid control-sample (German)	—	74·88	25·12	1·0104	26·2	12·98	0	21·0	3·74	—	1·16
Fresh pulp from 1929 crop (German)	—	92·4	7·6	1·0051	6·6	3·90	0	6·5	0·63	7·0	0·04
Do.	—	94·0	6·0	1·0040	5·2	2·56	0	5·2	0·50	5·5	0·04

J. G.

Determination of Formic Acid in Fruit Juices. A. Hanak and K. Kürschner. (*Z. Unters. Lebensm.*, 1930, **60**, 278–290.)—Ten c.c. of juice (or 10 grms. of fruit syrup diluted to 20 c.c.) are steam-distilled with 0·3 grm. of tartaric acid, so that after 45 minutes there is very little increase in the volume of liquid in the distillation flask, which should on no account exceed 20 c.c. Overheating is avoided by means of an asbestos shield, and 400 c.c. of distillate are collected in sufficient distilled water just to cover the end of the condenser adapter. The distillate is diluted to 500 c.c., 200 c.c. of which are titrated, and the total free, volatile acids calculated. The calculated amount of 0·5 *N* sodium hydroxide solution required to neutralise the acidity is then added to 100 or 200 c.c. of distillate (according to the amount of formic acid present), followed by 0·5 to 1 c.c. in excess. The mixture is warmed for 10 minutes to saponify any formic esters, and then evaporated with 0·2 grm. of sodium carbonate, without boiling, till 30 c.c. remain. A 0·2 *N* solution of potassium permanganate is added, so that

there is an excess of about 1 to 1·5 c.c.(3 and 5 c.c. being usually required for 100 and 200 c.c. of original solution, respectively). Oxidation is complete after 45 to 60 minutes, when 1 c.c. of 10 per cent. zinc sulphate solution is added to flocculate any suspended matter, and the solution diluted to 50 c.c. and filtered and the excess of permanganate determined colorimetrically. For this purpose the colour of 25 c.c. of filtrate is matched in a colorimeter against that of a 0·2 *N* solution of potassium permanganate. Since the sensitiveness of this colorimetric method is 0·1 c.c., and 1 c.c. of 0·2 *N* permanganate solution ≡ 2·76 mgrms. of formic acid, the method should be accurate within ±0·276 mgrms. Solutions containing 0·13 to 0·34 grms. of formic acid per 100 c.c. gave results with an error of 0 to +6 mgrms. Fincke's mercuric chloride method (*id.*, 1911, **21**, 1; Reisser, *id.*, 1916, **96**, 355), which is discussed critically, gave results 10 to 30 mgrms. low. The method was tested on apple juice (16·7 mgrms. per 100 c.c.), currant (40·45), bilberry (45·5), and other fruit juices. J. G.

Koryo (Millet Seed) Oil. S. Ueno and N. Kuzei. (*J. Soc. Chem. Ind. Japan*, 1930, **33**, 452B.)—The oil used was extracted with ether from the ground grains of millet, *Andropogon sorghum vulgaris*, Hack (Manchu). It amounted to about 3 per cent. of the grains, was a dark brown paste having an odour resembling ethyl alcohol. The constants of the refined oil were:—Sp. gr. at 20/4° C., 0·9206; n_D^{40}, 1·4659; acid value, 40·0; saponification value, 183·0; unsaponifiable matter, 5·43 per cent.; m.pt. of unsaponifiable matter, 67·8 to 80·7° C.; with iodine value, 68·4; iodine value of the oil, 114·0. The lead salt and ether method showed the fatty acids to consist of 71·6 per cent. of liquid acids (iodine value 120), and 28·4 per cent. of solid acids (iodine value 13·1). The unsaponifiable matter appeared to contain considerable amounts of hydrocarbons (m.pt., 60·5 to 61·8°), higher alcohols and sterols. The unsaturated acids consisted of oleic and linolic in about the proportion of 2:1. The saturated acids were mostly palmitic with traces of myristic and stearic acids. R. F. I.

Oil from the Seeds of *Asteriastigma Macrocarpa*. D. H. Peacock and Chit Thoung. (*J. Soc. Chem. Ind.*, 1931, **50**, 7–8T.)—The seeds of *Asteriastigma macrocarpa* are known in Burma as Kalaw seeds, a term which includes seeds of other species, most of which contain optically active fatty acids. (*Burma Forest Bull.*, No. 21.) The oil extracted by means of benzene, and dried, had: Sp. gr. 35° C., 0·9501; n_D^{29}, 1·4790°; $[\alpha]_D^{29}$ +52·8; iodine value, 112·3; saponification value, 195·6; and unsaponifiable matter, 3 per cent. The oil was saponified with alcoholic potash, the potassium salt extracted with ether, the recovered acids dissolved in alcohol, and treated with half the requisite quantity of lead acetate. The precipitated salt contained no ether-soluble fraction. The alcoholic solution of the rest of the fatty acids was treated with excess of lead acetate, and the acid recovered from the ether treatment of the precipitated salts had a negligible rotatory power and an iodine value of 90, and was proved to be oleic acid (5·7 per cent. on the original oil). The mixture of fatty acids was converted to the acid chlorides and then the amides. The acids were esterified, and four fractions

were obtained distilling (1) below 210°; (2) 210°–220°; (3) 220°–225°; (4) 225°–235° C. After redistillation the rotations were (1)+37°; (2)+37°; (3)+41°; and (4)+44°. These fractions were redistilled twice; and eventually, after further analyses, it is concluded that a saturated acid, possibly palmitic, is present, and also chaulmoogric acid, or possibly a mixture of chaulmoogric and hydnocarpic (although the latter could not be isolated) to the extent of about 75 per cent. of the original fatty acids. D. G. H.

Quantitative Determination of Cholesterol and Lecithin. Evaluation of Egg Products. J. Tillmans, H. Riffart and A. Kühn. (*Z. Unters. Lebensm.*, 1930, **60**, 361–389.)—The methods suggested by other workers for these determinations are critically discussed, and the following procedure (after Szent-Györgyi) recommended for *cholesterol*:—A solution of not more than 4 mgrms. of cholesterol in 2 c.c. of warm acetone is evaporated on the water-bath with 1 c.c. of a 2 per cent. solution of pure digitonin in 80 per cent. alcohol till 1·5 c.c. remains. After 15 minutes at room-temperature the mixture is filtered on a sintered-glass crucible enclosed in a jacket through which steam can be passed, the precipitate transferred with acetone and given 2 washes with ether, followed by 3 with warm chloroform, 2 with ether, 1 with acetone, and 1 with cold water, 1·5 c.c. at a time. The suction is adjusted to filter 1 drop per second, and the precipitate should always be covered with liquid to protect it from the air. Steam is then passed through the jacket, 10 (1·5 c.c.) portions of water added, and the washing finished, using the full suction of the pump. The suction and heating are continued, 10 c.c. of a clear 1 per cent. solution of potassium dichromate in concentrated sulphuric acid pipetted slowly on to the precipitate so as to fill one-fourth of the crucible, the crucible washed with 3 (1 c.c.) portions of cold water, sucked well and the total filtrate collected. After 1 hour oxidation is complete; 100 c.c. of water and 10 c.c. of 5 per cent. potassium iodide solution are added, and the liberated iodine titrated with 0·1 *N* sodium thiosulphate solution, using 10 drops of 1 per cent. starch solution for every 10 c.c. of chromic acid taken, allowance being made for the blank titration. The mean results of 15 experiments with pure cholesterol showed that the volume of thiosulphate solution equivalent to the chromic acid used up, divided by 8·7, gives the cholesterol content (in mgrms.) for 0·5 to 4 mgrms. The factor 7·9 was found for phytosterol. The m.pts. were 145° and 136° C. for the two sterols, recrystallised from absolute alcohol, and 114° and 126° C. for their acetates, respectively.

Egg-products are first dried on the water-bath with sand, extracted in a Soxhlet apparatus with ether, and the residue, after evaporation, dried at 100° C. and weighed. A weighed portion is then dissolved in warm acetone, the solution filtered, diluted to a suitable volume with acetone, and the above procedure followed. This gives "free" cholesterol. Another portion of the ethereal extract is then saponified on the water-bath for 1 hour with a 20 per cent. alcoholic solution of potassium hydroxide (or by heating with 20 c.c. of 5 per cent. sodium alcoholate for 4 hours), the residue dried, mixed with sand and extracted as before, to give the

cholesterol after saponification. The results obtained for 8 samples of egg-products are slightly higher than those of other workers, and a mean value of 239 mgrms. of cholesterol, calculated on the basis of an egg-yolk weighing 16 grms., was found (without saponification). After saponification the values were 10 to 80 mgrms. higher. Pastry is ground, 20 grms. extracted for 6 hours with ether, and the above method applied. Various products gave 10·2 to 25·6 mgrms. per 100 grms. of dry sample, whilst vermicelli, etc., containing dried or fresh egg contained 81 to 140 mgrms. per 100 grms. of dry material. Fats are melted and filtered, and 0·2 grm. dissolved in 2 c.c. of acetone. In such cases the digitonin precipitate should be washed with ether (5 times), warm chloroform (5), ether (3) and twice with acetone (1·5 c.c. each time). The results agree well with those of Bömer (*id.*, 1901, **4**, 1070), but are much higher than those of other workers, *viz.* 140 to 370 mgrms. per 100 grms. for butter, lard, beef-fat, etc.

Lecithin should be determined as follows (*cf.* Juckenack, *id.*, 1904, **8**, 94):— The powdered sample (10 grms.) is extracted for 3 hours with hot alcohol in a Soxhlet apparatus, and the residue left on evaporation of the extract is warmed with 15 c.c. of perhydrol and 5 to 10 c.c. of concentrated sulphuric acid till brown in colour. The cool mixture is diluted to 100 c.c., 25 c.c. neutralised with ammonia to methyl orange, and the lecithin phosphate precipitated in the cold in a volume of 60 c.c. with 5 c.c. of 1·5 per cent. strychnine nitrate solution and 15 c.c. of a mixture of 33·33 grms. of ammonium molybdate in 100 c.c. of water and 300 c.c. of dilute nitric acid containing 200 c.c. of acid of sp. gr. 1·4 (*cf.* Embden, *Z. physiol. Chem.*, 1921, **113**, 138). After 20 minutes the precipitate is filtered off on a weighed crucible, washed with 25 c.c. of the ice-cold mixed reagents (diluted 5-fold), and then with ice-water till free from acid. The weight of precipitate (dried at 100° C.) divided by 39 gives P_2O_5, and the method has an accuracy of 0·02 mgrm. for 0·5 to 4 mgrms. of P_2O_5. The agreement with Juckenack's method (*loc cit.*) is good, but the colorimetric method of Misson (*Chem. Ztg.*, 1908, **32**, 633) gives higher results. The contents of water, cholesterol and lecithin (found by the above method and by Juckenack's method, *loc. cit.*), are tabulated for 21 types of pastry-products containing eggs, together with tests for artificial colouring matters. A table is also given for the calculation of the egg content of a sample from the cholesterol content, 58·7 and 71·4 mgrms. per 100 grms. of ordinary and hard (*e.g.* macaroni) pastry, respectively, corresponding with 1 egg per 500 grms. of flour, and 151·5 and 164·2 mgrms. respectively, with 3 eggs. Storage of such trade-samples for 1 year produced no reduction in cholesterol or lecithin content, but home-made products showed a marked decrease, especially in lecithin content. This fall is attributed to the influence of exposure to light and to the water content. Tollens' reagent (*Z. Unters. Lebensm.*, 1904, **7**, 22) and Popp's ultra-violet light method (ANALYST, 1926, **51**, 540) were found unreliable for differentiating the various types of protein in pastry. In general, "free" lecithin, determined on an extract of the sample in petroleum spirit, was found to be less for preserved than for fresh samples, but the method is not reliable for the purposes of differentiation.

J. G.

Determination of Nitrite and Sulphite in the Presence of one another in Salt Mixtures and in Meat Products. E. Szabó. (*Z. Unters. Lebensm.*, 1930, **60**, 389–395.)—The salt is dissolved in a little boiled water, sodium carbonate added, and the sulphite precipitated by a cold solution of lead acetate or nitrate. The mixture is diluted to a known volume, filtered, and an aliquot portion taken for the colorimetric determination of the nitrite by means of *m*-phenylene-diamine. The precipitate is washed with cold water, suspended in cold boiled water, and the sulphur dioxide determined by acidification and distillation in a stream of carbon dioxide in the usual way. Alternatively, the sample may be dissolved in a dilute solution of sodium bicarbonate, and the sulphur dioxide determined by addition of a slight excess of 0·1 *N* iodine solution and back-titration with 0·1 *N* sodium thiosulphate solution ($Na_2SO_3+I_2+H_2O=Na_2SO_4+2HI$). The air is then removed by means of a stream of carbon dioxide, the solution acidified with 1 c.c. of 0·1 *N* sulphuric acid, and after 1 minute (during which period the beaker of liquid should not be disturbed, so as to retain the carbon dioxide and exclude air) the free iodine is titrated with the thiosulphate solution ($2HNO_2+2HI=2H_2O+2NO+I_2$). The stream of carbon dioxide serves to mix the solutions. Potassium iodide solution should be added in the second titration only if the amount of nitrite is in great excess. A maximum error of approximately ±1 mgrm. is recorded for both determinations in the case of solutions containing mixtures of 65 to 100 mgrms. of sodium sulphite and 25 to 100 mgrms. of sodium nitrite. Meat samples are extracted with sodium bicarbonate solution, and the extract should be free from substances which may interfere with the iodimetric titration. J. G.

Potentiometric Determination of Alkaloids by Means of Potassium Iodomercurate. L. Maricq. (*Bull. Soc. Chim. Belg.*, 1930, **39**, 496–502.)—An extension of the author's method (ANALYST, 1930, **55**, 284) is described in which the alkaloid is precipitated by means of potassium iodomercurate, the excess of which is determined potentiometrically by means of a standard solution of mercuric chloride. Four possible alkaloid iodomercurates of the type $(HgI_2)_nA,HI$ may be formed according to the number of molecules of mercuric iodide in the complex, and it is necessary to calculate an empirical coefficient for each alkaloid. Atropine is used as a solution of 0·01 to 0·02 grm. in 5 to 15 c.c. of 0·02 *N* sulphuric acid, and it is found that the factor 0·001446 gives results having a maximum relative error of 2 per cent. For hyoscyamine the same factor is used, and the maximum error is 16 per cent. For pilocarpine the factor is 0·00104 (error 1·1 per cent.), and there is evidence that, if the amount taken exceeds 0·01 grm., there is a high negative error which may, however, be eliminated by addition of a little solid mercuric iodide at the moment of precipitation, followed by vigorous shaking. This error, which occurs also with excessive quantities of atropine or hyoscyamine and may be eliminated by a similar procedure, is due to an insufficiency of mercuric iodide in the reagent used, resulting in a complex of the wrong composition which is redissolved by the hydriodic acid liberated by the reaction itself. For cinchonine and sparteine 5 to 15 c.c. of a solution of 0·005 to 0·015 grm. of alkaloid in 0·05

and 0·02 *N* sulphuric acid, respectively, are used, the factors being 0·00073 and 0·00058, and the errors 2·0 and 1·8 per cent., respectively. Since 1 c.c. of 0·005 *N* mercuric chloride solution corresponds with 5×10^{-6} grm. mol. of a monobasic alkaloid, the factors are given in each case by the product, molecular weight $\times 5 \times 10^{-6}$. The method is confirmed by the results which, in each case, correspond with the type of complex shown by other workers to be formed by the alkaloid concerned. J. G.

Identification of Alkaloids by Precipitation. C. C. Fulton. (*J. Assoc. Off. Agric. Chem.*, 1930, **13**, 491–497.)—A natural classification of the alkaloids, based solely on their precipitation from dilute solution, is described, the key to the classification being the relative sensitivity of different reagents to each alkaloid. Amines and similar basic substances precipitated by the same reagents are included in the scheme and, for convenience, the other reagents are compared with phosphomolybdic acid. The procedure employed is as follows: A drop of a solution either of the alkaloid salt in water or of the alkaloid itself in dilute acid (sulphuric) is treated on a microscope slide with a drop of 10 per cent. phosphomolybdic acid solution. If a heavy precipitate is formed, the alkaloid solution is diluted until no more than a distinct precipitate is obtained with the reagent. This standard solution, which is nearly always considerably weaker than 0·1 per cent., is tested similarly with other reagents.

Tests thus made on a number of alkaloids show that the reagents may be so arranged that the effect of many reagents can be predicted with certainty from the result obtained with the first one tried. For instance, if picric acid precipitates the alkaloid in the standard solution, then Mayer's reagent, gold chloride, and bromine water will also precipitate it; whilst if picric acid does not precipitate it, platinum chloride, potassium chromate, and potassium hydroxide will also fail. The alkaloids may be similarly arranged. In the following arrangement of some of the principal alkaloids and reagents, each alkaloid is precipitated in the standard solution by all the reagents preceding it and by none of those succeeding it: phosphomolybdic acid, caffeine, Wagner's reagent, atropine, gold chloride, morphine, Marme's reagent, cocaine, picric acid, diacetylmorphine (heroine), ammonium molybdate, quinine, platinum chloride, strychnine, potassium chromate, papaverine.

Extension of this scheme to all the alkaloids and reagents reveals certain inconsistencies, mostly due to oxidation phenomena, but these disappear if the alkaloids are arranged in six groups, into which many (probably most) of the amines also fall. By means of this arrangement, which is fully described, it is possible, in combination with microscopic tests, to identify 0·005 grm. of any pure alkaloid. Moreover, it is usually possible to tell if a certain alkaloid is detectable in presence of another or if the two can be separated by precipitation, and, if so, with which reagents. T. H. P.

Determination of Nicotine in Commercial Solutions and Insecticides. A. Sabatié. (*Ann. Falsificat.*, 1930, **23**, 544–547.)—Nicotine may be conveniently

determined polarimetrically, pyridine and similar bases, which are sometimes used as adulterants, being optically inactive. Twenty-five c.c. of the commercial solution are diluted to 100 c.c., and 25 c.c. of the diluted solution transferred to a long-necked flask connected with a condenser and with a steam generator. The nicotine is liberated by addition of 2 c.c. of sodium hydroxide solution (36° Bé.), and is distilled off in a current of steam, which is passed slowly at first in order to obviate frothing. Entrainment of drops of liquid is easily prevented by baffling the end of the delivery tube in the flask with a disc of rubber held in position by two pins stuck into the stopper. The nicotine is carried over in the first fraction of 250 c.c., but further fractions of the same volume are collected and examined polarimetrically in a 50 cm. tube. In solutions with concentrations up to 35 per cent. the specific rotation of nicotine $[\alpha]_D$ has the value $-80{\cdot}2°$.

The result may be confirmed: (1) by titrating the nicotine solution with 0·1 *N*-acid in presence of methyl red as indicator, and (2) by precipitating the base with potassium silicotungstate in solution acidified with hydrochloric acid, weighing the double salt dried at 120° C., and again weighing the residue of silica and tungstic acid left after calcination. Multiplication of the weight of double salt by 0·1011, or that of the mixed residue by 0·1139, gives the weight of nicotine.

If the nicotine solution is adulterated with pyridine, the results obtained by methods (1) and (2) are higher than that of the polarimetric determination. If ammonia has been added as adulterant, the acidimetric result is higher than the polarimetric and precipitation results. T. H. P.

Determination of Picric Acid in Solution. M. François and L. Seguin. (*J. Pharm. Chim.*, 1930, **132**, 433–438.)—In the absence of phenol, the necessary quantity of the liquid under investigation, containing about 5 or 6 cgrms. of picric acid, is made up to 50 c.c. with water, and an excess of ammoniacal copper sulphate solution added (copper sulphate, 10 grms., 50 c.c. of ammonia, made up to 250 c.c. with water) so that the liquid over the precipitate is distinctly blue. (Thirty c.c. are necessary for the precipitation of 0·1 grm. of picric acid.) After standing one hour, the liquid is filtered through two weighed 9 cm. papers, and the precipitate carefully washed with 10 c.c. of dilute (1 : 5) ammonia, dried in air without heating, and weighed. The weight of ammonium copper picrate is multiplied by 0·7563 to give the weight of acid. If desired, the ammonium copper picrate, $[C_6H_2(NO_2)_3OH]_2CuO.2NH_3$, may be converted into insoluble black copper oxide and ammonium picrate by means of hot water, the filtrate evaporated, and the residue of crystalline ammonium picrate weighed. In the presence of phenol, the same procedure may be used if the time of contact between the ammonium copper picrate precipitate and the mother liquor is limited to one hour, or the phenol may be eliminated by first adding 1 grm. of crystallised sodium carbonate, so that insoluble sodium picrate is formed, after which the phenol is removed by four extractions with chloroform (20 c.c. each time), and the picric solution is warmed to expel any chloroform, and filtered, and the determination continued as before. In the presence of complex materials such as bread, the liquid portion is filtered

off, the solids washed, and to 100 c.c. are added 5 grms. of white wool in small pieces, and after 24 hours another 5 grms., and so on, until 20 grms. have been used, when all the picric acid will have become fixed. After washing with water, the wool is extracted with ammoniacal alcohol (ammonia 22 per cent., 5 c.c.; alcohol 95 per cent., 50 c.c.; and water 45 c.c.). The resulting ammonium picrate dissolves in the liquid, and after the alcohol has been expelled, the picric acid is precipitated with ammoniacal copper sulphate as described above. D. G. H.

Reactions of Antipyrin and Pyramidone. Determination of Antipyrin in Pyramidone. M. Ribere. (*J. Pharm. Chim.*, 1930, **122**, 444–446.)—To 2 c.c. of 1 per cent. aqueous solutions of (*a*) antipyrin, and (*b*) pyramidone 12 drops of the reagent to be used are added. With 20 per cent. trichloracetic acid a white precipitate soluble in excess or on heating is formed with (*a*) and the reaction is negative with (*b*). Perchloric acid gives similar results. Ammoniacal silver nitrate is immediately reduced by (*b*) on warming, but not with (*a*). Six drops of acid mercuric nitrate solution (prepared by dissolving 2 grms. of mercury in 20 grms. nitric acid, and shaking until almost complete disappearance of nitric fumes), gives with (*a*) a white cloudiness, increasing to an abundant precipitate, transformed on shaking into a greenish coagulate, which dissolves on boiling and reappears, of a brick red colour, on cooling. A violet oxidation colour is formed with (*b*). If 20 per cent. trichloracetic acid is added to this acid mercuric nitrate reagent, as little as 0·1 per cent. antypyrin may be detected in pyramidone. One-tenth of a grm. of the suspected material is dissolved in 10 c.c. of water, and to 2 c.c. 6 drops of reagent are added. For over 25 per cent. of antypyrin the reaction is the same as for 100 per cent. Up to 15 per cent. the light white precipitate dissolves on heating to an orange yellow solution, depositing on cooling a slight brick red precipitate; for 10 per cent. a red coloration is obtained on warming, and for 1 per cent., 1 c.c. of reagent is introduced without mixing to 2 c.c. of the solution, and at the junction of the liquids a violet ring (due to oxidation of the pyramidone) is surmounted by a white cloudiness rapidly attaining 1 cm. in height. The limit for the test is about 0·1 per cent. D. G. H.

Biochemical.

Plasma Phosphatase. I. Method of Determination. Some Properties of the Enzyme. H. D. Kay. (*J. Biol. Chem.*, 1930, **89**, 235–247.)—The phosphatase of the blood, and particularly that of the blood plasma, has heretofore been relatively little investigated. Preliminary experiments by the author showed that normal plasma contained a weak glycerophosphatase active over a fairly wide *p*H range from 7·0 to above 9·0. To compare one plasma with another it was necessary to evolve a reliable quantitative method for the determination of this enzyme, and details are given of a simple method which has been devised for the determination of the phosphatase content of the blood plasma of man and of other animals. After many trials to work out a method it was decided not to endeavour

to determine the activity at the optimum hydrogen ion concentration, but to use separated plasma at its natural *p*H (of 7·5 to 7·6) plus substrate at about the same *p*H, to determine the actual *p*H at which the hydrolysis proceeded, and to calculate, from the amount of free phosphate actually liberated the amount that would have been produced had the reaction proceeded at exactly *p*H 7·6, all the *p*H determinations being made at room temperature. A plasma sample containing 1 unit of phosphatase per c.c. is one of which 1 c.c. will liberate 1 mgrm. of inorganic phosphate (expressed as P) from excess sodium β-glycerophosphate solution in 48 hours at 38° C., and a *p*H of 7·6, the hydrolysis to be conducted in absence of added buffer, the *p*H determinations to be made at room temperature, and other conditions as stated. In normal plasma the amount of the enzyme per unit quantity of plasma is small compared with the amount present in an equal weight of tissues such as kidney, intestine, or bone. In a determination of the phosphatase content of plasma it is necessary to avoid both haemolysis and the presence of leucocytes in the plasma, since both red and white cells contain more phosphatase than plasma. Certain properties of the plasma enzyme are described. It appears to have the same optimum *p*H for activity as that of the phosphatases of other mammalian tissues (*p*H 8·8 to 9·2). Magnesium ions act as a powerful stimulant to its activity, with an optimum *p*Mg. between 1 and 2. Calcium ions act as a mild inhibitor. The enzyme will hydrolyse all the phosphoric esters which have so far been presented to it, namely, hexosediphosphate, synthetic α- and β-glycerophosphates, pyrophosphate, glycophosphate and guanine nucleotide. The hydrolysis of each of these substrates, with the possible exception of hexosediphosphate, is stimulated by magnesium ions. In young normal children, and in young animals generally, the blood plasma has a higher phosphatase content than in the adult. P. H. P.

Gasometric Determination of Cysteine and Cystine. H. D. Baernstein. (*J. Biol. Chem.*, 1930, **89**, 125–131.)—A method for the determination of cysteine and cystine is described which depends upon the reducing properties of the sulphydryl group of cysteine when oxidised in acid (2 *N*) by a solution of iodine in potassium iodide. Cystine may be reduced quantitatively by nascent hydrogen to cysteine, which is then oxidised back to cystine. A known excess of iodine is allowed to react with the cysteine, and the excess is determined gasometrically by a hydrazine titration in the apparatus of Van Slyke and Neill. Nascent hydrogen evolved from Devarda's alloy in acid solution appears to be an ideal reducing agent for cystine. Under the conditions described pure cystine is quantitatively reduced to cysteine in 10 minutes, and the excess alloy is then filtered off without difficulty. A series of preliminary experiments established the fact that an exact relationship exists between iodine taken and nitrogen liberated from hydrazine in the gasometric apparatus. It is believed that any improvement in the method of cystine assay is of real value because of the importance of cystine as an essential constituent of food and protoplasm, its probable rôle in biological oxidation, and its relation to glutathione and perhaps insulin. The method

described avoids the danger of destruction incident to alkaline hydrolysis, and the errors due to solubility in the isolation and the weighing of the amino acid itself, and is applicable to the determination of cystine in pure proteins and to its liberation during protein hydrolysis. The method is simple, rapid and accurate to about 4 per cent. A mixture which contained 2·31 mgrms. of cysteine and 1·51 mgrms. of cystine gave on analysis 2·32 and 1·52 mgrms. respectively.

P. H. P.

Saponin from Spinach. O. Dafert. (*Z. Unters. Lebensm.*, 1930, **60**, 408–409.)—Very little saponin occurs in the leaf of spinach, and none in the stalk and seed. The haemolytic index of the root, due to its saponin content, is 1:1000 for sun-dried roots, and 1:400 if dried in an oven at 100° C. It rises from 1:85 for a plant 3 weeks old to a maximum of 1:800 after 7 weeks, and then falls to 1:280 after 20 weeks. The saponin is extracted from the scraped, sun-dried root in a 5-fold volume of 70 per cent. alcohol for 2 hours, followed by extraction for 1 hour with a 2-fold volume of alcohol. The extracts are filtered, the residue pressed, and the crude saponin then separates from the filtrate after 1 day in the ice-chest. It is filtered off, washed with absolute alcohol, the filtrate evaporated to one-third of its volume, cooled, and an additional yield of saponin is thus obtained. The total quantity is dried, extracted with ethyl acetate, and the saponin recrystallised from hot 96 per cent. alcohol in pointed white needles ($C_{42}H_{78}O_{24}$, haemolytic index 1:100,000, ash 1·2 per cent., m.pt. 260° to 262° C.). It is readily soluble in dilute methyl or ethyl alcohols or in alkalis or hot water, but is insoluble in chloroform, ether, petroleum spirit, benzene, xylene, acetone, or amyl alcohol. No second saponin was obtained. Hydrolysis with sulphuric acid in 50 per cent. alcohol, and treatment with charcoal gave a white amorphous sapogenin ($C_{24}H_{44}O_7$, m.pt. 208° to 212° C.), soluble in alcohols or in ethyl acetate, and insoluble in water, ether or benzene.

J. G.

Toxic Effect of Fish Liver Oils and the Action of Vitamin *B*. E. R. Norris and A. E. Church. (*J. Biol. Chem.*, 1930, **89**, 437–449.)—Three years ago in the biological assay of fish liver oils for vitamin *A*, it was noticed that relatively small amounts of some fish liver oils (less than 2 per cent. of the diet) had a very deleterious effect, and a systematic study was initiated to determine what factor or factors of the oil were toxic or injurious. Previously the toxic effects of cod-liver oil had been noticed, and efforts had been made to correlate the effect with hypervitaminosis of one of the fat-soluble vitamins, the excess of free fatty acids, etc., but the published results are very contradictory. The results of the present study show that some cod-liver oils produce symptoms similar to vitamin *B* deficiency when given in relatively large amounts to animals (rats) on a diet containing as high as 10 per cent. of yeast. The toxic effect of excess of cod-liver oil can be counteracted by giving large amounts of yeast. The toxic effect of large doses of some cod-liver oils is not due to hypervitaminosis caused by excess of vitamins *A* or *D*. Continued small doses of *iso*-amylamine in amounts

which may be found in cod-liver oil produce paralysis, convulsions and lack of growth; the toxic effect may be prevented or cured by added yeast. Continued small doses of choline also produce symptoms identical with vitamin *B* deficiency, which may be prevented or cured by added yeast. The quantitative determination of vitamin *A* in cod-liver oil is influenced by the amount of yeast in the basal diet, even when there is sufficient yeast to satisfy the vitamin *B* requirements under some conditions. It is pointed out that determinations of vitamin *B* by methods of increase in rat weight, where a cod-liver oil of unknown toxicity is incorporated in the diet, cannot be compared. These results might explain some of the discrepancies and variations in both vitamin *A* and *B* determinations. P. H. P.

Study of the Antimony Trichloride Colour Reaction for Vitamin *A*. III. Effect of Concentration of Reagent Used, and the Stability of the Chromogenic Substance to Light. E. R. Norris and A. E. Church. (*J. Biol. Chem.*, 1930, **89**, 421–435.)—The effect of concentration of the antimony trichloride reagent upon the colour produced with the chromogenic substance of cod-liver oil has been shown. At low colour values the variation of the observed colour with concentration of antimony trichloride is slight. With high concentrations of oil the rate of fading of the colour produced, and consequently the observed colour at 30 seconds is dependent upon the light used for measurement of the colour. Experiments were made to determine which wave-lengths of the visible spectrum were most destructive to the chromogenic substance of cod-liver oil. The results showed that the chromogenic substance of cod-liver oil or vitamin *A* is rapidly destroyed by light of wave-length less than $500m\mu$, and practically unaffected by light of wave-length greater than $500m\mu$. Tables and graphs show the results. It has also been shown that the presence of oxygen increases the rate of destruction of vitamin *A* by light. P. H. P.

Antineuritic Vitamin. A. G. Van Veen. (*Rec. Trav. Chim. Pays-Bas*, 1930, **49**, 1178–1179.)—By suitable treatment of yeast or rice-bran extracts with aromatic acid chlorides or, better, sulphochlorides, it is possible to remove many physiologically active and other substances without destroying the antineuritic vitamin, which, when it has been purified to a certain degree, may be obtained crystalline by utilising the slight solubility of its double salt with platinum or cadmium chloride. By this procedure, 75 kilos. of rice-bran yield, in a short time, about 140 mgrms. of crystalline, but not quite pure vitamin, which is readily purified further by recrystallisation. The product thus obtained responds only faintly to Pauly's reaction with diazotised sulphanilic acid, and, if crystallisation is repeated, the coloration given is scarcely distinguishable from that of the control, although the activity of the vitamin is equal to that of Jansen and Donath's preparation. T. H. P.

Stability of Vitamin *G* as Measured by its Growth-Stimulating Effect. N. B. Guerrant and W. D. Salmon. (*J. Biol. Chem.*, 1930, **89**, 199–211.)—Water-soluble vitamin *B* is composed of at least two factors, one of which, the

so-called growth factor, was thought from early work to be more resistant to destruction or inactivation by heat than the antineuritic factor. During attempts to concentrate and purify this supposedly stable factor numerous irregularities were met which seemed to show that destruction or inactivation had taken place during the treatment. The authors have, therefore, investigated the effect of heat, hydrogen ion concentration, oxidation, reduction, de-amination and ultra-violet irradiation upon the growth-promoting activity of vitamin *G* in yeast or yeast extract. Young albino rats were used for the tests. The results show that yeast and yeast extracts, autoclaved for 4 hours at 20 pounds, lost some of their growth-stimulating effect. Dry yeast heated for 4 hours at 120° C. showed a similar loss of activity. The destruction of vitamin *G* in yeast extracts by autoclaving apparently depends upon the *p*H of the extract when autoclaved. Autoclaving of alkaline extracts results in a greater destruction than a similar treatment to an acidic extract. The passing of gaseous oxygen through a hot concentrated yeast extract for 4 hours had very little effect upon its growth-stimulating qualities. The passing of hydrogen sulphide gas through a hot concentrated yeast extract for 4 hours had no effect upon its growth qualities. Whether partial deamination of yeast extracts decreased their growth-promoting effect seemed to depend upon side reactions, rather than upon deamination of the free amino groups. Yeast extracts concentrated to a thick syrupy consistency and irradiated at a distance of 20 cm. from a mercury arc showed some decrease in growth-stimulating effect. The total destruction was greater in alkaline than in acid solution, the result apparently being the combined effect of the separate action of alkali and irradiation.

P. H. P.

Bacteriological.

Acido-proteolytic Bacteria in Pasteurised Milk. C. Gorini. (*Compt. rend.*, 1930, **191**, 885–886.)—In the course of the control of pasteurised milk during last summer at the central dairy of Milan, it was noticed that the bottles kept at 22–30° C. showed varying behaviour. Whereas, in most cases, alteration of the milk occurred only after 48–54 hours, with formation of a firm, compact curd and an acidity of 5–7 per 1000, in other instances change took place after 28–34 hours, a soft, flocculent curd and lactic acidity of 2–2·5 per 1000 being developed. The firm curds contained a preponderance of lactic organisms and acido-proteolytic coccus forms, whereas the soft ones showed principally acido-proteolytic bacilli, which are facultative thermophilic organisms of the *Subtilis* group. The latter are among the most injurious micro-organisms to pasteurised milk and, in order to prevent their development during the cooling subsequent to pasteurisation, it is necessary to effect this cooling to below 10° C. as rapidly as possible. T. H. P.

Testing of Wood Preservatives. (*Nature*, 1930, **126**, 921–922.)—The following conclusions were arrived at by the conference held in June, 1930, at the Berlin–Dahlem Biologische Reichsanstalt, at which Austria, Denmark, Germany, Great Britain, Holland, Japan, Norway, Switzerland, and the U.S.A.

were represented, but these were regarded as in no way final, and a working committee was set up. The effectiveness of a wood preservative against fungi may be suitably estimated by the wood-block method, using Kolle flasks, and the inhibition point should be expressed as kilos. of preservative per cb. metre of wood. The agar method is only to be regarded as of value for preliminary investigation of a new material, and the inhibition point is the interval between that concentration of the material under test in the medium at which growth just occurs, and that at which it is prevented. The wood should be impregnated with two fungi, one of which should be *Coniophora cerebella*, whilst the exact species of the other has still to be selected. The fungi should be chosen after consideration of the use to which the timber is eventually to be put, and the choice will also obviously depend upon the species of wood used (frondose or coniferous). If the values are to be internationally comparable, different research institutes must use the same strains of fungi of known origin. The determination of the toxicity to fungi of a material must be supplemented by investigation into susceptibility to leaching and physical and chemical stability. Tests against spore infection are as important as tests against infection by mycelium. D. G. H.

Agricultural.

Colorimetric Determination of Phosphoric Acid in Hydrochloric Acid and Citric Acid Extracts of Soils. R. G. Warren and A. J. Pugh. (*J. Agric. Sci.*, 1930, **20**, 532–540.)—The soil is powdered (1 mm. sieve), 20 grms. boiled in 70 c.c. of hydrochloric acid, and the mixture allowed to stand on the water-bath for 48 hours, cooled, diluted, filtered and the filtrate diluted to 250 c.c. An aliquot (15 c.c.) portion is then heated on a sand-bath for 15 minutes with 0·5 c.c. (2 c.c. for peaty soils) of 20 per cent. sodium permanganate solution to oxidise organic matter, the liquid diluted to 30 c.c., 6 c.c. of 10 per cent. potassium ferrocyanide and 5 c.c. of 10 per cent. manganese sulphate solutions added, followed by dilute (1:1) ammonia until the blue colour changes to purple (*p*H 6·8 to 6·9). After the addition of 3·5 c.c. of 2 *N* sulphuric acid the mixture is diluted to 100 c.c., filtered, and aliquot portions taken for the actual determination. This procedure removes the iron and produces the optimum conditions for the phosphate reaction. If less than 0·02 per cent. of acid-soluble phosphoric anhydride is present, the oxidised solution should be evaporated, the residue dissolved in dilute acid, and the iron precipitated without removal of the silica. The factor 0·995 corrects for the volume of the ferrocyanide precipitate. Oxidising agents (*e.g.* ferricyanides) impart a colour to the solution and must be absent. For citric acid extracts 25 grms. of soil are shaken for 24 hours with 250 c.c. of 1 per cent. citric acid solution (plus sufficient acid to neutralise any calcium carbonate), and 75 c.c. of the filtered extract oxidised as above with 10 c.c. of hydrochloric acid and 12 c.c. of permanganate solution for 30 minutes, and heated with agitation for a further 30 minutes till no manganese precipitate remains. Iron is removed as before, using 4 c.c. of ferrocyanide and 1·5 c.c. of sulphuric acid solutions. For the actual determination

(after Fiske-Subbarow, ANALYST, 1926, **51**, 205) 10 to 50 c.c. of extract are diluted to 75 c.c., 10 c.c. of ammonium molybdate solution (25 grms. per litre of 0·5 *N* sulphuric acid) added, and then 4 c.c. of a solution of 0·5 grm. of aminonaphthol sulphonic acid—1:2:4 in 195 c.c. of 15 per cent. sodium bisulphite solution, to which 20 per cent. sodium sulphite solution is added till solution is complete. The mixture is shaken well, diluted to 100 c.c., and the colour matched after 15 minutes against that produced from 2 to 15 c.c. of a standard solution of potassium dihydrogen phosphate (1 c.c. ≡ 0·0001 grm. P_2O_5) under the same conditions. The method of Denigès (ANALYST, 1921, **46**, 460) may also be used, when 1 to 25 c.c. of extract are diluted to 90 c.c. and shaken with 1 c.c. of a mixture of 100 c.c. of 10 per cent. ammonium molybdate solution with 300 c.c. of dilute (1:1) sulphuric acid (stored in the dark), and 3 drops of stannous chloride solution freshly prepared by the action of 0·1 grm. of tin-foil and 1 drop of 4 per cent. copper sulphate solution, in 2 c.c. of hydrochloric acid, the mixture being diluted to 10 c.c. and filtered when reaction is complete. Comparison with 5 to 50 c.c. of standard treated similarly is made after 5 minutes in a volume of 100 c.c. Good agreement with the results given by the gravimetric method were obtained for a variety of soils, and the method overcomes the interfering effect on the colorimetric method of iron in clay soils. If a colorimeter is used, the deeper colours are preferable, with a colour ratio below 1:3, whilst with Nessler cylinders 25 c.c. and 4 c.c. of standard are the maximum amounts which may be used in the Denigès and Fiske-Subbarow methods, respectively. J. G.

Organic Analysis.

Determination of Ethylenic Nitriles. G. Heim. (*Bull. Soc. Chim. Belg.*, 1930, **39**, 458–461.)—The conditions determining selective bromination of β-γ-olefinic nitriles in the presence of their α-β-(unsaturated) isomers depend on the nature and concentration of the bromine and nitrile solutions (*i.e.* on the partition-coefficient of bromine between water and the immiscible solvent used), and on the time of reaction. The optimum conditions are obtained with 5 c.c. of a solution of the nitrile, in a suitable solvent, corresponding with 0·025 mol. per 100 c.c., and twice the theoretical quantity of 10 per cent. aqueous potassium bromide solution. The mixture is emulsified by shaking for 10 seconds, a 10 per cent. solution of potassium iodide added, and the displaced iodine titrated with 0·1 *N* sodium thiosulphate solution. Under these conditions the absorption of bromine by vinyl acetic nitrile was almost quantitative (99·8 per cent.), whilst α-β-nitriles (*cis*- and *trans*-crotonic nitriles and acrylic nitrile, and their homologues) absorbed only 0·9 to 3·0 per cent. The compositions of artificial mixtures of the two types of nitrile were determined with a mean error of 0·5 per cent. The crotonic nitriles absorb less bromine than iodine, vinyl acetic nitrile more (*cf.* Linstead and others, *id.*, 1929, **38**, 1269, 2139). J. G.

New Colour Reaction of Aromatic Amines. I. De Paolini. (*Gazz. Chim. Ital.*, 1930, **60**, 859–862.)—Addition of an aromatic amino compound to an alcoholic suspension of benzoyl peroxide gives, in the cold, but more rapidly on

gentle heating, a coloration which is usually reddish-brown or violet. This reaction, which is given by a considerable number of aromatic amines, substituted amines, and amino-acids, is due to the formation of quinonoid compounds, and it seems probable that the benzoyl peroxide gives rise first to perbenzoic acid.

T. H. P.

The Biuret Reaction. II. The Biuret Reaction of Di-acid Amides. M. M. Rising, J. S. Hicks and G. A. Moerke. (*J. Biol. Chem.*, 1930, **89**, 1–25.)—Recently, Rising and Johnson (*J. Biol. Chem.*, 1928, **80**, 709) described a study of the biuret reaction of acid imides; the work was an early part of an extensive investigation of the chemistry of the biuret reaction, the results of which, it is hoped, may uncover clues relating to the molecular structure of the proteins, which so characteristically form coloured products with the biuret reagents. Further work upon the chemistry of the biuret reaction is now reported. A discussion is given of the biuret reaction of the typical di-acid amides biuret, malonamide, and oxamide. The sodium copper salts of the first two of these were isolated and analysed, empirical formulae for them deduced, and structures proposed. The biuret reaction is also discussed of the substituted di-acid amides *N*-monoethylmalonamide, monoethyloxamide, and symmetrical diethyloxamide. Their sodium copper salts were isolated and analysed, empirical formulae deduced, and structures proposed. Schiff (*Ann. Chem.*, 1898, **299**, 257) stated that symmetrical diethyloxamide does not react with the biuret reagents; but when precautions were taken to prevent hydrolysis by the use of a nearly non-aqueous reaction medium containing an excess of alkali and of diethyloxamide, the biuret reaction was found to occur. Biuret, oxamide and malonamide readily show the reaction; mono- and symmetrical di-alkylated oxamides show the reaction, but unsymmetrical di-alkylated and tri-alkylated oxamides do not; *N*-mono-alkylated malonamides show the reaction, whilst further alkylation of the malonamide molecule prevents it. These findings aid in the prediction, with some degree of accuracy, of the behaviour of di-acid amides toward the biuret reagents. The theory of the biuret reaction is extended with regard to the atoms concerned in the biuret reaction of di-acid amides. A molecule which is sensitive to the biuret reaction is both an acid and a base, and the reaction of such a molecule with alkali and cupric ion involves salt and complex ion formation. Apparently 4 ionisable hydrogen atoms take part in the reaction. A typical acid imide molecule contains 1 such hydrogen atom, and hence 4 imide molecules are to be found in the biuret reaction product of an acid imide, whilst a di-acid amide with 2 ionisable hydrogen atoms forms a product containing only 2 amide molecules. The bare essentials in the matter of atoms for the occurrence of the biuret reaction of a di-acid amide seem, therefore, to be:—(1) Two acid hydrogen atoms in each molecule for salt formation, and (2) one or more amine nitrogen atoms in each molecule for complex ion formation. Apart from the "essential atoms," several factors influence the behaviour of the di-acid amides toward the biuret reagents; one of these may be termed the ionising power of the amide as an acid. If the delicate balance between acidity

and basicity in the amide molecule is disturbed, the reaction may not occur. In the oxamide series the authors have correlated the progressive decrease in acid strength attendant upon alkylation with decreased tendency to show the biuret reaction, and it is shown that the occurrence of the biuret reaction is prevented earlier in the biuret and malonamide, than in the oxamide, series. An explanation is offered for the inhibition of the biuret reaction of di-acid amides by multiple substitution of alkyl groups in their molecules, and by separation of the amide groups by carbon or nitrogen atoms. P. H. P.

Determination of Starch in Finished Goods and Yarns. D. A. Derrett-Smith. (*J. Text. Inst.*, 1930, **21**, 583T.)—The cloth is heated with dilute sulphuric acid for $2\frac{1}{2}$ hours, the liquid filtered, the filtrate neutralised and treated with an alkaline solution of copper, and the resulting cuprous oxide washed and dissolved in a standard solution of ferric ammonium sulphate. This is then titrated with weak standard potassium permanganate solution, the amount of starch originally present being then calculated from the number of c.c. required. Eleven determinations may be carried out by this method in 3 to 4 hours.

The details of the method are as follows: The weight of cloth to be taken should be such as to correspond to a titre not exceeding 35 c.c. of $N/25$ potassium permanganate solution. (In the case of a full-bleach cloth with medium starching, 1 grm. is suitable.) It is cut up into squares of about $\frac{1}{8}$ inch, or in the case of yarn, into lengths of $\frac{1}{4}$ inch, and placed in a dry 175 c.c. conical flask, having a loose glass pear stopper. Thirty c.c. of water are added, followed by 25 c.c. of $2N$ sulphuric acid. The flask is then immersed for exactly $2\frac{1}{2}$ hours in a boiling water-bath kept at constant level. The material is filtered off by suction through a sintered glass funnel, the fabric transferred to the funnel, and the flask rinsed twice with 5 c.c. The solution is made nearly neutral to methyl orange by adding slowly about 22·5 c.c. of 2 N sodium carbonate solution, is then heated to boiling and treated with a boiling solution of 95 c.c. of alkaline copper solution (sodium carbonate, anhydrous, 129·7 grms.; sodium bicarbonate, 50 grms.; water to 1000 c.c.) and 5 c.c. of copper sulphate solution (100 grms. in 1000 c.c.), the flask then being immersed in the boiling water bath for half-an-hour. On to the filtered and washed cuprous oxide are poured two successive portions of 15 c.c. of the iron sulphate solution, followed by two washings with $2N$ sulphuric acid. The filtrate is then titrated direct with $N/25$ potassium permanganate solution. In all determinations a "blank" test should be carried out simultaneously on the fabric before starching. If 1 grm. of fabric is taken, 11·36 c.c. of $N/25$ permanganate are equivalent to 0·99 per cent. of starch on the dry starched fabric containing 6 per cent. of moisture. The method can also be applied to grey, boiled and bleached flax yarns. R. F. I.

Inorganic Analysis.

Analytical Research on Protargol (Determination of Alkalinity and Silver. A. L. Dragenesco and E. Weinberg-Sachetti. (*J. Pharm. Chim.*, 1930, **12**, 536–539.)—*Alkalinity.*—Protargol (0·25 grm.) is dissolved in 50 c.c. of

water, and 1 grm. of sodium thiosulphate (neutral) is dissolved in the liquid which is then titrated with $N/10$ sulphuric acid, using phenolphthalein as indicator, until the colour matches that of a similar solution which does not contain phenolphthalein. The function of the thiosulphate is to change the deep brown colour of protargol in water to light yellow. *Silver.*—Silver is determined by titration with $N/10$ thiocyanate, the organic matter having been destroyed either by calcination or by wet oxidation with sulphuric acid and permanganate; the latter method gave the more consistent results. S. G. C.

New Method for the Determination of Mercury in Mercuric Cyanide. E. Cattelain. (*J. Pharm. Chim.*, 1930, **12**, 529–531.)—Mercuric cyanide is immediately reduced in dilute hydrochloric acid solution by a mixture of hydrogen peroxide and hypophosphorous acid, giving metallic mercury. In this respect it has been found to differ markedly from other salts of mercury, which, under the same conditions, yield mercurous chloride, as shown by Vanino and Treubert (*Ber.*, 1897, **30**, 1999). In order to obtain mercurous chloride from mercuric cyanide by reduction with hypophosphorous acid and hydrogen peroxide, it is first necessary to decompose the cyanide radicle. The method given is as follows: —To the mercuric cyanide solution (10 c.c.) is added sodium chloride solution (10 c.c., 10 per cent.), ammonia (2 c.c., sp. gr. 0·925), and saturated potassium permanganate (10 c.c., a large excess is stated as being necessary). The solution is maintained at 80° C. for half-an-hour. The excess of permanganate is then reduced by adding ethyl alcohol (2 c.c.) and heating for 5 minutes; the heating is continued for another 5 minutes after adding concentrated hydrochloric acid (5 c.c.) to dissolve the manganic hydroxide produced. The solution is cooled. Hydrogen peroxide (20 c.c., 10–12 vol.) and hypophosphorous acid (2 c.c.; 50 per cent., sp. gr. 1·274) are added, and, after the lapse of 5 minutes, the precipitate of mercurous chloride is filtered off on a Jena sintered-glass crucible (1 G4), washed with cold water, and dried at 100° C. The process is supported by one test result. S. G. C.

"Direct Green-*B*." A New Sensitive Reagent for Copper. P. Sisley and David. (*Bull. Soc. Chim.*, 1930, **47-48**, 1188–1192.)—The solution to be tested is brought to *p*H 6 to 9, and 0·1 to 10 c.c. of a 0·1 per cent. solution of direct green-*B* (No. 593, *Colour Index*, cf. *Allen's Organic Analysis*, Vol. VI, p. 200) added to 25 c.c. It is convenient to work with 4 pairs of 25 c.c. test-tubes containing 0·1, 0·5, 2·6, and 10 c.c. of the dye solution, respectively, in order to obtain different ratios of dye to copper. One tube of each pair is heated for 15 minutes on the water-bath, and the change in colour from blue-green to violet-rose is compared with that obtained from a solution containing a known amount of copper sulphate, the tube corresponding with the greatest concentration of dye and a ratio of dye to copper within the range 5 to 50 being selected for the comparison. This gives an estimate of the copper present, while the unheated portion serves as a control. With the optimum ratio (10:1), 0·1 mgrm. of copper per litre is detectable. The reaction proceeds slowly in the cold, and is inhibited by acid, but is unaffected

by the nature of the anion. Iron, chromium and mercury precipitate the dye, without, however, influencing the colour-change, and silver gives a brown-yellow colour. Neutral salts (1 to 10 per cent.) which diminish ionisation, retard the reaction, and in the presence of organic colloids which mask the reaction, the solution should be evaporated and the ash tested. Water distilled in copper apparatus was found to give a positive reaction. J. G.

Separation of Lead as Chromate from Mercury and from Copper. H. Fink and J. Schormuller. (*Z. anal. Chem.*, 1930, **82**, 361–365.)—From *Mercuric ion.*—The faintly acid solution is treated with an adequate excess of sodium acetate and 50 c.c. of 5 per cent. sodium chloride solution, boiled, and precipitated with 5 c.c. of hot 5 per cent. dichromate solution (bulk, 100 c.c.). When cold, it is filtered through a porous porcelain crucible for gravimetric, or paper for iodimetric, work; the precipitate is washed with cold, 1 to 2 per cent. acetic acid. The filtrate is acidified with hydrochloric acid and heated with alcohol, cooled, and treated with hydrogen sulphide; the mercury is determined as sulphide. From *Mercurous ion*—The solution, treated with sodium chloride as in the preceding case, is stirred and treated with bromine-water until the mercurous chloride has re-dissolved. After addition of sodium acetate, the above process is applied. From *Copper.*—The method has been used by Hodgson for the determination of lead in brass and like alloys (*Chem. News*, 1919, **118**, 37). The procedure given above can be used, the addition of sodium chloride being unnecessary. W. R. S.

New Method for the Separation of Nickel and Cobalt. G. Schuster. (*Ann. Falsificat.*, 1930, **32**, 485–487.)—Cobalt and nickel are precipitated by sodium hydroxide and hydrogen peroxide as cobaltic hydroxide and nickelous hydroxide, respectively; it is stated that the nickel hydroxide can be dissolved from the mixed precipitate in an ammoniacal solution of ammonium chloride. In the one quantitative test experiment described, sodium hydroxide (3·5 c.c., 10 per cent.) and hydrogen peroxide (2 c.c., 10–12 vol.) were added successively to the boiling neutral solution of cobalt and nickel sulphate (50 c.c.) and the precipitate filtered and washed with water. The precipitate was digested for 10 minutes on the filter with 30 c.c. of the ammoniacal ammonium chloride solution (ammonia (22° Bé) 20 grms.; ammonium chloride, 10 grms.; water not stated), the outlet of the funnel meanwhile being closed by a rubber cap. The residual cobalt hydroxide was washed with 10 c.c. of the ammoniacal liquid and the cobalt determined by reduction in hydrogen after calcination and also by the α-nitroso-β-naphthol method. The nickel in the filtrate was determined gravimetrically by means of dimethylglyoxime.
S. G. C.

Rapid Method for the Determination of Selenium. E. Benesch and E. Erdheim. (*Chem. Ztg.*, 1930, **54**, 954.)—The solution, in which the selenium may be present either as selenious or selenic acid, is diluted to about 400 c.c., acidified with hydrochloric acid (about 5 c.c.), and a very large excess (30 grms.) added of hydrazine hydrochloride or hydrazine sulphate (preferably the former on

account of its greater solubility). The solution is boiled for half-an-hour, and then kept on a water-bath until the precipitate of selenium, which has changed from the red to the black variety during the boiling, settles out. The precipitate is filtered off on a weighed filter which has been dried at 110° C., washed with hot water, and the whole dried at 110° C. and weighed. Selenium, which is present in samples in powder form, is brought into solution as follows:—The sample is well mixed, in a nickel crucible, with iron oxide (3 grms.), potassium chlorate (3 grms.) and sodium bicarbonate (4 grms.). The mixture is heated until it sinters and afterwards extracted with water; an aliquot part of the filtered extract is used for the above determination. Details for the recovery of the unused hydrazine are given. The three test results which are given show a satisfactory recovery of selenium, selenic acid or selenium being the starting material. The tests made do not cover the possible influences of other elements. S. G. C.

Volumetric Determination of Sodium. A. Blenkinsop. (*J. Agric. Sci.*, 1930, **22**, 511–516.)—The solution, containing not more than 12 mgrms. of sodium (preferably as chloride), is evaporated, and 15 c.c. added of a mixture (filtered after 24 hours) of 10 grms. of uranyl acetate ($2H_2O$) in 6 c.c. of 30 acetic acid and 60 c.c. of water with 30 grms. of zinc acetate in 3 c.c. of acetic acid and 32 c.c. of water (*cf.* Barber and Kolthoff, ANALYST, 1928, **53**, 456). The mixture is stirred, the precipitated $(UO_2)_3ZnNa(CH_3COO)_9,6H_2O$ filtered off in a Jena G-4 filter-funnel after 1 hour, and washed 3 times with 2 c.c. of a saturated solution of the precipitate in 95 per cent. alcohol. A solution of the uranyl zinc sodium acetate in 10 c.c. of hydrochloric acid is transferred to a flask, the air removed by means of a current of carbon dioxide, and the uranium reduced by the action of an excess of 0·02 *N* titanous chloride solution for 2 minutes at 40° C. The excess is determined by back-titration with iron-alum solution in the presence of 20 c.c. of 2 per cent. hydrofluoric acid, and 10 c.c. of 10 per cent. potassium thiocyanate solution till a permanent red colour is obtained. It is convenient just to over-run the end-point and to re-titrate again with the titanous chloride solution till the red colour disappears. Then 1Fe=2·133 U=0·06865Na, or weight of sodium×0·01495=weight of uranyl salt, or 1 c.c. 0·02 *N* $TiCl_3$=0·0000767 Na, the titanium solution being standardised against pure ferrous ammonium sulphate which has been carefully oxidised with potassium permanganate. The hydrofluoric acid stabilises the uranous compound, without affecting the ferric salt, by the precipitation of the amorphous green uranous fluoride. The accuracy is 0·1 mgrm. of sodium, and is unaffected by calcium, magnesium and relatively large amounts of potassium. Iron and aluminium (as phosphates) may be rendered insoluble by ignition without loss of sodium, or may be removed by precipitation with ammonia and ammonium carbonate, and removal of the reagent by volatilisation. J. G.

Microchemical.

Tests in Capillary Tubes applied to the Identification of Nitrates and Nitrites. F. L. Hahn. (*Mikrochem. Emich-Festschrift*, 1930, 143–147.)—

Capillary tubes, 1–2 mm. in diameter and 5–6 cm. long, drawn out at each end to 0·1–0·2 mm. diameter and 1 cm. long, are used for the diazo test for nitrites and the diphenylamine test for nitrates. The reagent is allowed to run up the capillary, so that it fills 0·5–1 cm. of the wider part of the tube, which is then sealed at both ends. A number of capillaries may be filled and kept sealed until they are used, when the tip of the fine capillary at the end containing the liquid is broken off. The tube is warmed above the liquid with a micro flame, so that a small drop of liquid is expelled; this is removed, and the tip of the capillary dipped in the test solution, which is sucked up as the tube cools, when it is re-sealed. The sealed capillary can be heated, if necessary, for the reaction to take place.

Diphenylamine test.—The reagent is prepared from 20 mgrms. of a mixture of 1 part of diphenylamine and 20 parts of sodium chloride, in 1 c.c. of water and 2 c.c. of concentrated sulphuric acid. The mixture dissolves on heating. Using 1 c.mm. of the test solution, 0·01γ of nitrate can be detected, in a dilution of 10^{-5}.

Diazo test.—Using 1 c.mm. of test solution, $10^{-4}\gamma$ of nitrite can be detected, in a dilution of 10^{-7}. For the test for nitrate by conversion into nitrite similar capillary tubes are used, except that they are drawn out to a finer tip at one end only. A little finely powdered lead formate is added, and the test solution is allowed to run up the fine end when both ends are open. The capillary is then sealed at both ends, heated for 5–15 minutes over a water-bath, when the fine end is opened and the diazo reagent solution added, as before. Using 1 c.mm. of test solution, $4\times10^{-3}\gamma$ of nitrate can be detected, the dilution is 4×10^{-6}.

Precipitation test for nitrite.—The reagent used is 2-4-diamino-6-hydroxy-pyrimidine (Merck's "Rosit"), the free base is used in 2 *N* acetic acid solution, or the sulphate or chloride is dissolved in a little mineral acid (0·1 grm. per c.c.). Equal volumes of the reagent and test solution are drawn up into the test capillary, with the wider end left unsealed, the sediment is centrifuged into the narrower end, and when no precipitate is visible with the naked eye the microscope is used. Using 1 c.mm. of test solution $<0\cdot01\gamma$ of NO_2' can be detected in a dilution of $<10^{-5}$. J. W. B.

Micro Vacuum Sublimation of Synthetic Compounds used in Medicine. R. Eder and W. Haas. (*Mikrochem., Emich-Festschrift*, 1930, 43–82.)—The different micro vacuum sublimation methods are described and discussed, and for 0·01–0·05 grm. of material Eder's method (ANALYST, 1913, **38**, 426) is recommended, working at pressures of 18–12 mm. with an oil or sulphuric acid heating bath. The sublimation should take about 1 hour. For amounts of material of about 0·2 grm., they use Diepolders' method. Using the two methods, the temperature at which sublimation begins and the optimum temperature, and also the microscopic appearance of the sublimate of a large number of substances, were observed. The substances include veronal, adaline, bromural, isopral, neuronal, antipyrin, phenacetin, acetanilide, orthoform, tutocain, salol, salicylic acid, luminal, dial, phanodorm, sandoptal, allylisopropyl-bartituric acid, nirvanol, pyramidol, antifebrin,

lacto-phenin, citrophen, dulcin, saccharin, novocaine, anaesthesin, β-eucain, psicain, stovain, holocain, alypin, salophen, aspirin, and salacetol. The results are summarised in two tables, and photographs are given of the microscopic appearance of crystalline sublimates. J. W. B.

Crystallographic Examination of the Micro Sublimates of Synthetic Compounds used in Medicine. W. Haas. (*Mikrochem., Emich-Festschrift,* 1930, 83–119.)—The crystalline sublimates of the compounds named in the preceding abstract are examined in detail in ordinary light, and in parallel and converging polarised light. The examination includes the measurement of refractive indices, determination of the optical character, and whether the crystals are mono- or bi-axial, the interference colours and general characteristics. The results are summarised in tabular form. J. W. B.

Microchemical Identification of some Barbituric Acid Compounds. L. van Itallie and A. J. Steenhauer. (*Mikrochem., Emich-Festschrift,* 1930, 166–169.)—The tests used in the toxicological examination of material for barbituric acid compounds are described. The test substance is dissolved in potassium hydroxide solution, but an excess of alkali is to be avoided. The barbituric acid compound is precipitated with acetic acid, and with ammonium phosphate, and the formation of the crystals is observed. From the various results of the reactions with thallium acetate, ammoniacal silver solution, ammoniacal copper solution, a saturated solution of bromine and water, and baryta water, it is possible to identify the individual compounds. Drawings are given of the different crystals, and the results of the tests are summarised in a table. J. W. B.

Application of Microchemical Methods to the Analysis of Pigments of Paintings. H. Hetterich. (*Mikrochem., Emich-Festschrift,* 1930, 152–162.)—The micro qualitative methods of Emich, carried out on a microscope slide, together with the "spot" test methods of Feigl, are applied to the rapid accurate analysis of very small samples of pigments in paintings. Details are given of the analysis of the pigments used in the blue, the green and the red portions of an old picture. J. W. B.

Physical Methods, Apparatus, etc.

New Method for the Optical Determination of Atmospheric Ozone. A. I. Duninowski. (*Compt. rend.,* 1930, **191**, 859–861.)—By measuring the atmospheric absorption in the visible region of the solar spectrum, with the help of a prism spectroscope with equatorial mounting and a photographic registering apparatus, values have been obtained for the thickness of the ozone layer of the air to within about 0·2 mm. The results obtained at Montpellier during the period August–December, 1929, indicated, for this layer, thicknesses varying from 2·0 to 3·6 mm. T. H. P.

Ultra-Violet Absorption of Honeys. J. Stitz and J. Koczkás. (*Z-Unters. Lebensm.*, 1930, **60**, 420–425.)—The Bunsen-Roscoe absorption extinction coefficient (e) is given in terms of the intensities of incident light (I_0) and transmitted light (I), the concentration (c), and the thickness of the layer of solution examined (x) by $(1/cx) \log I_0/I$. By means of the Judd Lewis sector photometer and the Hilger quartz spectrograph e was determined for wave-lengths (λ) of 5500 to 2350 Å from the measured values of $\log I_0/I$, with a relative error of $\pm 2{\cdot}2$ per cent. A layer 1 to 4 cm. thick was used, the source of illumination being a tungsten iron arc with electrodes 3 mm. apart in front of a 0·02 mm. slit. Absorption curves, in which e is plotted as ordinate against λ, are shown for 6 varieties of genuine honey, a characteristic curve always being obtained, with a sharp maximum at 2700 Å corresponding with $e = 0{\cdot}13$ to $0{\cdot}145$ for a 1 cm.-layer of a 4·5 to 6·5 per cent. solution. Since water has almost negligible powers of absorption, addition to honey of more than the amount usually present (14 to 22 per cent.) produces a marked decrease in e. Glucose, fructose and sucrose give higher, but relatively low, absorption maxima, the value for fructose being the greatest. For commercial sucrose e is slightly higher than for the pure product. Dextrin, on the other hand, has an extremely high absorption in the same region (*i.e.* 2700 to 2800 Å). Addition to honey of 22 per cent. of a sucrose solution containing 47·2 per cent. of sucrose lowered e by 0·3. The total absorption of honey cannot be determined from the individual absorptions of its sugar constituents alone, since the nature and concentration of the ash, colouring matter and proteins has a considerable influence. J. G.

Reviews.

ELEMENTARY ANALYTICAL CHEMISTRY—QUALITATIVE AND QUANTITATIVE. By FRANK CLOWES, D.Sc., Lond., and J. BERNARD COLEMAN, A.R.C.S. Revised by FRANCIS ARNALL, Ph.D., M.Sc., F.I.C., and F. N. APPLEYARD, B.Sc., F.I.C. 11th edition. London: J. & A. Churchill. 1930. Price 6s.

Clowes and Coleman's well-known standard book on elementary analytical chemistry, both qualitative and quantitative, has now reached its 11th edition, and has been revised and brought up to date by Dr. Arnall and Mr. Appleyard.

Although, primarily, this book is written for students working for qualifying examinations, the field covered is so large that it might well be used for the higher examinations of the University of London, such as the Intermediate Science and Pharmacy examinations of that University. In this edition considerable new matter has been introduced, mainly in the section on Volumetric Analysis, also more modern methods of group separation have been substituted. Otherwise,

the order of previous editions is generally followed, and comprises five divisions, embodying Qualitative Analysis, Volumetric Analysis, Gravimetric Analysis, Inorganic Preparations, Organic Compounds, together with an Appendix containing a Table of Atomic Weights, and one of Logarithms. Exercises for practice are also given.

The whole conception of the work is well framed, and this also applies to the separate divisions. The work is very free from mistakes, and is presented in a way that a student can readily understand. A large field is covered in a comparatively small space, and this is effected without in any way impairing the value of the work. The student will find this book a real help in his study of elementary analytical chemistry. The revised edition marks a decided advance on previous ones, and should conduce to the continued popularity of an already well-known book.

ERIC VOELCKER.

MIKROCHEMISCHES PRAKTIKUM. By FRIEDRICH EMICH. 2te auflage mit einem abschnitt über TUPFELANALYSE, by FRITZ FEIGL. Pp. xii+157, with 83 illustrations. 1931. Munich: J. F. Bergmann. Price M.12.80.

Although there are a number of books on branches of applied micro-chemistry, such as toxicology, analysis of blood, and plant micro-chemistry, comparatively few have been written on the subject as a whole. Prof. Emich has written the only text-books covering the whole range of micro-chemistry, with the exception of organic quantitative work, which is sufficiently described by Pregl in his book "Quantitative Organic Micro-analyses." Prof. Emich's two books, the "Lehrbuch" and the "Praktikum," treat the same subject somewhat similarly, except that the "Lehrbuch" contains more detailed theory, whilst the "Praktikum" is meant for a student's handbook and a book for use in the laboratory. The "Praktikum," which was first published in 1924, has now been brought completely up-to-date, and, as micro-chemistry has advanced considerably in the last few years, the book contains much new matter.

The first part is concerned with apparatus and methods. The microscope and its use is described only briefly, since more detailed descriptions are available in books on chemical microscopy. The apparatus and methods of qualitative analysis are described in detail, with the aid of drawings, so that the apparatus can easily be constructed by anyone with a slight skill in glass blowing, and with the aid of a laboratory mechanic.

Prof. Emich describes the different methods of micro recrystallisation, fractionation, boiling point and melting point, determinations and sublimation, and there is a new section on liquid striation which describes recent work of the author and some of his pupils.

The quantitative inorganic section, which in the new edition is much enlarged, is particularly valuable, as no other text-book deals with this subject. The new

"filter stick" filtration devised by the author is carefully described; this is both the simplest and most accurate method of micro-filtration, which, with a little practice, even those who are not trained chemists can use with success.

The second part of the book consists of the description of 76 practical examples applying the general methods of the first part. These include qualitative tests for all the common inorganic elements and radicles individually, the analysis of mixtures, and the tests for elements in organic compounds. The reactions and some preparations are given of many of the more common organic compounds and groups, and Rast's micro molecular weight determination is described. The quantitative examples include the gravimetric determination of barium, potassium, aluminium, and nickel, and mixtures of calcium and magnesium, and silver and copper, and Pregl's electrometric determination of copper. There is an increasing tendency in inorganic qualitative work to use specific tests, by the "spot" method, and in a section at the end of the book, Dr. Feigl, the leading authority on this method of analysis, describes the method in general, and gives a few examples of the tests, which are usually colour tests, involving the use of an organic reagent.

A very valuable section of the book is the appendix describing the apparatus and where it may be obtained. Three lists are given; the first containing the most essential apparatus, and the other two describing various desirable additions. Much of the apparatus is very simple, and, in the preface, Prof. Emich points out that any chemist who owns a microscope can carry out qualitative micro-chemical tests, and anyone with a micro balance can do the quantitative inorganic work.

The book is written in a clear and interesting style, and contains many illustrations. It is well printed, and the use of small type serves to distinguish the work for more advanced students from the important tests and determinations which every micro-chemist should carry out. As a new and revised edition of Pregl's book has recently been published, it is particularly useful that Prof. Emich's book has also been revised, so that the most recent general work in all branches of micro-chemistry is now available in text-book form.

JANET W. BROWN.

INTRODUCTION TO PHYSIOLOGICAL CHEMISTRY. By MEYER BODANSKY, Ph.D. 2nd Edition, re-written and re-set. London: Chapman & Hall. 1930. Price 20s. net.

This text-book of biochemistry is fairly comprehensive, and the present edition brings it thoroughly up-to-date. Though general in scope, those parts having reference to human physiology are stressed; it is, therefore, more suited to the medical student than to the student of general biochemistry, and would, in fact, form an admirable companion to any of the well-known text-books of physiology.

The style is clear, and complex relationships are explained by the use of excellent diagrams; at the same time the author does not claim finality, and the reader is constantly referred to original authorities.

Among much that is good one criticises apologetically, but the last paragraph on p. 406 embalms a hoary error in attributing the main effect of adrenaline to carbohydrate rather than to fat metabolism, in spite of the work of Cori and Cori, to whom the author nevertheless refers.

Sulphur metabolism receives short shrift, the important contributions of Hele and his co-workers being omitted.

The chapters on nutrition and calorimetry are particularly good.

M. STEPHENSON.

THE SPIRIT OF CHEMISTRY. AN INTRODUCTION TO CHEMISTRY FOR STUDENTS OF THE LIBERAL ARTS. By ALEXANDER FINDLAY, Professor of Chemistry, University of Aberdeen. Pp. xvi+480. Illustrations. London: Longmans, Green & Co. 1930. Price 10s. 6d.

"It is not the particular facts of a science that constitute its vitality, but the generic facts or conceptions to which they have elevated the mind. Facts are the body of science, and the idea of those facts is its spirit." Such is the quotation which Professor Findlay has chosen as the motto for his title-page. It is an excerpt from Samuel Brown's *The History of Science*; it serves as the text for Professor Findlay's short dissertation on "The Aim and Scope of Chemistry," which concludes with the observation that the study of chemistry "is not only a means of cultivating the mind and of training and strengthening the scientific habit of thought, but it also brings us into closer relations with, and gives a fuller understanding of, the physical universe in which we live. In the study of chemistry one must seek not only the knowledge by which material results may be achieved, but the understanding of its laws and theories by which alone intellectual satisfaction can be secured." The author says that his book has been written for the increasingly large number of university students who take chemistry as a subject of study in the prosecution of a scheme for achieving general culture rather than as a branch of professional study. He has written it so as to "appeal to the imagination and intellectual interests of those who are not destined for a scientific career, but who desire to understand something of the intellectual progress of recent years and to gain some knowledge of a branch of science on which much of our present-day civilisation is based."

The work will have a much wider circle of readers than is indicated in the foregoing remarks, because it is cast in a form which will be greatly appreciated by all senior students of chemistry, as well as by those professional chemists who desire to maintain the inspirational energy obtainable by reviewing from time to time the historical development of their special branch of science. By taking the more important aspects of theoretical and practical chemistry for treatment in separate chapters and treating of them severally from the historical point of view, bringing the account of each down to the present day, the author has quickened the interest alike in historical facts and present-day knowledge. An

example will help to show how wide is the net used by Professor Findlay to catch all the material suitable for illuminating his text: Chapter X deals with "the Gases of the Atmosphere and the Phenomenon of Combustion"; it begins with an illustration of ancient Egyptians using the foot-bellows, and it ends with a picture of a neon lighthouse. Interesting biographical notes accompany the information as to the contributions made by all the chief persons who have played their part in the growth of chemistry, and the brilliant achievements of the better known philosophers, whose names are known to everyone, are not allowed to entirely obscure those of men whose discoveries are but rarely referred to in the more popular histories of science. Jean Rey, Stephen Hales, Döbreiner (of Jena), Perrin (of Paris), Thomas Northmore, John Mayow, Daniel Rutherford (the botanist), and many other of the less well-known persons who have contributed to enrich chemistry find their appropriate place in Professor Findlay's presentation of the "Spirit of Chemistry." The interest is greatly increased by the profusion of illustrations; there are over eighty figures in the text, and nearly seventy portraits and other pictures. The general reader and the student will feel grateful for the list of books, given in a selected bibliography, which may be read to augment the matter in each of the twenty-nine chapters. There is an excellent index, and the volume is printed in a manner to sustain the reputation of the publishers. Once again, Professor Findlay has laid his fellow chemists under a debt of gratitude by treating his subject so as to commend their science to all men of education.

WILLIAM KIRKBY.

FÄLSCHUNGEN. Edited by Dr. SIEGFRIED TÜRKEL. Pp. 93. Graz: Ulr. Moser. 1930. Price M.12.

This book, which is one of a series of publications on scientific criminology, consists of a collection of contributions, by several authors, on frauds and forgeries, and is issued under the editorship of Professor Türkel, of the Police Headquarters in Vienna. Its aim is to direct attention to ways in which spurious articles are prepared for sale, rather than to give full particulars of the methods of detecting the frauds.

The first section, written by Dr. Türkel, deals with the methods of forging or reconstituting natural history specimens, such as skeletons, minerals, fossils, butterflies, etc. This is followed by a section by Dr. Penn on seals and the methods of tampering with them. It is, perhaps, hardly advisable to give such full working details of how to take copies from seals on documents—even the composition of the most suitable amalgams and alloys for making moulds from the seal is given—and it would have been better to have devoted more space to the methods of detecting the fraud. Reference is made to various chemical and optical methods of examination, including the use of ultra-violet light, but there is no mention of the work of Osborn, who has dealt very fully with the subject in his "Questioned Documents."

Other sections deal with the fraudulent imitation of old book-bindings and drawings, and there is an interesting account of a forged letter of Schubert ingeniously built up by cutting words from reproductions of two genuine letters in a dealer's catalogue, pasting these on a sheet of paper, so as to form a new letter, and then copying this by means of tracing.

The last three sections of the book deal with the examination of oil paintings by microscopical and microchemical methods, with the *X*-ray photography of pictures (illustrated by a series of interesting plates), and with the identification of paintings. The methods, briefly described and summarised, are, in the main, those with which English chemists have been made familiar by the work of Professor Laurie, to whom, by the way, only the briefest references are made.

The book, as a whole, should prove a useful guide to some of the more obscure paths followed by the forger, but it would gain much by the addition of full bibliographies to all the sections, and it is a blemish to issue a reference work of this type without an index. EDITOR.

Publications Received.

THE MICROSCOPIC EXAMINATION OF CATTLE FOODS. By S. T. PARKINSON and W. L. FIELDING. Ashford: Headley Bros. Price 6s. 6d.

PRACTICAL PHYSICAL CHEMISTRY. By A. FINDLAY. 5th Edition. Longmans. Price 7s. 6d. net.

THE MANUFACTURE OF ARTIFICIAL SILK (RAYON). By E. WHEELER. Chapman & Hall. Price 12s. 6d. net.

BACTERIOLOGICAL TECHNIQUE. By J. W. H. EYRE. 3rd Edition. Baillière, Tindall & Cox. Price 21s. net.

QUANTUM CHEMISTRY. By A. HAAS. Translated by L. W. CODD. Constable. Price 6s.

ABRIDGED SCIENTIFIC PUBLICATIONS FROM THE KODAK RESEARCH LABORATORIES. Vol. XIII. Rochester, New York.

THE RELATIVE VALUES OF COD-LIVER OILS FROM VARIOUS SOURCES. By J. C. DRUMMOND and T. P. HILDITCH. (Empire Marketing Board) H.M. Stationery Office. Price 1s. net.

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