THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held at the Chemical Society's Rooms, Burlington House, on Wednesday, February 3rd, the President, Dr. J. T. Dunn, being in the chair.

Certificates were read for the first time in favour of:—Alan Arthur Douglas Comrie, B.Sc., A.I.C., Edwin William Stanley Press, B.Sc., A.I.C., and Muriel Roberts, B.Sc., F.I.C.

Certificates were read for the second time in favour of Albert Green, M.C., M.Sc., Ph.D., F.I.C., John Farrar Hardwick, B.Sc., A.I.C., Ernest Stephen Hawkins, B.Sc., A.R.C.S., F.I.C., Joseph Robert Johnson, F.I.C., M.Inst.M.M., Arthur Pillans Laurie, M.A., D.Sc., F.R.S.E., and John Morgan Tucker, B.Sc., A.I.C.

The following were elected Members of the Society:—Thomas Whittaker Lovett and William Charles Wise, B.Sc.

Notice was given that the Annual General Meeting of the Society would be held on Friday, March 4th, at 3 p.m., instead of on Wednesday, March 2nd, and the Anniversary Dinner at 7.30 p.m., on March 4th, at the Trocadero Restaurant.

The following papers were read and discussed:—"A New Method for the Determination of Lead in Organic Substances, with Special Reference to Dyestuffs," by N. L. Allport, A.I.C., and G. H. Skrimshire; "(i) Some Analytical Applications of Sodium Hydrosulphite, II.," and "(ii) A Rapid Method of Dissolving Lead Alloys Preparatory to the Determination of Tin and Antimony," by B. S. Evans, M.C., Ph.D., F.I.C.; "The Phloroglucinol Method for the Determination of Mechanical Wood Pulp in Paper," by H. B. Dunnicliff, M.A., D.Sc., F.I.C., and H. D. Suri, M.Sc.; "Investigations relating to Milk Standards under the Burma Food and Drugs Act," by E. H. Bunce, F.I.C.

NORTH OF ENGLAND SECTION

THE Seventh Annual General Meeting of the Section was held in Manchester on February 13th, 1932. The attendance was thirty-two, and, in the absence of the Chairman, Dr. Dunn presided.

The Secretary read the report and the financial statement for 1931, which were adopted.

The following appointments were made:

Chairman: John Evans. *Vice-Chairman:* Prof. W. H. Roberts. *Committee:* J. W. Haigh Johnson, J. Haslam, H. Heap, S. E. Melling, H. E. Monk, and J. Wood. *Honorary Auditors:* U. A. Coates and W. Marshall. *Honorary Secretary:* J. R. Stubbs.

The following paper was read and discussed:—"Castor Seeds in Feeding Stuffs," by F. Robertson Dodd, F.I.C.

The Calcium Fluoride Method for the Determination of Fluoride, with Special Reference to the Analysis of Nickel-Plating Solutions*

By S. G. CLARKE, Ph.D., A.I.C., and W. N. BRADSHAW, B.Sc.

One type of modern nickel-plating solution contains a high concentration of nickel sulphate, together with additions of potassium or sodium chloride to maintain anode efficiency, and of potassium or sodium fluoride and boric acid to act as buffering agents. The solution may also contain small quantities of iron, etc., as impurities. No difficulties arise in the determination of the nickel, sulphate, etc., and the problem of the determination of the borate in this type of solution has recently been satisfactorily solved by Willard and Ashworth (*Amer. Electroplaters' Soc. Monthly Review*, August, 1929, p. 7). A process for the determination of the fluoride has been published (Hammond, *J. Ind. Eng. Chem.*, 1924, **16**, 938), in which the lead chlorofluoride precipitation method is employed; the method is not easy to carry out, and has, in our hands, given a distinctly incomplete recovery of the fluoride. In a search for a suitable method we investigated the possibilities of the calcium fluoride precipitation method, and we have found how this can be used to provide a reasonably rapid and accurate method for the determination of the fluoride in these nickel-plating solutions. Our work further shows how, in the absence of interfering substances, the traditional calcium fluoride process can be simplified and rendered more rapid and accurate.

The calcium fluoride method continues to occupy a prominent position in textbooks since it has fewer disadvantages than other methods for the determination of fluoride. In a modification of this method, by Rose (*Ann.*, 1849, **72**, 343), some calcium carbonate is made to precipitate with the calcium fluoride, with the object of rendering the precipitate less difficult to filter. It is clear that this procedure restricts the method to solutions from which heavy metals, *e.g.* nickel, have been

* Communication from the Research Department, Woolwich.

removed. Moreover, the addition of sodium carbonate, which is recommended for producing the precipitate of calcium carbonate, may reduce the concentration of the calcium ions in the solution, at the time of precipitation, to a very low value, perhaps beneath that required for reducing the solubility of the calcium fluoride to the minimum. We have found that calcium fluoride can be precipitated and filtered practically quantitatively with ease, even in the absence of calcium carbonate or other co-precipitant, thus avoiding the subsequent troublesome and often incomplete removal of this from the precipitate. This ease of precipitation and filtration can be achieved by paying attention to the conditions under which the calcium fluoride is precipitated, and also to the method of filtering. The method which we advocate provides for an excess of calcium chloride for reducing the solubility of the calcium fluoride. For filtering off the precipitate we suggest the use of a paper-pulp filter* as absolutely essential, not only because this retains the precipitate of calcium fluoride more effectively than a folded filter paper, but also so that the washing process may be carried out with the minimum of washing liquid. It is probably from a lack of attention to these points that the calcium fluoride method has acquired a reputation for a tendency to give unsatisfactory results. The statement made in the recent textbook of Hillebrand and Lundell (*Applied Inorganic Analysis*, 1929), that ammonium chloride in the solution "holds up" the precipitate of calcium fluoride, has not been confirmed. We have obtained at least as good results from precipitation in presence of ammonium chloride as in its absence. Indeed, the presence of ammonium chloride is essential in applying the calcium fluoride method to solutions containing sulphate, *e.g.* nickel-plating solutions, because, by virtue of the greatly increased solubility of calcium sulphate in ammonium chloride solution, the precipitation of calcium sulphate may be substantially prevented.

Precipitation of Calcium Fluoride in Absence of Sulphates.—Experimental work on the following lines was undertaken to find the best conditions for precipitating fluoride as calcium fluoride, and it is shown that the precipitation can be successfully carried out from solutions having a wide range of *p*H values, containing fluoride either alone or with nickel chloride, boric acid, and considerable quantities of ammonium chloride.

The Standard Fluoride.—Some difficulty was at first experienced in obtaining a pure fluoride on which to base the work. Commercial "pure" sodium fluoride is liable to contain, among other substances, sodium fluosilicate, the solubility of which is very near that of sodium fluoride; the salt is extremely difficult to recrystallise, because both the solubility and the solubility gradient with temperature are low; in any case, its satisfactory recrystallisation demands large platinum vessels. Potassium fluoride, being a hydrated salt and somewhat hygroscopic, was considered unsuitable. A possibility which had to be abandoned was that of using diluted "A.R." hydrofluoric acid standardised by titration; this acid, on analysis, was found to contain about 1 per cent. of silicon as hydrofluosilicic acid, which, since it behaves on titration with alkali in the cold as a dibasic acid, vitiates

* The preparation of these filters from "ashless" filter paper macerated with water is described in, *e.g.* the following books: Picard, *Steel Analysis*, 1914, p. 5; Hackney, *Quantitative Inorganic Analysis*, 1930, p. 15.

the calculation of the total fluorine as hydrogen fluoride. We adopted, as a standard material, sodium fluoride prepared as follows:

A fairly strong solution of potassium fluoride was prepared in a platinum dish and filtered through a gutta-percha funnel, the filtrate being allowed to drop slowly into a well-shaken, equimolecular, roughly half-saturated solution of sodium chloride contained in a pyrex flask. The finely crystalline sodium fluoride, which rapidly separated out, was filtered off without delay, washed with water, and dried at 100° C. Its weight remained unchanged at 500° C., showing that it was anhydrous. Tests showed that it was practically free from chlorides.

Precipitation of Calcium Fluoride from Neutral Solution.—Solutions having the volumes shown in Table I were made up to contain various amounts of sodium fluoride. These solutions were rendered slightly acid with hydrochloric acid; a few drops of methyl red indicator (0·02 per cent. in 50 per cent. alcohol) and 10 c.c. of calcium chloride solution (10 per cent. $CaCl_2$) were added, followed by dilute ammonia until the indicator just changed to yellow. The liquids were boiled for about one minute, cooled in running water for fifteen minutes, and filtered through small compressed pulp filters. The precipitates were washed four times with cold ammonium nitrate solution (5 per cent.) and twice with cold water; about 10 c.c. of liquid was used for each washing. The filters were heated in weighed platinum capsules at a moderate temperature (400 to 600° C.) until all the carbonaceous matter had burned off, and then at about 800° C. for 10 minutes. In some cases the residue of calcium fluoride was converted to calcium sulphate. The results are shown in Table I.

TABLE I

Fluorine added. Grm.	Volume of solution before precipitation. c.c.	Weight of residue (CaF_2). Grm.	Fluorine found. Grm.	Weight of residue ($CaSO_4$). Grm.	Equivalent to fluorine. Grm.
Nil–Blank	50	[0·0008]		[0·0018]	
0·0100	50	0·0216–0·0008	0·0101	0·0375–0·0018	0·0099
0·0200	50	0·0424–0·0008	0·0202	0·0744–0·0018	0·0202
0·0400	50	0·0837–0·0008	0·0404	0·1468–0·0018	0·0404
Nil–Blank	100	[0·0011]		[0·0023]	
0·0100	100	0·0201–0·0011	0·0092	0·0349–0·0023	0·0093
0·0200	100	0·0407–0·0011	0·0193	0·0712–0·0023	0·0192
0·0400	100	0·0815–0·0011	0·0391	0·1430–0·0023	0·0392

The high "blank" obtained in this series of experiments was most probably caused by carbon dioxide being present in the water and by ammonia being used and gaining access to the solution during the boiling, etc. By using a washing liquid containing 1 per cent. of acetic acid and 5 per cent. of ammonium nitrate, the "blank" was reduced to about 0·0003 grm., as shown in Table II. This washing liquid was adopted throughout the subsequent work.

TABLE II

Fluorine added. Grm.	Volume of solution before precipitation. c.c.	Weight of residue (CaF_2). Grm.	Fluorine found. Grm.	Weight of residue ($CaSO_4$). Grm.	Equivalent to fluorine. Grm.
Nil–Blank	50	[0·0003]		[0·0005]	
0·0100	50	0·0200–0·0003	0·0096	0·0344–0·0005	0·0095
0·0200	50	0·0408–0·0003	0·0197	0·0713–0·0005	0·0197
0·0400	50	0·0815–0·0003	0·0395	0·1431–0·0005	0·0398

Precipitation of Calcium Fluoride from Acid Solution.—Table III shows the results obtained in the precipitation of calcium fluoride from solutions containing various concentrations of acetic acid. The solutions (50 c.c.) containing sodium fluoride were made just alkaline to brom-phenol blue, and acetic acid was added to give the concentrations shown; the method of precipitation, etc. already outlined was followed.

TABLE III

Fluorine added. Grm.	Acetic acid. Per Cent.	Weight of residue (CaF_2). Grm.	Fluorine found. Grm.	Weight of residue ($CaSO_4$). Grm.	Equivalent to fluorine. Grm.
Nil–Blank	1	[0·0002]		[0·0003]	
0·0100	1	0·0198–0·0002	0·0095	0·0339–0·0003	0·0094
0·0200	1	0·0408–0·0002	0·0197	0·0711–0·0003	0·0197
0·0400	1	0·0810–0·0002	0·0394	0·1409–0·0003	0·0392
0·0100	10	0·0194–0·0002	0·0094		
0·0100	25	0·0198–0·0002	0·0095		
0·0398	10	0·0810–0·0002	0·0393		
0·0398	25	0·0810–0·0002	0·0393		
0·0398	50	0·0810–0·0002	0·0393		
Nil–Blank	25	[0·0002]			

Precipitation of Calcium Fluoride from Solutions containing Boric Acid, Nickel Chloride and Ammonium Chloride.—The results recorded in Table IV were obtained. In each experiment the fluoride was precipitated by the addition of 10 c.c. of calcium chloride solution (10 per cent. of $CaCl_2$), the fluoride solution containing 1 per cent. of acetic acid.

TABLE IV

Added.		Found.			
Fluorine. Grm.	Grm.	CaF_2. Grm.	Fluorine. Grm.	$CaSO_4$. Grm.	Equivalent to fluorine. Grm.
0·0200	0·02 H_3BO_3	0·0397	0·0193		
0·0200	0·20 ,,	0·0397	0·0193		
0·0200	0·5 NH_4Cl	0·0402	0·0196		
0·0200	5·0 ,,	0·0423	0·0206		
0·0200	0·20 H_3BO_3; $5NH_4Cl$	0·0406	0·0198	0·0700	0·0195
0·0400	0·02 H_3BO_3	0·0794	0·0387		
0·0400	0·20 ,,	0·0810	0·0394		
0·0400	0·20 H_3BO_3; $5NH_4Cl$	0·0821	0·0400	0·1429	0·0399
0·0400	2·0 $NiCl_2.6H_2O$	0·0822	0·0400	0·1422	0·0397
0·0400	2·0 $NiCl_2.6H_2O$; $5NH_4Cl$	0·0840	0·0409		
0·0400	2·0 $NiCl_2.6H_2O$	0·0825	0·0402		
0·0400	2·0 $NiCl_2.6H_2O$; $5NH_4Cl$	0·0827	0·0402		

In the last two experiments precipitation took place from 10 per cent. acetic acid solution instead of from 1 per cent. solution.

THE INTERFERENCE OF IRON AND ITS AVOIDANCE.—In planning a process for the determination of fluoride in nickel-plating solutions attention had to be given to the interference of (ferric) iron. In proportion to the amount of ferric salts present, the precipitation of calcium fluoride is either partly or completely prevented. Attempts to overcome this interference by the addition of organic hydroxy-compounds, *e.g.* mannitol, lactic acid, citric acid, etc., were unsuccessful. It was found, however, that quantitative precipitation of the fluoride could be effected if the iron in the solution was reduced to the ferrous condition, the following

method being adopted:—To the solution containing 10 per cent. of acetic acid, were added 10 c.c. of calcium chloride solution (10 per cent.) and 1 c.c. of hydrazine hydrochloride solution (20 per cent.). The solution was heated to boiling. The brown colour of the ferric acetate (which is not precipitated) rapidly became bleached, and the calcium fluoride then precipitated. After boiling for about half a minute, the solution was cooled in running water for 15 minutes, and the filtration, etc., of the calcium fluoride carried out as usual. Table V contains the results obtained.

TABLE V.

	Added.				Found.	
Fluorine. Grm.	Iron (as $FeCl_3$). Grm.	Nickel (as $NiCl_2$). Grm.	Boric acid. Grm.	Ammonium chloride. Grm.	Calcium fluoride. Grm.	Fluorine. Grm.
0·0400	0·10	—	—	5·0	0·0811	0·0395
0·0400	0·05	0·5	0·20	5·0	0·0811	0·0395
0·0400	0·05	0·5	0·20	5·0	0·0821	0·0400

When the amount of iron is small, it can be removed from the solution by precipitation as ferric hydroxide, without causing any loss of fluoride. This method would appear to be more convenient than the above in the determination of fluoride in nickel-plating solutions, and will be referred to later in that connection.

PRECIPITATION OF CALCIUM FLUORIDE FROM SOLUTIONS CONTAINING SULPHATE.—The low solubility of calcium sulphate in water renders it practically impossible to precipitate calcium fluoride quantitatively in a pure condition by the addition of calcium chloride to a solution containing fluoride and sulphate. This is especially true of a nickel-plating solution, because the ratio of sulphate to fluoride is large. We have found, also, that there is a noticeable tendency for calcium sulphate to be adsorbed by a precipitate of calcium fluoride when this is formed in a solution only partly saturated with calcium sulphate. We have, however, been able to take advantage of the enhanced solubility of calcium sulphate in ammonium chloride solution to work out a feasible process for precipitating calcium fluoride from a solution containing much sulphate. By employing a regulated excess of calcium chloride, only a small quantity of calcium sulphate is precipitated with the calcium fluoride; a correction can be made for this by deducting from the weight of the precipitate the weight of the calcium sulphate, calculated from a determination of sulphate in it. The best results were obtained by precipitating the calcium fluoride from a neutral or slightly ammoniacal solution; good results, in the presence of sulphate, were not obtained when solutions containing acetic acid were used. Details of the method adopted are incorporated in the following process proposed for the determination of fluoride in nickel-plating solutions.

DETERMINATION OF FLUORIDE IN FLUORIDE-BORATE NICKEL-PLATING SOLUTIONS.—Ten c.c. of the solution are diluted to 25 c.c., and 5 c.c. of ammonia* (sp. gr. 0·88) are added. The liquid is boiled for about half a minute and filtered through a small filter paper (*e.g.* Whatman, 7 cm., No. 41). The beaker is rinsed out four times with 5 c.c. of 5 per cent. ammonia on to the filter, which is allowed thoroughly to drain each time. The precipitate, if any, of ferric hydroxide is

* This will provide the necessary amount of ammonium chloride.

rejected, and the filtrate, having a volume of about 45 c.c., is made slightly acid with concentrated hydrochloric acid, and 2·5 c.c. of calcium chloride solution (10 per cent., made from anhydrous salt) are added slowly, drop by drop (this quantity suffices for the amount of fluoride normally to be found in these nickel-plating solutions, *i.e.* up to about 10 grms. of sodium fluoride per litre; see note below); the solution is then made just alkaline with ammonia, boiled for about half a minute, and cooled in running water for fifteen minutes. The precipitate of calcium fluoride (with adsorbed calcium sulphate) is filtered off on a small well-compressed pulp filter and washed four times with 5 per cent. ammonium nitrate solution containing 1 per cent. of acetic acid, and then twice with cold water. The total amount of washing liquid used should not exceed 50 c.c. The filter, with the precipitate, is transferred to a platinum capsule, heated at about 400–500° C. until the carbonaceous matter has been destroyed, and then ignited at 700–800° C. for ten minutes. After weighing, 5 c.c. of concentrated hydrochloric acid (free from sulphate) are added to the residue in the dish, which is then heated until all the acid has evaporated. This treatment is repeated until the residue has been completely attacked and a clear solution can be obtained in the 5 c.c. of acid. The 5 c.c. of solution are transferred to a conical flask, diluted to about 100 c.c., and heated to boiling, and 20 c.c. of barium chloride (10 per cent.) solution are added slowly. The barium sulphate is allowed to settle, and is then filtered off, washed, ignited, and weighed. The equivalent weight of calcium sulphate is deducted from the weight of the original precipitate, and the weight of calcium fluoride is thus obtained.

TABLE VI

	Added.					Found.					
No.	Fluorine,	Nickel sulphate.	Boric acid.	Iron, as ferric sulphate.	Calcium chloride (10 per cent. solution).	Impure Calcium fluoride.	Barium sulphate.	Calcium sulphate. (calc.)	Nett Calcium fluoride.	Fluorine.	Fluorine (corrected).
	Grm.	Grm.	Grm.	Grm.	c.c.	Grm.	Grm.	Grm.	Grm.	Grm.	Grm.
1	0·0100	2·4	0·2	0·01	2·5	0·0195	0·0045	0·0026	0·0167	0·0081	0·0101
2	0·0200	2·4	0·2	0·01	2·5	0·0419	0·0080	0·0046	0·0373	0·0182	0·0202
3	0·0400	2·4	0·2	0·01	2·5	0·0891	0·0175	0·0101	0·0790	0·0385	0·0405
4	0·0800	2·4	0·2	0·01	3·5	0·1750	0·0300	0·0174	0·1576	0·0768	0·0788
5	*0·0216	2·4	0·2	—	2·5	0·0454	0·0095	0·0055	0·0399	0·0194	0·0214
6	*0·0288	2·4	0·2	—	2·5	0·0622	0·0138	0·0080	0·0542	0·0264	0·0284
7	*0·0592	2·4	0·2	—	2·5	0·1298	0·0300	0·0174	0·1124	0·0547	0·0567†

* Amount of fluoride added to test solutions unknown to the analyst.

† Low result indicates that insufficiency of calcium chloride was added.

Table VI shows results which were obtained with solutions made up to simulate nickel-plating solutions containing 240 grms. of nickel sulphate and 20 grms. of boric acid per litre, with different amounts of sodium fluoride added; to some of the solutions iron was added, in greater amount, however, than would be expected in a nickel-plating solution, in order to verify its non-interference in the process. It will be noticed that the results are lower than the theoretical by a fairly constant amount, *viz.* 0·002 grm.; this seems due to a solubility effect, probably introduced by the high nickel and sulphate concentration of the solution, and it is, therefore, suggested that a correction of this order should be applied to the results obtained.

The addition of 2·5 c.c. of calcium chloride solution (10 per cent.) is satisfactory for the precipitation (in a volume of 50 c.c.) of fluorine from 10 c.c. of nickel-plating solution containing not more than 10 grms. of sodium fluoride per litre, which is a concentration not usually exceeded in practice. In the event of the solution containing a higher concentration of sodium fluoride, it would be necessary to increase the amount of calcium chloride for the precipitation; in Experiment 4 (in the table), in which 0·08 grm. of fluorine was present in 10 c.c. (corresponding with 17·7 grms. of sodium fluoride per litre), 3·5 c.c. of calcium chloride were used. It is recommended, however, that this process should be carried out as described, and if the result comes out at more than 10 grms. of sodium fluoride per litre, the determination should be repeated, using slightly more calcium chloride for the precipitation. The amount of calcium chloride for the precipitation must be controlled with reasonable care, to keep the correction for calcium sulphate in the final precipitate as low as possible, yet (at the same time) to give a sufficient excess of calcium ions in the solution for the fluoride precipitation.

Scientific Documentary Evidence in Criminal Trials

By C. AINSWORTH MITCHELL, D.Sc., F.I.C.

(*Read at the Summer Meeting, North of England Section, July* 4, 1931)

When your Honorary Secretary (Mr. J. Stubbs) asked me to read a paper at your Summer Meeting, and this request was repeated by our President, I felt that it would be ungracious not to respond, and I, therefore, agreed to give a survey of the scientific evidence which may be based on documents in criminal trials and to illustrate this by cases within my own experience.

Scientific documentary evidence is, of course, circumstantial in character, and it is a common practice for counsel for the defence in criminal trials to decry all circumstantial evidence, notwithstanding the fact that it is often more trustworthy than personal testimony. Apart from that, if it were excluded, there would be few convictions even of dishonest tradesmen, and fewer still of the inveterate poisoner, who is the last person to advertise his intentions or to let his acts be seen.

The rules that govern documentary evidence are the same as those governing any other expert evidence. For example, since the leading case of *Seaman* v. *Netherclift* (1876), a witness on oath is in a privileged position, whether he asserts that a sample has been grossly adulterated, or that a signature is a forgery. There are also certain judicial decisions which have been given in connection with documents, and are also applicable to scientific evidence in general. For instance, Mr. Justice Finlay, in the case of *Rex* v. *Henry* (1929), allowed a tracing to be shown to the jury, and in the case of *Rex* v. *Podmore* (1930) the Lord Chief Justice held that a photograph upon which marks had been made with the object

of directing the attention of the jury to certain details in the original document, was admissible, since it went to strengthen the evidence.

Many of the scientific data upon which evidence as applied to documents can be based have been accumulated comparatively recently, and new cases frequently present problems for which no solution can be found in textbooks. These may best be considered under the headings of the various materials, beginning with ink.

EVIDENCE RELATING TO INKS.—*Differentiation of Writing Inks.*—The first occasion on which evidence as to differences in ordinary blue-black writing inks on a document was given in this country was at the trial of Brinkley in 1907 (*Rex* v. *Brinkley*). In that case a forged will was the motive for an unintentional murder (if that is not a contradiction in terms), for the prisoner had tried to poison a man whose name appeared as a witness on the will, but who had stated that he had signed only what he had been told was a petition for an outing. A bottle of stout, dosed with prussic acid, was left in this man's lodgings, where it was drunk by his landlord and landlady, both of whom died. An important point in the evidence was whether the statement about the signing of a petition for an outing in a public-house was true, and accordingly the ink used in that public-house was compared with the ink in the signature upon the will. Chemical tests could not be made, since the President of the Probate Court refused his permission for the will to be touched, but, fortunately, an optical examination was sufficient for the purpose. The ink ("Azuryte") from the public-house contained a particularly brilliant blue dye, which enabled it to be distinguished readily from most other inks in common use at that time; and by first matching the inks on the will under the microscope, and then preparing broad colour bands which could be compared by means of a tintometer, it was possible to demonstrate in Court that the ink from the public-house agreed exactly with the ink in the signature on the alleged will, and also that there were three separate inks upon that document. Brinkley admitted this, but tried to explain it away by saying that he had given two bottles of ink to a little girl. He was convicted and executed.

Prior to the war, English ink manufacturers were using a large variety of blue dyes as provisional colouring matters, and this was helpful for distinguishing between different inks, whether by optical or chemical methods (see ANALYST, 1908, **33**, 80), but for some years past the Board of Trade has consistently refused to allow any dyestuffs to be imported for ink-making, with the result that the provisional colouring matter in writing ink is usually the same "Ink Blue" (Soluble Blue), and chemical tests must now be based, in the main, on the different proportions of dye, tannin substances and iron in the inks.

Osborn's comparison microscope, which has been devised since the Brinkley case, is a valuable instrument for comparing colours and recording their Lovibond values, although it is not advisable to attempt to demonstrate its use to a jury; the colour-strip method, as used in that trial, is still the simplest way of showing in Court differences of colour in inks on a document.

Gradation of Colour.—The colour of an ink will naturally vary with such conditions as the length of time the ink has remained exposed to the air in an inkpot, or the time it was left upon paper before blotting. These points are of considerable

importance in judging whether the whole of a given piece of writing was done at the same time, as may readily be seen on comparing successive entries in hotel registers. It was a significant fact that in the case of *Bishop of Lincoln* v. *Wakeford* (1921) the disputed words "and wife" in the two entries in the hotel book agreed exactly in gradation of tone with the preceding words, "J. Wakeford." It would have been an exceptionally skilful forger who could have twice completed those entries in ink of exactly the correct shade.

Photographic Differentiation.—Another optical method of distinguishing between two writing inks, one of which is richer in blue units than the other, is by means of photography on an ordinary "process" plate. When such difference in colour is present, two inks which, when examined under the microscope, appear to match one another, may show a pronounced difference when photographed, the ink richer in red appearing much darker in the print than in the original document. This method afforded conclusive evidence in the case of *Rex* v. *Cornwallis* (1919), in which a woman produced a letter acknowledging the receipt of £200, with a final "0" added to the amount in a different ink.

A remarkable case in which a photographic reproduction suggested forgery was that of *Hawes* v. *Skelton* (1924), in which a will was discovered in the pocket of the overall of a woman who was bathing a dog. The signature on this will showed different colours, and had manifestly been re-touched, but the Judge (Mr. Justice Horridge) was not satisfied that these abnormalities were sufficient to condemn the document, and deferred the case for a scientific opinion. The bottle of ink with which the document was alleged to have been signed was produced in Court. It was said to contain a mixture of three different inks bought at a sale, and experiments showed that this mixture was capable of accounting for all the abnormalities found on the document. It produced writing of different colours according to the depth to which the pen was dipped into the ink, and the ink ran badly from the nib, necessitating re-touching of the writing. As a result of this evidence the Judge pronounced in favour of the will.

AGE OF INK IN WRITING.—The change of colour which rapidly takes place when ink begins to oxidise on paper may sometimes afford proof that writing is recent. Thus, in a claim brought against an insurance company by a clothier for the alleged loss of his stock by fire, it was found that entries in a stock book, which purported to be two years old, darkened perceptibly in the course of three or four days, and must, therefore, have been comparatively recent. The colour readings in tintometer units were taken on successive days during the period of the test, and were checked by another observer, so that there was no doubt as to the progressive change in colour. But, at best, evidence of this kind is unsatisfactory, since it is subjective in character, and cannot be supported by photographic proof or checked by subsequent examination.

Chemical evidence of the age of ink is much more convincing than colour records, since the test can be repeatedly checked, as I have shown in my discussion on the subject (ANALYST, 1920, **40**, 247). In the case of *Rex* v. *Pilcher* (1911) special permission was obtained from the President of the Probate Court for chemical tests to be applied to the inks upon a will purporting to be thirteen years old. All the inks on the document readily ran over the paper when treated with

reagents, and formed blue smudges, whereas the inks on the older counterfoils of cheque books of the deceased woman, which dated back for six or eight years, remained practically unaffected by the reagents under the same conditions; hence the inks on the disputed will could not have been as old as its date. After this evidence had been given, Colonel Pilcher confessed that he had uttered the will, knowing it to be a forgery, and he was sentenced to three years' imprisonment.

ANACHRONISMS IN INKS.—On several occasions fraudulent claims for old-age pensions have been exposed owing to the fact that the entries in family Bibles, produced as evidence of age, were written in blue-black ink containing an aniline dye, whereas aniline dyes had not been discovered at the dates mentioned. Such dyes appear to have been introduced into writing fluids in this country about 1880, but the earliest instance I have yet found in old ledgers was an entry written in 1885.

A more uncommon anachronism was disclosed in connection with two documents in the case of *Rex* v. *Rogers* (1930). It would need the pen of a Thomas Hardy to do justice to the story unfolded at that trial of a man who had foisted forged documents on to an ignorant old woman, an old-age pensioner, and had received £5 from her. She was a descendant of William Penn, the Quaker, and claimed to be entitled to his estates. The two documents, which she was led to believe would establish her claim, were genuine old parchments, one of which related to a conveyance of land by the Earl and Countess of Yarmouth in 1688. In each instance the name of "William Penn" appeared after the signatures of the other witnesses, and was apparently in the same kind of iron-gall ink which had disintegrated with age, leaving a residue of oxidised iron compounds. But the inks of the William Penn signatures, unlike all other writing on the documents, contained no iron, and when treated with a minute drop of hydrochloric acid, became milky. This was found to be due to silver, which had become brown —in other words, the ink had the characteristics of a silver marking ink. This discovery became still more significant when counsel for the defence asked whether the forgery could have been perpetrated by William Ireland, who was responsible for the wholesale forgery of Shakespearian documents at the close of the eighteenth century. Ireland afterwards wrote a full confession of his methods, and, from the description of his ink, he was evidently using a preparation containing a silver salt.*

The answers to the question raised about Ireland were: (1) Ireland did not use a steel pen for his forgeries, as had manifestly been used in the Penn forgeries

* *The Confessions of William Henry Ireland, containing the Particulars of his Fabrication of the Shakspeare Manuscripts* (1805), p. 39. "One of the journeymen, looking at the manuscript, informed me that he could give me a mixture that would resemble old ink much more than that which I had used; and, in consequence of my request, he immediately mixed together in a phial three different liquids used by bookbinders in marbling the covers of their calf bindings. These ingredients, being shaken up, produced a fermentation; when, the froth having subsided, the liquid was of a dark brown colour. The young man then wrote his name with this mixture, but it was very faint on the paper; however, on holding it for a few seconds before the fire, the ink gradually assumed a very dark brown appearance. . . . It was with the same ink I afterwards wrote the Shaksperian manuscripts. Their scorched appearance originated in my being compelled to hold them to the fire, as before stated; and, as I was constantly fearful of interruption, I sometimes placed them so near the bars as to injure the paper; which was done in order to complete and conceal them as speedily as possible from any unexpected person who might come suddenly into the chambers." (*Cf.* Plate, Fig. 4.)

(Fig. 1); steel pens were not invented until 1808. (2) Ireland confined his attentions to fifteenth and sixteenth century documents; in those days there would have been no motive for producing autographs of William Penn. (3) There were no characteristics in the writing to suggest that Ireland had had anything to do with the forgery, which was a clumsy imitation of the genuine writing, as may be seen by comparison with the signature on one of the genuine Penn documents brought into Court for comparison (see Plate, Figs. 2 and 3).

The prisoner was found guilty of uttering the forged documents, and was sentenced to a term of imprisonment.

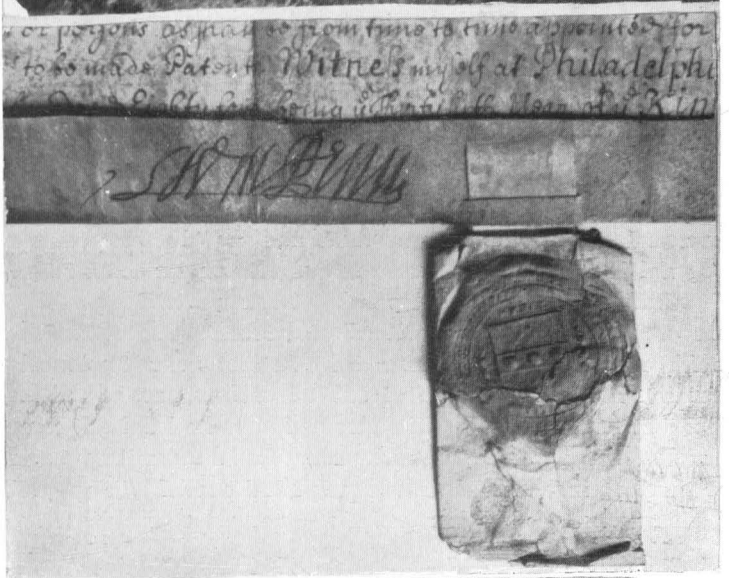
Fig. 1. Enlargement of forged signature of William Penn—showing the effect of a steel nib.

INK IN CREASES IN PAPER.—The way in which a paper has been folded may be significant, especially when there is writing across the fold. If the writing has been added since the folding of the paper, it will sometimes be found that the ink has spread slightly along the crease, owing to the paper having become more porous through a break in the surface sizing after repeated folding in the same place. In a case tried in 1929, a fact of this kind afforded proof that some writing on an agreement was not so old as the document itself.

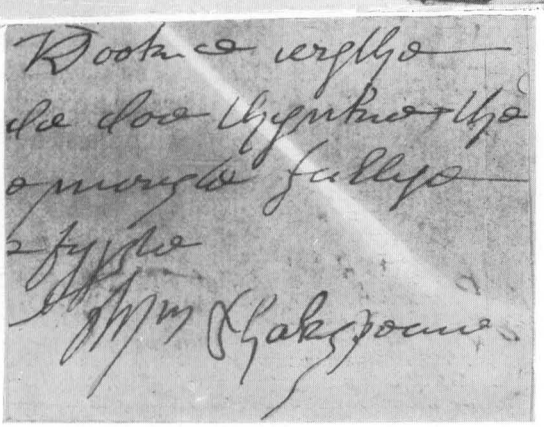
SEQUENCE OF STROKES IN WRITING.—The conditions under which it is possible to be certain that a stroke in writing which appears to be on the top of another stroke really is uppermost have been discussed in a previous paper (Mitchell and Ward, ANALYST, 1927, **52**, 580). Briefly, the rule is that if one of the pigments in the intersecting lines is a thin film of colour, as in stamping ink or blue-black writing ink which has been blotted immediately after writing, it is not possible to express an opinion which line was made first; but, if the pigment is sufficiently dense, as in printing ink, or if a layer of solid pigment forms, as when iron-gall inks oxidise on paper, definite conclusions may be drawn. These conditions were present in the case of *Rex* v. *Cohen*, discussed in a former paper (ANALYST, 1920, **45**, 252). In another case (*Lonnen* v. *Lonnen*) a codicil to a will, which two witnesses swore that they had seen signed, was upset by the fact that at two points the writing of the codicil intersected the writing of the signature of the testator,

2

3



4



2. Forged signature of William Penn.
3. Genuine signature and seal of William Penn, when Governor of Pennsylvania, July 31st, 1684 (Penn MSS, Library, Society of Friends).
4. One of Ireland's forgeries of Shakespeare's writing and signature, on the fly-leaf of a book "*The Catholikes' Supplication*," 1603 (Brit. Museum, Stowe, 996, 576, *a*. 34). The darkening of the paper was due to scorching during the heating of the silver ink (*cf.* p. 147).

and could be demonstrated by means of enlarged photography to be uppermost, thereby proving that the codicil had been inserted after the will had been signed.

With black lead pencil writing the sequence of strokes can always be determined by the lines of the silver striations (due to the impurities in the graphite or to the added clay), which are seen when the strokes are examined in oblique lighting (see ANALYST, **1922**, **47**, 379).

DIFFERENTIATION OF PENCIL PIGMENTS.—In some cases microscopical differentiation of pencil marks is possible (*J. Soc. Chem. Ind.*, **1919**, **38**, 381T), but great caution is necessary, since it is possible, by wetting the lead or varying the pressure, to alter the way in which pigment is deposited on the paper, and the series of strokes containing approximately the same amounts of pigment must be chosen for the comparison. The differentiation of old graphite pencils from modern composite pencils is usually practicable, however, owing to the fact that the silver striations of the former are irregular and interrupted, whilst those of the latter are like strings of beads.

Chemical tests depend upon the fact that graphites contain widely differing amounts of iron and chlorides, and, in exceptional cases, there is sufficient titanium to respond to a micro-chemical test. Coloured pencils can usually be distinguished from one another without much difficulty (see ANALYST, **1922**, **47**, 385).

COPYING INK PENCILS.—An outline of the methods of distinguishing between the pigments of copying ink pencils on paper will be found in THE ANALYST (**1917**, **42**, 3). There is more scope than with ordinary blacklead pencils, owing to the fact that there are usually three constituents present—kaolin clay, violet dyestuff, and blacklead, and that the proportions of these affect the reactions given by the writing. In the case of *Rex* v. *Wood* (1907), in which charred fragments of paper were found in the grate of the house where a woman had been murdered, it was possible to prove by a series of tests that the pigment on the paper agreed with that of a copying ink pencil in the possession of an artist who was accused of the murder. Ultimately he admitted having written the letter, but was acquitted of the murder.

The possibility of such pigments resisting the action of sea-water for some weeks was raised in the case of *Macbeth* v. *King* (1916), in which the genuineness of a stave of wood with a message supposed to have originated from a steamship, presumably sunk by a torpedo, was a point at issue. Experiments described to the Court showed that copying ink pencil writing on a piece of oak would not be obliterated by six weeks' exposure to sea-water and air.

The behaviour of copying ink pencil writing towards various solvents had to be determined in the case of *Rex* v. *Podmore* (1930), in which a scrap of paper, about two inches square, which was found behind a barrel in a garage at Southampton where a man had been murdered, led ultimately to the conviction of the murderer. This fragment was caked with dirt and soaked in oil, and had been repeatedly trodden under foot, and the problem was to remove the dirt and oil, without also removing the pigment of the copying ink pencil. After numerous experiments with various makes of copying ink pencil, petroleum spirit was found to be suitable for the purpose, and a message from a man calling himself "W. F. Thomas" was

left upon the paper. Until then, it was not known that anyone of the name of "Thomas" (an alias of Podmore) had been in any way connected with the victim.

The use of ultra-violet light made it possible to read much more of this message than could be brought out by an ordinary photograph, but, to avoid any suggestion of the use of the imagination, the evidence on this fragment at the trial was restricted to the words and characters which the jury could see for themselves.

INDENTATIONS IN PAPER.—Another document produced at this trial was a leaf from a note-book showing indentations which had, presumably, been made by the pressure of a pencil on another leaf of the book subsequently torn out. By means of photography with the use of oblique lighting to illuminate the edges of the indentations, words relating to bogus orders, with the initials of "Thomas," were rendered visible, and this formed a further link in the evidence which led to the conviction of Podmore.

SECRET WRITING.—The search for secret writing in a document is usually only needed on special occasions, such as the detection of espionage in war time or irregular communication with the outside world by prisoners. The substances which are capable of being used as invisible inks are almost innumerable, but, broadly speaking, they fall into two classes—those forming a coloured compound on treatment with a mordant, and those which become visible when examined by special optical methods such as ultra-violet light. A discussion of the subject will be found in my book, *The Scientific Examination of Documents*, p. 153 (Chas. Griffin & Co.), and in Lucas's *Forensic Chemistry*, 2nd Ed., p. 105 (E. Arnold & Co.).

In the earlier trials of German spies during the war (*Rex* v. *Kuepferle* (*Times* Report, May 15, 1915), *Rex* v. *Müller and Hahn*, and some others), the primitive method of writing with lemon juice was employed, and evidence was required to prove that steel pens in the possession of the accused had deposits upon them consistent with their having been dipped into lemon juice, whilst cut lemons gave reactions for iron at points where, apparently, something had been inserted into them. In later trials, more elaborate methods of secret writing were employed, as may be gathered from the story of these spy cases in Felstead's *German Spies at Bay* (Hutchinson).

The knowledge of the use of saliva for secret writing, and of its development with a dilute solution of ink, was common property long before the war (*cf.* Dennstedt and Voigtländer, *Lehrbuch der gerichtlichen Chemie*, 1907, p. 122). In a study of the action of saliva upon iron-gall ink (ANALYST, 1920, **45**, 256) I have shown that it behaves like an oxydase, accelerating the oxidation of the ferrous tannate and forming a compound which is distinct from that which forms normally when ink is oxidised by exposure to the air. The process of the development with ink of writing done with saliva thus appears to be partly physical (absorption of dye where the fibre of the paper has been indented) and partly chemical (accelerated oxidation of the ink).

TYPEWRITING.—Fortunately it is not generally realised, especially by those who are given to writing anonymous letters, that typewriting can be identified with that produced by a particular machine, with a much greater degree of certainty than the identification of handwriting. Apart from the fact that each

manufacturer uses a different fount of type, which is usually varied in successive models, every machine has its individual faults of alignment and displaced and imperfect letters, by means of which its identity, and, in certain cases, its age, can be established.

The best methods and instruments for recognising and demonstrating such identity are described by Osborn in his *Questioned Documents* (2nd Ed., p. 437, *et seq.*). Identity in the relative positions of various letters is shown by means of photographs taken under glasses ruled in standard squares, and the typing produced by battered letters is easily recognised, even by an unintelligent jury, when shown in an enlarged photograph. Osborn cites many examples of cases in which the evidence derived from a study of typewritten documents has been incontrovertible, and these might be supplemented by numerous cases in this country. Among the most recent of these is *Rex* v. *Parry* (1930), in which a former tax collector was convicted of inducing an old man and his wife to make a successful fraudulent claim for the repayment of income tax, by which he also benefited. He denied that the fraudulent returns had originated from his office, but the typing upon them agreed in all its characteristics with that upon admitted letters typed on his machine, and this was one of the facts which led to his conviction.

PRINTED MATTER.—Much of what has been said about the differentiation of typing applies also to printed characters. The founts used by different printers have distinctive differences, and parts of certain letters tend to become defective with use. Evidence on such points is required when the authenticity of a printed document, such as a passport (*vide* infra), is questioned.

SEALS.—The methods of forging seals have been fully described by Türkel in his *Fälschungen*, p. 14 (*cf.* ANALYST, 1931, **56**, 141). Photographic methods can be used for detecting such forgeries, and in some cases chemical evidence may be decisive. For example, in the case of *Rex* v. *Fink* (1911), the defence set up in a case in which a cheque had been forged was that the forgery had been committed after the cheque had been posted in a sealed envelope. Chemical tests, however, were applied to the wax of Major Fink and to the seal on the letter, and gave practically identical results for colouring matter, ash and sulphate, whereas the corresponding figures given by the wax from the post office where the letter was posted, and by six samples of red sealing wax, bought at random, were totally different.

STAMPS AND POSTMARKS.—As an illustration of the kind of information that may be gathered by minute examination of the stamp and postmark on an envelope, reference may be made to a case in which an anonymous letter, bearing a German postmark and registration mark, was received in a London office. Microscopical examination showed that only that part of the postmark upon the stamp was genuine, the remainder of the circle upon the paper having been crudely completed. Ultimately it was found that the letter had originated from within the office, and that the stamp and registration number had been detached from a genuine registered German letter (the receipt for which had been duly signed), and then put on to another envelope containing the anonymous letter, after which the missing part of the postmark had been replaced by hand, probably in Indian ink.

COMPOSITION OF THE PAPER.—The composition of the paper of a document may give useful suggestions, as in a case in 1914, in which the crudely forged notes of an Oriental bank were found to consist of pure flax. This suggested that the forgeries had probably emanated from Russia, as was subsequently found to be the case. Chemical tests, including the difference in the liberation (by the sizing) of iodine from potassium iodide and the determination of the acidity, afforded proof that the forged American passport produced by the German spy, Brekoff (*alias* Rowland), was not genuine, and evidence to that effect was given at his trial in 1915. In addition to chemical differences in the papers, the dyestuffs in the paper seals attached to the two documents were chemically different, and there were differences in the type in which the passports were printed. Among the printing defects was one which could easily be seen by the jury, for in the German version the American eagle had been docked of one of its tail feathers. There were also two other cases in which similar evidence was required.

ARTIFICIAL WATERMARKS.—The usual method of fabricating a watermark is to stamp the paper with the required device in a wax medium. Such spurious watermarks can, as a rule, be removed by treating the paper with a suitable solvent, as was found to be possible with certain notes which were being widely circulated. The fraud can also generally be made manifest by examining the document in ultra-violet light, when the artificial watermark will often show a pronounced fluorescence.

CHARRED DOCUMENTS.—I have discussed the methods of deciphering charred documents in previous papers (*Discovery*, 1924, **5**, 336; ANALYST, 1925, **50**, 174). Photographic methods, in which the charred fragment leaves an imprint of printed matter on a sensitised plate, depend upon the fact that printing ink which has been burned produces certain products which reduce silver salts, whereas charred cellulose has no action. In the communications mentioned above it was shown that different printing inks behave differently in this respect.

The other method of deciphering charred fragments is to continue the calcination further, for which purpose I have found the use of two pieces of wire gauze held in crucible tongs to be the most suitable, since the calcination can be controlled so that a coherent ash of the required colour is left. The lampblack of printing inks being less combustible than the char of paper, is left in its original black characters on white ash, whilst the alumina forming the bases of coloured printing inks remains in white on dark gray or blackish ash. Ordinary writing ink leaves a brownish residue of iron oxide, and the graphite of blacklead pencils and of copying ink pencils can be obtained as a residue on either white or grey ash.

The reason why so little writing was visible in the charred fragments in the Wood case (*supra*) was that the pigment of the copying ink (Swan) pencil which had been used contained no graphite.

This calcination method was found effective in a case in 1923, in which it afforded confirmation of the statement of a prisoner that he had accidentally burnt a bundle of bank notes of high value (*cf.* ANALYST, 1925, **50**, 178). Experiments made at that time showed that banknote paper contains so little filling material that it would be quite possible for a bundle of notes to be burnt and to leave very little coherent ash.

EVIDENCE ON HANDWRITING.—In the Podmore case, as in many other cases, questions of the identification of handwriting were raised. Sooner or later every chemist who undertakes the scientific examination of documents will be faced with the difficulty that his work must be incomplete unless it also deals with the examination of the writing on the documents; in fact, the Government Analysts of several of the Dominions and Colonies have been compelled by circumstances to take the subject of handwriting into consideration. In the interests of justice this is a move in the right direction, for much of the obloquy attaching to handwriting experts has been due to the fact that the earlier experts were frequently trained observers of minute detail (some were engravers), but they had not had a scientific training, and thus were prone to draw deductions which were not warranted by the observed facts. For instance, it was not uncommon for a handwriting expert to swear positively in the box that a given piece of writing was written by a particular person, whereas all that his observations justified was the inference that the characteristics of the writing agreed in form or writing habit with those of that person. Hence, when (as sometimes happened) it was found that more than one person shared those characteristics, a mistake had to be admitted, and discredit was brought upon the whole system of comparing handwriting.

Essentially, the judgment of handwriting is an analytical process, and depends upon the same fundamental rules of inductive logic (isolate, vary, measure) as are used in chemical analysis, but it is only exceptionally that a categorical conclusion can be drawn. The real value of the evidence is that it assists the Court to decide in which direction the balance of probability lies. For example, in the Podmore case the demonstrable facts were that the characteristics of the writing on the dirty fragment of paper and of the indented writing in the order book agreed with those of admitted writing of the accused. If it was not his writing, either it must have been a deliberate imitation of it, or there must have been two persons writing in exactly the same way and having the same initials, in the garage at Southampton at about the same time. It was for the jury to decide to what extent such a coincidence would be probable.

Or, again, in the case of Archdeacon Wakeford, the writing in the hotel register of the disputed words "and wife" agreed in all respects with admitted writing, even including the formation of a straight stroke as a sign for "and"—a habit which, until it was discovered in a volume of his manuscript, was unknown to those best acquainted with him. The only possible conclusion, other than that the writing was that of the accused, was that it was the work of an abnormally skilful forger, and it was then for the Court to decide whether such a forger could have been present at the hotel on two unexpected occasions, and could have added the words in such a way as to give the correct colour tones of the inks, as well as to reproduce the exact formation and style of the genuine writing.

TRACED FORGERIES.—Exceptionally, it is possible to state a conclusion in more positive terms than has been indicated, as, for example, in the case of *Beckerkunst* v. *Cohen* (1929), in which a forged will was produced, signed in an abnormal way by a woman whose condition was proved by medical evidence to be such that firm writing such as that of the signature in the document would have been impossible. In

this case the results of comparison of the signature on the will with other signatures of the deceased woman supplemented the medical evidence, but there are instances when the evidence of documents by themselves may be conclusive. For example, in the case of *Rex* v. *Henry* (1929) a will was produced in terms which were practically identical with those on a draft of the will produced some months previously and then photographed. The formation of the words and their coincidence in position on the paper were explicable only on the assumption that one was, in part, a tracing from the other. When, subsequently, the production of the draft was again required, a third document, purporting to be this draft, was produced. This differed materially from the original draft, which had been photographed, and the space left where a piece had been cut from the edge of the former differed in size and shape from the corresponding space cut out in the latter; this space might have become larger in the interval between the times when the production of the draft will was demanded, but it was an impossibility for it to have shrunk.

On rare occasions the model which has served for a tracing may be discovered, and when it is, it may convert probability into certainty.* The classic instance of this type of forgery is the American case of Rice-Patrick, in which the signatures on four pages of a will agreed so closely with one another, that it was obvious that they must have been tracings, since no one would reproduce all the lines of the words in practically the same relative positions (see Osborn, *Questioned Documents*, p. 293).

When considering indications of tracing, there is one possibility which must not be overlooked. Solicitors sometimes indicate, by pencilling in a name, the place on a document where the signature is required, and sometimes a client whose intelligence is not of a high order will attempt to copy the pattern. In the case of *Oliver* v. *Oliver* (1930) there were features in a signature which, had they been in a letter and not upon a legal document, would have been conclusive that the signature was not genuine. The lines were wavering and hesitating, there were frequent breaks in unusual places, the formation of the letters was abnormal, and there were traces of graphite at the edges of some of the strokes. The significance of these points, however, was discounted by the fact that none of them was inconsistent with the other hypothesis, namely, that they were the results of an attempt to make a slavish copy of a signature previously outlined by a solicitor in blacklead pencil.

Discussion

Mr. A. Lucas confirmed Dr. Mitchell's statement that chemists who attempted to confine their work to the microscopical and chemical examination of documents were eventually compelled to take up the examination of handwriting. He gave a few examples of cases in his own experience, illustrating points which had been raised in the paper. In one of the cases cited, an ink that was alleged to be a mixed ink was proved not to be a mixture. In another case an anachronism was discovered in the composition of paper, which contained wood cellulose, although the date upon the document was about 60 years before that material was used as an ingredient of paper. Mr. Lucas also confirmed the value of Osborn's comparison microscope for the examination of documents.

* In the case of *Rex* v. *Brown* (1931), which was *sub judice* when this point was mentioned, a signature showing indisputable indications of marks made with a dry point was found, and the coincidence of this signature with one on the document in dispute was conclusive evidence that the latter was not genuine.

The Biological Method for the Detection of Arsenic

By A. F. LERRIGO, B.Sc., F.I.C.

In view of the recent case of poisoning through arsenical wall-plaster (see p. 163), the following survey of the literature of the arsenic-liberating moulds may prove of interest; I made it some years ago in an attempt (abortive, as it proved) to investigate certain possibilities of the phenomenon.

1. "The capacity of the metalloid arsenic for combining with hydrocarbon radicles . . . has not been manifested . . . either in the mineral kingdom or as a result of the vital activities of living organisms. The special conditions under which this attraction becomes effective have been, without exception, established by the art of the chemist. All the organic arsenicals are synthetic products" (*Organic Compounds of Arsenic and Antimony*, by G. T. Morgan, 1918). The writer then states, in a footnote, that: "A possible exception to this generalisation is noted on p. 45 in reference to the production of di-ethyl arsine by the growth of moulds on carpets and wall-paper containing arsenical pigments. It is conceivable that this phenomenon might be realised with naturally-occurring arseniferous materials altogether apart from the intervention of human activities, but hitherto this likely formation of organic arsenicals has not been realised."

Numerous cases are on record of poisoning due to wall-papers bearing arsenical colouring matters, such as Schweinfürth Green (aceto-arsenite of copper) and Scheele's Green (copper hydrogen arsenite); Kirchgasser (*Vierteljahr. gericht. Chem.*, N.F., **9**, 96) has described twenty-six such cases. According to Allen (*Organic Analysis*, 4th Ed., Vol. VII, p. 355), Selmi has shown that a volatile arsine, possessing a strong toxic action, is formed by the contact of arsenious acid and albuminous matter; and T. Husemann (*Arch. Pharm.*, 1881) suggested that such a compound was formed from the size or paste used in fixing arsenical wall-paper.

The former worker (F. Selmi, *Gazz. Chim. Ital.*, 1881, **11**, 437) described a volatile arsenical compound which gave a crystalline hydrochloride, and which he had found in the urine of dogs that had been receiving small doses of arsenic. The alleged formation of these volatile bodies was said to account for the reported disappearance of arsenic from corpses, but Mario Tonegutti (*J. Chem. Soc.*, 1909, *A.*, ii, 700), investigating the putrefaction of protein substances containing arsenic, declared that it was not accompanied by the evolution of arsenical gas, although small quantities of organic arsenic compounds appeared, these having a basic character and being volatile at 50 to 60° C.

In nearly all the wall-paper cases, the poisonous effects were attributed entirely to the presence in the air of the room of arsenical dust. Fleck (*Z. f. Biol.*, 1873, **8**, 444), investigating the presence of arsenic in the air of rooms having arsenical wall-papers, declared that, in addition to arsenical dust, there was present arsenious hydride, which was produced by the action of organic matter (starch paste and gelatin) and moisture on the free arsenious acid contained in the arsenic colour.

N. P. Hamburg (*Pharm. J.*, (3), 1874, [iii], **4**, 81) passed the air of a similar room into silver nitrate solution after removing all dust, and found the presence of both arsenic and silver sulphide indicated. H. C. Bartlett (ANALYST, 1880, **5**, 81) passed the air with hydrogen containing a little ammonia on to silver nitrate paper, and obtained a dark stain.

Emmerling (*Ber.*, 1896, **29**, 2728), investigating a case due to arsenic-containing curtains, declared the improbability of the volatilisation of arsenic by the agency of micro-organisms. This was contested by B. Gosio (*Ber.*, 1897, **30**, 1024), who described the action of certain moulds which were able, under suitable conditions, to synthesise volatile arsenic compounds. He found that the most active was *Penicillium brevicaule*; other moulds having a similar action were *Mucor mucedo*, *Aspergillus glaucus* and *A. virens*. In a later paper (*L'Orosi*, 1900, **23**, 361) Gosio described a method of utilising this action of *P. brevicaule* for the detection of small quantities of arsenic in organic materials, relying on the intense garlic-like odour of the arsenical gases evolved. He placed the sample, or a portion of it, upon sliced potato or bread, and sterilised the whole, after which it was inoculated with sterile water containing, in suspension, spores of this particular mould, and then incubated at 37° C. Gosio apparently used this method with success in examining mineral waters, viscera, physiological secretions and minerals.

F. Abba (*Centrbl. Bakteriol.*, 1898, [ii], **4**, 806) repeated and confirmed some of Gosio's work, and the test is sometimes described under his name; he found the biological method to be more delicate than that of Marsh. Abel and Buttenburg (*Chem. Centrbl.*, 1900, **1**, 428) described several other moulds capable of volatilising arsenic, and stated that they could detect 0·001 mgrm. by Gosio's method. The extreme delicacy of this qualitative test was further confirmed by W. Scholtz (*Chem. Centrbl.*, 1899, ii, 1032), and again by Valerio and Stryzowski (*Chem. Centrbl.*, 1901, i, 63). A. E. Bell (*Pharm. J.*, [iv], **17**, 484) and M. Segale (*Z. physiol. Chem.*, 1904, **42**, 175) have also investigated certain aspects of this biological reaction, while the necessity for the presence of carbohydrate has been emphasised by Neppi (*Scienza Pratica*, 1908, **1**, 82).

G. Markmann (*Chem. Centrbl.*, 1900, ii, 1187) declares that if sulphur or phosphorus be present, the garlic odour is masked; Maasen (*ibid.*, 1902, i, 1245) and O. Rosenheim (*J. Chem. Soc., Proc.*, 1902, 138) point out that compounds of tellurium and selenium are attacked by *P. brevicaule* and other organisms, giving off volatile bodies. In the case of tellurium the odour is similar to that from arsenic; several organisms are known which decompose tellurium and selenium compounds, but not arsenic. Knaffl-Lenz (*Arch. expt. path. Pharm.*, 1913, **72**, 224) was unable to find a mould giving volatile compounds with antimony.

Among the species reported to be capable of liberating gaseous arsenic compounds are *Stemonitis* (Biourge, *Étude Monographique*, 1923). According to Thom (*The Penicillia*, 1930, 92), however, Biourge had misunderstood Thom's conception of *P. divaricatum* (Thom) which Depoorter, in 1921, found to be the most active form, enabling him to detect as little as 0·00008 per cent. of arsenic.

Discussing the question of the degree of tolerance of mould fungi for arsenic, Thom (*loc. cit.*, p. 93) reports that in the Bureau of Chemistry of the Agricultural Department, U.S.A., small mould colonies developed on a 0·5 per cent. solution

of arsenious oxide exposed to the air for five months, and that a strain allied to *P. purpurogenum* was subsequently isolated from the growth.

This peculiar faculty of liberating volatile arsenic compounds is not confined to the so-called "arsenic moulds." V. Puntoni (*Ann. d'Ig. Roma*, **1917**, **27**, 293) records that persons taking arsenicals, such as sodium cacodylate, by ingestion, exhale a penetrating odour resembling garlic, which is due to the bacterial decomposition of the arsenic compound.

2. Various conflicting statements have been made as to the exact nature of the arsenic compound volatilised by *P. brevicaule*. G. Markmann (*loc. cit.*) and others refer to it as arsenious hydride, but Biginelli (*Atti Real. Accad. Lincei*, 1900, [v], **9**, 210) decided that it was di-ethyl arsine. In this he was supported by Neppi (*loc. cit.*), but Klason (*Ber.*, **1914**, **47**, 2634) stated that the gas evolved was di-ethyl cacodyl oxide $(AsEt_2)_2O$, and that arsine was never produced.

It was recorded by Gosio (*loc. cit.*) that on passing the evolved gases into a 10 per cent. solution of mercuric chloride in dilute hydrochloric acid, a crystalline mass was formed at the point where the gas entered the liquid. Biginelli (*loc. cit.*) gave the composition of this body as $AsHEt_2.2HgCl_2$. Another worker (*Il Policlinico*; through *Repertoire*, **14**, 88) declared that this substance was metallic arsenic, and that, by collecting and weighing it, quantitative results could be obtained; this statement appears to lack confirmation.

Most of the published work on this subject is in agreement as to the poisonous nature of the volatilised arsenic compounds; Gosio proved it to his own satisfaction at the expense of some rabbits; but Huss (*Z. Hyg.*, **1914**, **76**, 361) declared that "the arsenic-containing gas possesses little toxicity."

A survey of the literature available suggests to my mind the possibility that *P. brevicaule* and its allies may volatilise arsenic in more than one form; this would be quite in keeping with the nature of the phenomenon and would account, to some extent, for the apparently contradictory statements of certain of the investigators.

3. Originally it was my intention to see if the biological method could be utilised practically in detecting and determining small quantities of arsenic in organic materials, without any preliminary destruction of organic matter, particularly with reference to arsenic existing in organic combination.

Unfortunately, with the only two cultures of *Penicillium brevicaule* which were tried (they were obtained from the Lister Institute), absolute failure was experienced in producing any mould growth accompanied by liberation of arsenic. On this point the opinion was advanced by one authority that the moulds in question lose their specific property after a time, and require special cultural conditions to restore it.

Attempts were, therefore, made to obtain mould growths on various nutrient media, as well as on foods (raw potato, fish, etc.) in the presence of small and varying amounts of arsenious oxide. In no case was any volatile arsenic compound detected, either by means of mercuric chloride or by the characteristic garlic odour. The matter was dropped at this stage, but two other suggested points were investigated.

The first was whether, in the event of the mould action being enzymic, pepsin or taka-diastase had any similar action. Artificial digestions were carried out with each, in the presence of varying amounts of arsenic, but no positive results were obtained. The second point was raised in the case of *Rex* v. *Greenwood*, when it was suggested by the defence that arsenical weed-killer, sprinkled on a garden path, could result in volatile arsenic compounds being produced by the agency of soil bacteria. Experiments were made with several samples of garden soil, to which arsenic had been added, but no volatile arsenic could be detected.

CITY ANALYST'S DEPARTMENT,
44, BROAD STREET, BIRMINGHAM.

Woods Used by the Ancient Egyptians

BY KENNETH P. OAKLEY

As no one can hope to have specialised knowledge in all branches of science, it is almost essential for the modern archaeologist to obtain the co-operation of technical experts.

Is it too much to suggest that owners and curators of archaeological collections should have the materials of which their specimens are made scientifically examined, so that the misleading statements, at one time so common on museum labels, might be avoided? The accompanying photomicrographs, illustrating the structure of two of the commonest woods employed by the ancient Egyptians, may be of interest to those engaged in such research on Egyptian materials.

These photomicrographs show sections of specimens in my own collection. The sections were cut and examined for me by Mr. J. C. Maby, then of the Forest Products Research Laboratory (Oxford Branch), by whose kind permission I am able to reproduce them. They illustrate a special application of the technique recently described by him (ANALYST, 1932, 3).

FIG. 1.—A transverse section cut from a wooden model of a building-cradle (used in raising blocks of stone) found in a "Foundation Deposit" under the Temple of Queen Hatshepsut at Dêr el-Baḥarî, and dating from about 1500 B.C. The wood is that of *Ficus sycomorus* (?), which was one of the most important indigenous trees growing in Ancient Egypt. Although this wood was extensively used in Egypt when small lengths only were required, it has serious limitations, and its coarse grain and light spongy texture make it unsuitable for long straight planks or beams. The hulls of many Nile boats in Egypt were made of this wood, cut up into short rectangular blocks and built "brickwise."

FIG. 2.—A transverse section cut from a block of wood which originally formed part of the side of a Xth–XIth dynasty coffin (*circa* 2200–2000 B.C.) found at Dêr el-Baḥarî. The wood is coniferous, and hence must have been imported from abroad. It is, in fact, almost certainly the wood of Lebanon cedar (*Cedrus libani*, Barrel).

FIG. 3.—A transverse section from a piece of coffin wood (probably XIIth dynasty, *circa* 2000 B.C.), which has also been identified as *Ficus sycomorus*.

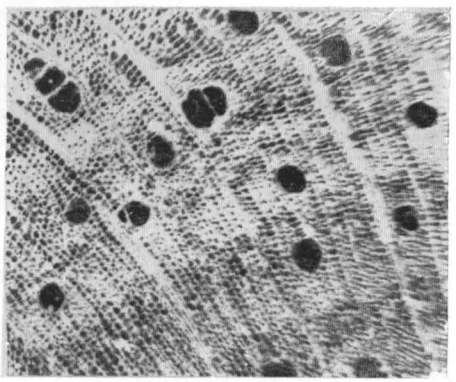


Fig. 1

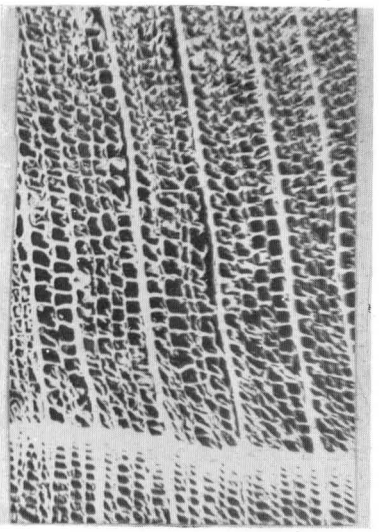


Fig. 2

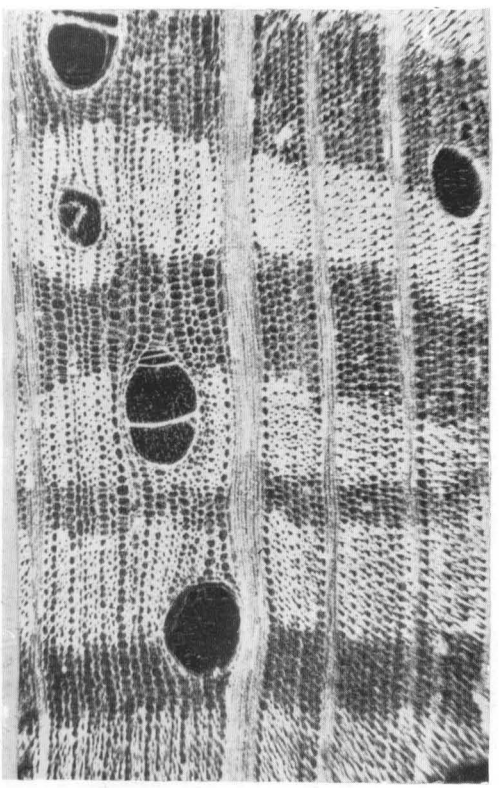


Fig. 3

Fig. 1. *Ficus sycomorus* (?). Cut from near centre of tree. Transverse section × 50 linear.
Fig. 2. *Cedrus libani*, Barrel. Transverse section × 50 linear.
Fig. 3. *Ficus sycomorus*. Cut from near the outside of the tree. Transverse section × 50 linear.

As Professor P. E. Newberry pointed out in his Presidential Address to the Anthropological Section of the British Association, 1923, Lebanon cedar is not the durable and priceless wood that some writers have thought it to be, although it was certainly superior to any wood native to Egypt itself, and hence in ancient times formed relatively valuable timber. The wood is reddish in colour, and has a silky grain. It is apt to shrink and warp badly under some conditions. Nevertheless, it was one of Egypt's most valued imports, because the one thing that the country lacked was large timber. As early as the Ist dynasty coniferous wood (almost certainly *Cedrus libani*) was being shipped from Palestine to the Valley of the Nile, and throughout Egyptian history the trade was continued, with the result that Byblos, the port of the Forest of Lebanon, became one of the chief maritime trading centres on the Syrian coast.

The following is a summary of the identifications of the woods from which the Egyptian specimens in my collection were made, and I am much indebted to Mr. L. A. Boodle, of Kew, to Mr. J. C. Maby and to Mr. B. J. Rendle, of the Forest Products Research Laboratory, Oxford, for their assistance in the identification of these woods.

Wood.	*Object made of the wood.*	*Date about*	*Source of the wood.*
Ficus sycomorus	XIIth dynasty, coffin	2000 B.C.	Egypt.
Ficus sycomorus	XIIth dynasty, tomb statuette	2000 B.C.	Egypt.
Ficus sycomorus	Model building-cradles	1500 B.C.	Egypt.
Cedrus libani	X–XIth dynasty, coffins	2200–2000 B.C.	Lebanon.
Tamarix nilotica (?)	Model mattock, from Dêr el-Baḥarî	1500 B.C.	Egypt.
Acacia seyal or Acacia nilotica (?)	Boning-rod with flax string	1st cent. B.C.(?)	Egypt.
Buxus sempervirens	Mummy labels, with Greek script	3rd–4th cent. A.D.	N. Africa (?).
Tilia, sp.*	Mummy label, with Greek script	,, ,,	Some country north of Egypt.
Fagus sylvatica†	Mummy label with Coptic script	,, ,,	,, ,,

* Lime. † Beech.

A Simple Apparatus for the Rapid Determination of Combustible Vapours in the Atmosphere

By L. C. McNAIR, B.Sc., and H. C. GULL, M.Sc.

Associated with the industrial use of inflammable organic liquids are frequently their poisonous character and the risks of explosion. It is useful, therefore, to have a simple and rapid method whereby the concentration of the vapour of such liquids present in the atmosphere of a room or tank may be determined with reasonable accuracy. For this purpose the Haldane apparatus (Foster and Haldane, 1905, *The Investigation of Mine Air*, p. 100) has been largely used.

In order to meet the desire for an apparatus which embodies the principle of that of Haldane, but is more portable, the instrument to be described was devised. This matter was brought to our notice some three years ago, and the

present modification of the Haldane apparatus is the outcome of experiments made in this laboratory during the last two years.

It consists of two glass vessels of approximately 100 ml. capacity: one of known volume to contain the sample of air under examination (Vessel A, Fig. 1), the other a control vessel, B. In Fig. 1, for the sake of clearness, the manometer

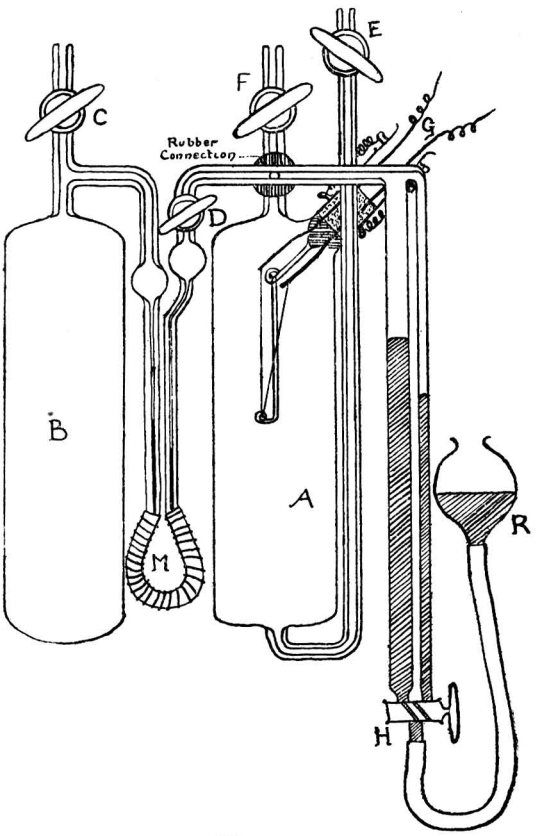


Fig. 1.

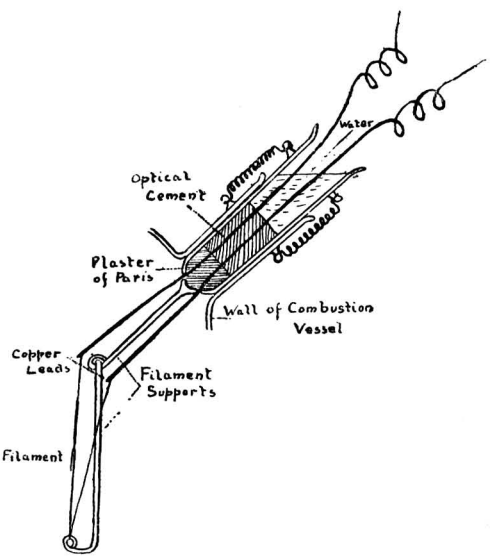


Fig. 2.

and burettes are shown displaced to the right, whereas in the actual apparatus they are in front of vessels B and A, respectively. Both vessels are immersed in a water-bath which is stirred by a current of air. The control vessel, B, is fitted with a stopcock, C, opening to the air, and is connected with the sample vessel by the manometer, M, containing coloured water, and the cock, D. For the construction of the manometer, capillary tubing of approximately 0·5 mm. bore is used, and the two limbs are connected at their lower ends by a piece of rubber tubing bound with copper wire to prevent kinking. A small bulb, of 0·5 ml. capacity, is blown in the upper part of each limb, to minimise the risk of the coloured water being drawn out of the manometer by a considerable difference in pressure between the two vessels.

At its lower end the sample vessel, A, is fitted with an inlet tube and stopcock, E, and at the top with an outlet tube and cock, F. This vessel is in

connection with the manometer and with a graduated burette consisting of two branches; the larger, with a capacity of 5·5 ml., is graduated in tenths of a ml., and the smaller, with a capacity of 1 ml., in hundredths of a ml. A three-way tap, H, at the lower end of the double burette, allows either section to be connected independently with the reservoir, R. Mercury or water may be used in the reservoir and burettes. The overall dimensions of the apparatus are 10 in. × 5 in. × 3 in.

It has been found advisable to have the arrangement for combustion as a separate entity. A side tube, 1 cm. in diameter, is sealed into the upper end of the sample vessel. Into this is ground a glass stopper, G, carrying a platinum filament, together with its supports and electrical connections, by means of which the inflammable vapour in the sample vessel is burned to carbon dioxide and water. Fig. 2 illustrates the construction of this stopper in greater detail. To the lower end of the stopper is sealed a short length of thin glass rod, terminating in a hook from which is suspended another piece of rod to support the centre of the filament. The ends of the filament are twisted round two stout copper wires which pass to the interior of the stopper through two small holes in the glass, one on either side of the support; there they are held in place by a small plug of plaster of Paris, followed by a non-porous layer of shellac cement or sealing wax of high melting point. The remaining space in the stopper, which is open to the air, may be filled with distilled water, to keep the copper wires quite cold in use. Two springs are used to keep the stopper in place, and a little tap grease is used as a lubricant to ensure a gas-tight joint. This arrangement allows the platinum filament to be easily replaced should a "burn-out" inadvertently occur.

In use, it is essential that the insides of both vessels be kept moist. When carrying out an analysis, all taps are opened, and the reservoir, R, is lowered until the liquid level in the burette to be used is near the lower end of the burette. Taps D and H are then closed, and the air under examination is aspirated through the apparatus, by applying suction at F, until the original air in the sample vessel has been replaced. It usually suffices to aspirate about 400 ml. Taps C, E, and F, are then closed, and D and H are opened. The water-bath is well stirred for a minute or so, and the liquid level in the burette is adjusted so that the water levels in the two manometer limbs are equal. The burette reading is noted, and taps D and H are closed. The filament leads are connected with a battery in series with a small variable resistance, and the filament is raised to a dull red heat for five minutes. At the end of this period the current is stopped and the apparatus is allowed to cool for six minutes, the water-bath being stirred meanwhile. Tap H is first opened, and then D, cautiously, and the liquid level in the burettes is readjusted so as to bring the liquid levels in the manometer back to equality. It has been found that the times given are the least that are necessary to ensure satisfactory results. It should be noted that a considerable pressure is developed in A during combustion, and all taps must be closed and be quite gas-tight before switching on the current.

In order to test the apparatus, mixtures of benzene and air, and of hexane and air in known concentration, were prepared and examined in the following way:

A suitable quantity of the liquid solvent was weighed in a thin-walled, sealed

glass bulb and dropped into a clean dry bottle of 11,200 ml. capacity, together with 100 ml. of clean mercury. The bottle was fitted with a closely-fitting glass stopper (a gas-tight joint being ensured with a little tap grease), fitted with a three-way tap, and held firmly in position with a clamp. Some two litres of clean air were then pumped into the bottle, the tap closed, and the bottle shaken vigorously until the glass bulb was broken. A small mercury manometer was connected with the bottle and the pressure in it was measured. The bottle was then connected with the inlet tube of the analysis apparatus by means of a short length of glass tube, rubber connections being reduced to a minimum, and the gas in the bottle was allowed to flow through the combustion vessel, replacing the air therein. An analysis was then carried out as described above. The composition of the gas issuing from the bottle was calculated from the weight of solvent used, capacity of the bottle, pressure and temperature of the gas in the bottle, and the barometric pressure. It was found that the inflammable vapour in these mixtures was partly removed by contact with rubber or water. Results of these test analyses are given below.

In blank tests, a contraction of 0·01 per cent. was always obtained. This was due, not to leaks, but, possibly, to the formation of oxides of nitrogen, since, after combustion, these were detected in the air from the combustion chamber. A deduction was made from all observed contractions to allow for this small error.

RESULTS OF ANALYSES

Substance.	Calculated. Per Cent. (by vol.).	Found. Per Cent. (by vol.).	Contraction observed in vessel of 98 ml., less 0·01 ml. ml.
Benzene	1·18	1·18	2·90
,,	0·807	0·816	2·00
,,	0·023	0·022	0·055
Hexane	1·05	1·04	4·56
,,	0·107	0·107	0·470
,,	0·025	0·025	0·110

The results agree with those calculated. Quantities greater than 2·5 per cent. of benzene or 1·2 per cent. of hexane could not be determined, as a contraction greater than 5·5 ml. could not be measured in our burette.

Mixtures rich in inflammable vapour should first be tested in a stout glass bulb, fitted with a stout filament for firing, to see if an explosion is possible.

In conclusion, it may be stated that several forms of this apparatus were constructed and found unsuitable in one respect or another. Among these was an apparatus with a brass combustion chamber, which gave consistently high results, due to interaction between carbon dioxide and the walls of the vessel, in the presence of moisture.

We desire to tender our thanks to the Government Chemist for permission to publish this work.

THE GOVERNMENT LABORATORY,
CLEMENT'S INN PASSAGE, STRAND, W.C.2.

Official Appointments

THE Minister of Health has confirmed the following appointment:

ERIC VOELCKER, F.I.C., A.R.C.S., as Public Analyst for the Borough of Banbury (December 7th, 1931).

The Minister of Agriculture and Fisheries has confirmed the following appointments:

HAROLD LOWE, M.Sc., F.I.C., as Agricultural Analyst for the Counties of Anglesey and Caernarvon and the County Borough of Chester (February 23rd, 1932).

JOHN EVANS, F.I.C., as Agricultural Analyst for Cardigan (February 23rd, 1932).

J. G. LUNT, F.I.C., as Deputy Agricultural Analyst for the County Borough of Leicester (February 23rd, 1932).

ALBERT E. PARKES, F.I.C., F.C.S., as Agricultural Analyst for West Ham (February 23rd, 1932).

W. H. ROBERTS, M.Sc., F.I.C., as Agricultural Analyst for the County Borough of Southport (February 23rd, 1932).

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.

GASEOUS ARSENIC FROM WALL-PLASTER

ON January 19th an inquest was held at Cinderford, Forest of Dean, on the bodies of two children who had died after a mysterious illness. The parents and two other children were also affected.

Professor H. A. Scholberg, of Cardiff University, said that he had made a microscopical and bacteriological examination of the lungs of the boy. He attributed the death to bronchial pneumonia and blood poisoning. In this case the jury returned a verdict of death from "natural causes," and were of opinion that there was not sufficient evidence to show that the arsenic found had contributed to the death.

At the inquest on the girl, Mr. R. H. Ellis, F.I.C., County Analyst, said that he had found arsenic in certain organs of the body (*viz.* intestines, liver, kidneys and lungs), the total amount (as arsenious oxide) being 2·65 mgrms. He had also analysed samples of the wall-paper and of the plaster. In the paper from a dry part of the wall he had found 8·3 parts per million of arsenious oxide; in samples from a part where the mould was most pronounced there were 2·3 parts per million, and in the plaster there were 91 parts. An unused roll of wall-paper, purchased at the same time, contained 4·4 parts of arsenic per million. He had found definite traces of arsenic being given off in gaseous form from the wall that was affected by mould, and it was significant that the arsenic content of the mouldy wall-paper was only half that in a portion of the new paper, and only a quarter of that in a sample of the same paper taken from a dry part of the wall. In his opinion, the arsenic in the paper was present as an impurity, and he attributed the trouble to

the plaster, and not to the paper. The arsenic in the plaster, which was composed of coke breeze and cement, would dissolve in the moisture coming through the wall from the bank of soil outside, and the mould would then grow on the paper and would liberate the arsenic in the form of a very deadly organic compound.

Mr. Ellis added that other tests made by him showed that four of the six members of the family had traces of arsenic in their systems.*

The jury returned a verdict that death was due to dysentery and to exposure to arsenic, which was generated in the house in a gaseous form. They added, as a rider, that the house was not fit for human habitation in its present condition, and should be inspected by the Medical Officer of Health before the family were allowed to return to it.

* Mr. Ellis has informed the Publication Committee that consideration was given to the possibility of the quantity of arsenic found being present without any question of poisoning, but the distribution of the quantities found was also taken into account and considered in connection with the pathological condition of the organs. One of the chief factors which led to the conclusion formed was the fact that the amount of arsenic found in the lungs was greater in parts per million than in any other part of the body, except the large intestine, and this agreed with the condition of the lungs.

The problem of proving the presence of the arsenic in the air was more difficult, and an attempt to detect it by simple aspiration gave negative results. Experiments were, therefore, made by exposing filter papers, saturated with silver nitrate, on the walls of the house, and these were left for 7 and 9 days, respectively. When these filter papers were destroyed, in the usual way, and the amount of arsenic was determined by the electrolytic Marsh test, small mirrors of arsenic were obtained.—EDITOR.

SUMMONS ISSUED UNDER MILK AND DAIRIES (AMENDMENT) ACT, 1922

ON January 9th, 1932, a dairy farmer was summoned before the Reigate (County) Bench for selling milk to which water had been added, proceedings being taken under Section 4, Sub-section 1, of the above Act, instead of under Section 2 of the Food and Drugs (Adulteration) Act, 1928. The samples in connection with which summonses were issued were taken in course of delivery, and contained 8·41 and 8·28 per cent. of solids-not-fat, and 3·49 and 3·89 per cent. of fat, respectively. In the opinion of the Public Analyst, the freezing points of the samples showed that the solids-not-fat were originally high, not less than 9·1 per cent., and indicated that the samples contained not less than 7 and 9 per cent. of added water. Samples of the mixed milk (in two churns) from the herd (24 cows), taken at the farm 24 hours later, were found to contain 9·16 and 9·39 per cent. of solids-not-fat, and 3·73 and 4·39 per cent. of fat, respectively. A conviction was recorded and a fine of £2 was imposed.

Department of Scientific and Industrial Research

REPORT FOR THE YEAR 1930–1931*

The Report of the Privy Council for Scientific and Industrial Research (pp. 1–8) notes the reduction of expenditure effected and deals with the personnel of the Advisory Council, Research Boards and Standing Committees. During the year the responsibility of the British Museum Laboratory has been transferred to the trustees of the Museum. Twenty-six British patents have been applied for, in connection with the various lines of research, and the report of the Research Committee, set up by the Imperial Conference in 1930, is noted. *The Report of the Advisory*

* Obtainable at Adastral House, Kingsway, W.C.2. Price 3s. 0d. net.

Council (pp. 9–20) covers the history of the growth of the Department's organisation; its contact with industry through the Research Associations, established by firms in an industry or group of related industries, and by the research stations of the Department; the application of new inventions, and general conclusions reached. The next section of the Report is the *Summary of Work* (pp. 21–93), followed by an account of the *Research Associations* (pp. 94–129) and *Appendices* (pp. 130–175), including a list of publications.

Summary of Work

National Physical Laboratory.—The annual report for the Laboratory has been already published (Analyst, 1931, **56**, 661).

Fuel Research.—Work on the constitution of coal and on methods for examining it proceeds, and the work in hand includes the study of pitch and its application to briquetting, domestic heating and testing of combustibility of cokes and other solid fuels; internal combustion engines, and tests of various fuels and blends, and development of gas producers for use with motor lorries; the study of pulverised fuel and some aspects of atmospheric pollution, and the production of lubricating oils from coal.

Food Investigation.—The Report for the year 1930 has been published (see Analyst, 1931, **56**, 531).

The Low Temperature Research Station deals largely with gas-storage of ripe fruit. The two gas-stores for English Bramley's Seedling apples have proved so successful that others are being opened.

Retarding the Ripening of Fruit.—Bananas have been treated for retarding ripening. It has been shown that acetaldehyde is a normal intermediate product in the breakdown and oxidation of sugar to carbon dioxide and water in plant tissues generally, and in over-ripe fruit partial inactivation of the respiratory mechanism results in accumulation of acetaldehyde. Also, by packing the fruit in an atmosphere containing a regulated amount of acetaldehyde, the breakdown of the sugar is thrown out of its normal course.

Canning.—The problems connected with corrosion of tin plate cans by fruit juices and syrup have been investigated (see Analyst, 1931, **56**, 315), and the prolonging of the canning season for soft fruits and vegetables by cold storage is suggested as applicable to many products. The colour and flavour of peas can be preserved by parboiling or blanching in water and then freezing and storing indefinitely at -20° to -10° C.

Pig Products.—The smoking of bacon has been found to retard the onset of rancidity.

Torry Research Station.—Carbohydrate Metabolism of Fish.—The nature of vitamin *A* present in the fats of fish and the colour test for it are being studied, and the claims made by Mittelmann, namely, that the oil prepared from fresh cod livers autoclaved in sealed tins did not, on first opening of the tins, respond to the antimony trichloride test, but recovered its chromogenic power on standing, have been investigated and partly substantiated.

Ditton Laboratory.—The distribution of temperature in a mass of 110 tons of fruit, when refrigerated in different ways, is being mapped. Isolation of the volatile substances given off by apples during storage has been found to be beset with technical difficulties, but is being attempted, and the nature of the waxy coating on apples is also being investigated.

Forest Products Research.—Among much other work, the conclusions arrived at by the International Conference on Standardisation of Wood Preservatives (Analyst, 1931, **56**, 127), wherein it was agreed that wood block tests are

more likely to give a true value for the toxicity of a preservative than agar tests, have resulted in certain standard methods being worked out in co-ordination with other research institutes, the first step being the choosing of a single vigorous strain of *Coniophora cerebella*.

CHEMICAL RESEARCH.—CORROSION.—In this section quantitative measurements have been extended to the corrosion of iron and mild steel when immersed in salt solutions saturated with oxygen, and determinations have for the first time been made on the rate of the hydrogen evolution from steel in potassium chloride solutions. Exploratory work on the protection of magnesium alloys has involved an examination of more than 500 coatings, from which 12 of the more promising have been selected for further trials. Among such selected coatings were those containing a chromium basis derived from either chromic acid or an alkali chromate. These earlier experiments were followed by the important discovery that protective films of selenium are produced when sheets of light magnesium alloys are immersed in acidified solutions of selenium compounds (selenious acid, sodium selenite, etc.). Such selenium films exhibit a noteworthy resistance to intermittent spraying with salt water.

LOW-TEMPERATURE TAR.—The presence of anthracene and its homologues has been confirmed in the anthracene fraction of low-temperature tar, and the new 2:3:6:7-tetramethylanthracene and the corresponding tetramethylanthraquinone have been synthesised, the former being identified in the low-temperature tar. Homocatechol, iso-homocatechol, resorcinol and quinol have also been identified. To the methods used for separating tar constituents has been added distillation of the least volatile neutral oils and phenols by means of high-vacuum plants. At temperatures not above 120° C. the greater part of the crystalloid portion of these fractions distils over, leaving the resinenes and resinols.

CHEMOTHERAPY.—Two promising indications of therapeutic activity have been revealed by a series of fluorene derivatives containing arsenic, and a new group of arsenicals analogous to tryparsamide.

BRITISH MUSEUM LABORATORY.—*Removal of Stains:* The value of chloramine-T for removing "foxed" and other mildew and mould stains from water colours has been confirmed, and vinyl acetate seems likely to be a valuable strengthening element for porous and friable material.

Among the antiquities from Ur of the Chaldees was a silver dish which has been renovated by a solution of citric acid, still the most efficient treatment for bronze and silver objects alloyed with copper.

Other sections of the Report deal with *The Geological Survey and Museum of Practical Geology; Building Research; Steel Structures Research; Electro-Deposition; Water Pollution: Radio Research; Dental Investigation; Illumination; Lubrication; Fabrics; Geosophysical Survey; Atmospheric Pollution;* and *X-ray Analysis of Crystals.*

RESEARCH ASSOCIATIONS

Among the subjects dealt with are:

RESEARCH ASSOCIATION OF BRITISH PAINT, COLOUR AND VARNISH MANUFACTURERS.—With the co-operation of the British Colour Makers' Association a table of fastness has been prepared, which is rapidly being accepted by the industry as a basis of colour evaluation of pigments, and it is claimed that no difficulty will be found in correlating results with those obtained under other defined conditions.

Value of Iron Oxide as Pigment.—An investigation of the precipitated iron oxides shows that the value of iron oxide as a pigment depends on the colour-staining power and other factors (described collectively as paint-making property), depending chiefly on the degree of reactivity between pigment and oil medium. A

series of products obtained by heating precipitated iron oxides at different temperatures for different times was examined by *X*-ray methods, and it was shown that the structure developed by the oxide is very sensitive to small changes in degree of heat treatment, and that, up to a point, these are correlated with pigment property.

Lithopones, mechanical mixtures of barium sulphate, titanium oxide and the co-precipitated products have also been examined; and although the *X*-ray patterns of the mechanical and co-precipitated mixtures do not differentiate between these products possessing very different painting properties, the figures are possibly confused through growth or diminution of crystal size as a result of heating.

British Cotton Industry Research Association.—An observation likely to be practically important is that certain fast colours, themselves unaffected in shade during bleaching, are capable of inducing very rapid attack by the bleach liquor of the cotton on which they are dyed, so that in a bleached shirting or other cloth the stripe dyed with such a colour rapidly disintegrates in wear, if not before. Much work on the re-classification of dyestuffs is involved in the light of such information.

Wool Industries Research Association.—With mixtures of cellulose, leather, rubber and wool, all in suitable form, products resembling leather were produced, and after the preparation of bulk samples, their uses for gloves, boot uppers, leather coats, etc., are being explored. Although a special fabric treatment is involved, wool has been found particularly suitable for such purposes.

British Launderers' Research Association.—Strongly adhering dirt particles are found to be those which, in minute form, are adsorbed on the fibre surface, and not those attached to the fibre by grease. Grease, except when present in large quantities, presents no special difficulty in removal; but for such articles as butchers' or engineers' overalls it is probable that an organic solvent, suspended in a dilute solution containing only just sufficient of an emulsifying agent, will prove helpful. Under suitable conditions the solvent will be deposited only on the greasy or oily areas of the immersed fabric. "Breaking emulsion" methods of treatment may prove economical in the cleansing of such fabrics.

Research Association of British Flour Millers.—New crop wheat examination is now a permanent feature of the work. Two Reports have been issued, summarising 6 years' study of baking methods, including relations between yeast percentage and time and temperature of fermentation, etc. The importance of fermentation time and tolerance of a flour is emphasised. Methods of determining flour ash, and hydrogen-ion concentration have been improved, and one for finding the hydrogen-ion concentration of dough has been developed, as well as one for measuring the rate of movement of water through wheat. The factors producing "low-gradeness" in flours, and the part played by oxidation on storage, have been studied.

British Association of Research for the Cocoa, Chocolate, Sugar, Confectionery and Jam Trades.—*"Bloom" on chocolate* may be due to formation of a film of minute crystals of cocoa butter, or to crystals of sugar. The methods found for preventing fatty bloom have been successful, both for ordinary chocolates and those with nutty or fatty centres, although, in the latter case, an appreciable cost is added to manufacture.

British Food Manufacturers' Research Association.—The dominant factor influencing the formation of patchy separation of liquid or fat, and appearance of air spaces and films in potted meat and fish pastes has been found. The closing of paste jars under vacuum was found to remove entirely the greyish discoloration due to the action of residual air, and the purplish discoloration on food in glass containers has been traced to contamination with minute quantities of metals. The causative agent of green discoloration of cooked pickled foods has also been investigated, and methods are suggested for overcoming the discoloration of sausages on exposure to air. D. G. H.

Connecticut Agricultural Experiment Station

ANNUAL REPORT FOR THE YEAR 1930*

THIS Station was the first Agricultural Experiment Station in U.S.A. to be officially charged with food control work by the State legislature. The present law, enacted in 1907, was preceded by a comprehensive and practical statute, passed in 1895, which aimed at preventing adulteration of foods generally. Under this old law the first food report was issued by the Station in 1896. Similar duties were imposed upon the Stations in Kentucky in 1898, in North Dakota and Wyoming in 1903, and in Maine in 1905. Prior to 1895 there were a few special laws governing the manufacture and sale of certain foods, notably vinegar, butter and molasses.

The revision of the food law, made in 1907, contemplated chiefly an inclusion of drugs in the scope of inspection and control, and for the last twenty-three years the annual report on foods has included also an account of drug inspection.

Most of the samples examined are submitted by the Dairy and Food Commissioner, although the Station may, and does, examine samples of both foods and drugs collected by its own agent. In all cases, however, instances of adulteration or misbranding are reported to the Commissioner for corrective action as required by law.

Among the points of interest dealt with by Dr. E. M. Bailey in the present report are the following:

CARBONATED BEVERAGES, ETC.—The provision of the carbonated beverage law, requiring not less than 5 per cent. of sugar in beverages of this type, is always met, and generally exceeded. Failures to declare artificial colours and flavours in beverages where such ingredients are used are rare. Saccharin is seldom found. A number of beverages sold under distinctive names, "Pepsi-Cola," "O. C. Kola," "Coca Cola," and "Braser," contained caffeine in approximately the proportions found in tea infusions, as ordinarily prepared.

CHERRY CIDER.—A product was rather widely sold during the past season under the name of "cherry cider." Artificial colour and flavouring material and a benzoate were regular constituents of the samples examined. In one instance the base of the product appeared to be apple cider to which colour and flavour had been added to simulate the character of cherry.

ORANGE JUICE.—The emphasis placed upon the nutritional advantages of fruit juices has resulted in a large increase in consumption of drinks consisting wholly, or in part, of fruit juice, more especially orange juice. In many instances orange juice is prepared in the presence of the customer and the genuine character of the beverage is unquestioned, but when served from stock solutions the consumer may receive a beverage considerably diluted as regards actual fruit juice. This is well illustrated by the following comparative analyses. Sample 1 is genuine orange juice prepared in the presence of the inspector, whilst samples 2 and 3 are so-called "orange juice," considerably diluted and fortified with sugar and citric acid, and artificially coloured. Analyses are on filtered juice, and are in terms of grms. per 100 c.c.

	1 (genuine).	2 (diluted).	3 (diluted).
Solids	9·40	—	—
Sucrose	4·36	14·51	14·67
Invert sugar	3·32	0·82	0·94
Total sugars	7·68	15·33	15·61
Acidity, as citric acid ..	0·76	0·99	0·97
Ash	0·41	0·08	0·07
Nitrogen	0·052	—	0·017

* *Bull.* No. 329, 1931.

The characteristics of genuine orange juice are seen to be a relatively high ash and a considerable amount of invert sugar. The sugar distribution in the artificial products will, however, vary, according to the degree of acidity and the length of time that the acid has had an opportunity to act upon the sucrose present. The chief characteristic of the genuine juice is the ash content, which itself is further characterised by its potassium and phosphorus content, in the above cases undetermined.

The proportion of orange juice which should be present in a beverage not claimed to be entirely juice, but laying stress on orange juice content, is not a matter of concordant opinion or practice at the present time. Some State Regulations allow as little as .5 per cent., whilst others require 15 per cent. It would appear that the higher figure is more nearly in accord with what the consumer may reasonably expect in a beverage such as orangeade. This proportion might, or might not, hold for other fruit juices, according to the character of the fruit in question.

Since orange juice, as ordinarily prepared by reaming or other means accomplishing a similar result, contains some pulp, abuses may arise from the inclusion of excess of pulp, and thus create a false impression of fruit juice content.

Considerable attention has been given to the problem of a fair and rational classification of carbonated and still beverages, both by state control officials and by the Standards Committee, but conflicting trade practices and other difficulties have thus far prevented any satisfactory solution to the question.

COFFEE, ETC.—A sample of "Café des Invalides," a product labelled as containing about 7/8 coffee, the remainder being other vegetable substances, was examined. The addition of non-coffee material is designed, in part, to reduce the caffeine content below that of ordinary coffee. A mechanical separation of the ingredients on the basis of 1 grm. showed 0·892 grm. of coffee, 0·092 grm. of vegetable material, which appeared to be pea hulls, and 0·016 grm. of chicory. The caffeine content was 1·10 per cent. The label declaration of 7/8 coffee is borne out by the examination of the product.

Yermat.—A sample of *Yermat*, prepared by The Yerba Maté Corporation of Chicago, was submitted for analysis. This is a carbonated infusion of the dried leaves of Yerba Maté (*Ilex paraguayensis*), sweetened, and flavoured with an essential oil. It contained 8·5 per cent. of sugar and 4 mgrms. of caffeine in each 100 c.c. of solution. The leaves of this plant, sometimes called Paraguay tea, are used in the preparation of an infusion largely consumed in South American countries as a beverage.

IMITATION MAPLE FLAVOUR.—One sample of imitation maple flavour was submitted by the Dairy and Food Commissioner. The brand name was *Elpam*. The accompanying literature showed plainly the imitation character of the product, but the identity of the flavouring principle was not established. An attempt was made to isolate choline and trigonelline by the method of Jahns (*Ber.*, 1885, **18**, 2518) to show the presence of fenugreek, but, although the presence of organic bases was indicated, they were insufficient in quantity to make isolation and identification possible.

SAUSAGES.—Five samples of Frankfurt, and one of Bologna, sausages were examined, and four were found to be illegal because of undeclared cereal or cereal in excess of 3·5 per cent.

In the manufacture of sausage it is permissible to use 3 per cent. of water or ice, and somewhat more in the case of those types of sausage which are smoked or cooked; but in no case should more water be introduced than is necessary to facilitate satisfactory manufacturing processes or to make the products palatable. In the instance of Frankfurt sausages, for example, 10 per cent. of added water appears to be an acceptable margin in control practice.

A study of the ratio of water to protein in the cuts of meat generally used in sausage making indicates that the proportion is 4 to 1. The procedure for estimating excess moisture is to determine total moisture and nitrogen in the samples. The nitrogen, multiplied by 25 (basis of $N \times 6{\cdot}25 = \text{protein}$), should not be exceeded by the total moisture in the sample by more than 3 per cent. in the case of pork sausage, or by more than 10 per cent. in sausage of the Frankfurt type.

Samples of Frankfurt sausages, submitted by a local packer, afforded an opportunity to try this method and to check our results in some cases with those obtained in other control laboratories. Eight samples were analysed. The uncertainty in comparing results from different laboratories hinges particularly upon lack of evidence that the samples submitted to the several laboratories were sufficiently alike in composition to make strict comparisons. In one case of disagreement, results calculated to the water-free basis, showed that the solids in the two samples worked upon were substantially different in amount. If the uniformity of samples is assured, the other sources of disagreement are differences in methods used. Results for nitrogen should be satisfactorily close, but moisture may vary according to the procedure followed. In our work, drying for 16 hours at 100° C. in an air-oven was found to compare very closely with results obtained by the Bidwell-Sterling distillation method.

One sample in this series, upon which results for added water from three laboratories are available, shows data as follows:—This laboratory, 9·3 per cent.; laboratory A, 11·3 per cent.; laboratory B, 10·7 per cent. The results (this laboratory) in detail are as follows:

	Per Cent.	Per Cent.	Per Cent.		Per Cent.
Water (drying oven) ..	60·2	59·8	59·4	avg.	59·8
(distillation method)	—	—	—		59·6
Nitrogen	2·01	2·03	2·03	avg.	2·02
Protein ($N \times 6{\cdot}25$)	—	—	—		12·63
Protein × 4	—	—	—		50·5
Added water (59·8—50·5)	—	—	—		9·3

On the basis of these data it would appear that only one of these results would raise any question whether the limit of 10 per cent. of added water had been exceeded. How strict an interpretation can be placed upon the margin between the 10 per cent. tolerance and the highest result reported, *viz.* 11·3 per cent., will depend upon what evidence is available that the sample is representative of the batch involved, and on the coefficient of error in determining moisture and nitrogen in the laboratory concerned.

MARKET MILK.—The large proportion of samples (214 of 785 examined) found to be, in part, skimmed, is explained by the fact that many were found in places where milk was dispensed either by dipping, or from quart bottles rather than from individual service bottles. The law requires that milk, when served by any hotel, restaurant, lunch room, fountain or any other place of public entertainment, shall be served in the original bottle, the cap of which shall not be removed except in the presence of the customer. Commenting upon this provision of the statutes, the Dairy and Food Commissioner says in his biennial report: "So gratifying are the results of the original or individual bottle law that a sampling campaign is now being carried on to demonstrate, particularly to the 'fountain' interests, the extremely low quality of milk used in mixed drinks. Hundreds of samples indicate that milk so used contained much less than the legal standard of butter fat."

It was with this in mind that the inspection was extended by the Commissioner to include tests for milk fat in milk drinks dispensed by "fountains." This inspection was confined, however, to so-called "milk shakes," in the preparation of which, other materials are not added in amounts sufficient to reduce the fat content of the drink substantially below that in the milk used in its preparation.

Sixty-five samples were examined, which may be classified as follows:—Below 3·25 per cent. of fat (legal standard for milk), 39; above 3·25 per cent. of fat, 26. In the first group, the fat content ranged from 0·9 to 3·2 per cent., and averaged 2·5 per cent. In the second group, the range was from 3·3 to 7·2 per cent., and the average 4·0 per cent. High percentages of fat indicate the use of top milk. There is no official definition of what constitutes the article known as a "milk shake," but it has been ruled that skimmed milk should not be used in the preparation of drinks sold as chocolated milk or milk chocolate and, by analogy, it would appear that whole milk should be used in the preparation of milk shakes.

SALAD DRESSING, MAYONNAISE.—Mayonnaise salad dressing is essentially an oil dressing further characterised by varying amounts of egg-yolk or whole egg, and contains vinegar or lemon juice and seasoning materials. The U.S. Department of Agriculture, in its standard for this product, specifies for the major ingredients not less than 50 per cent. of oil and a percentage of egg-yolk and oil together of not less than 78.

The estimation of the egg content in materials of this sort is made upon the basis of accepted average values for lipoid phosphoric acid, which is present in egg-yolk, but absent or negligible in the white. It is evident that the application of a uniform factor for the estimation of the egg content of various brands of market mayonnaise dressings involves necessary reservations. The accepted value for the lipoid phosphoric acid content of whole dry egg is 1·38* per cent., and for dry egg-yolk 1·78* per cent. Assuming 25 per cent. of solids for fresh whole egg, and 50 per cent. of solids for fresh egg-yolk, these percentages become 0·35 and 0·89, respectively.

The following table gives the results of a few typical analyses:

ANALYSES OF MAYONNAISE DRESSINGS

Manufacturer and brand.	Starch.	Halphen test, on fat.	Solids.	Ash.	Protein (N x 6·25).	Carbohydrate, including fibre, by diff.	Fat (Roese-Gottlieb).	Salt.	Acidity (as Acetic acid).	Lipoid P_2O_5.	Distribution of egg and oil, estimated.		
											Egg-yolk, fresh.	Oil.	Oil and egg-yolk.
Booth, *Booth's*	none	+	92·33	0·87	0·88	3·21	87·37	0·68	0·29	0·020	2·25	86·62	88·87
Duke Products Co., *Duke's*	none	—	87·91	1·21	1·50	0·82	84·38	1·05	0·55	0·039	4·38	82·92	87·30
Easton, *Easton's* ..	none	+	86·29	1·24	1·75	2·46	80·84	0·91	0·49	0·060	6·74	78·60	85·34
Heinz, T. H., Co., *Heinz*	none	—	86·44	1·04	1·88	0·00	83·52	0·76	0·34	0·060	6·74	81·27	88·01
Ivanhoe Foods, Inc., *Shady Lane* ..	present[1]	+	72·58	1·71	1·75	5·17	63·95	1·40	0·58	0·056	6·30	61·85	68·15
Kraft-Phoenix Cheese Corp., *Kraft* ..	none	+	85·76	1·18	1·44	1·99	81·15	0·92	0·33	0·052	5·84	79·20	85·04
Leggett, *Premier* ..	none	+	63·82	3·06	3·75	1·73	55·28	1·83	1·37	0·138	15·50	50·11	65·61
Preston Market Co., Inc., *Premar* ..	none	—	87·60	1·43	1·25	3·09	81·83	1·15	0·43	0·019	2·13	80·60	82·73
Swift & Co., *Gem* ..	none	+	79·17	2·07	1·44	5·43	70·23	1·71	0·66	0·027	3·04	69·22	72·26

[1] Corn starch declared.

EXAMINATION OF ANIMAL TISSUE FOR ARSENIC.†—An examination made recently, when it seemed advisable to utilise all the procedures noted, is recorded here because of its interest from the standpoint of closely agreeing analytical results.

The material was finely comminuted to insure reasonable uniformity of sample. A portion was first subjected to the Reinsch test and a copious steel grey deposit

* Hertwig, *Proc.A.O.A.C.*, 1924, **8**, 2, 118.

† By C. E. Shepard and E. M. Bailey.

on copper foil was obtained. Thin strips of the foil were then introduced into a capillary tube, and, by careful heating, a sublimate was formed which revealed the characteristic octahedral crystals of arsenic trioxide when viewed with a microscope. The crystals may be seen distinctly in the capillary tube, and it is not necessary to resort to manipulation to obtain them on a slide before making the microscopic examination. This test alone establishes a strong presumption of the presence of arsenic, but, since antimony deposits as a film on copper foil, and yields a sublimate which, although generally amorphous, may consist of, or contain, octahedral crystals, further tests to identify arsenic are necessary. This test was carried out on the original materials, without destruction of organic matter, the copper foil being placed in a suspension of the tissue in water acidified with hydrochloric acid. A weighed quantity of material was then boiled with sulphuric acid and nitric acid until organic matter was destroyed, and the solution was made up to definite volume with water. Aliquot parts were taken for subsequent determinations.

The Gutzeit test furnishes qualitative evidence of arsenic, and the method may also be conducted on a quantitative basis. Aliquot parts from each of the solutions, representing 0·4 grm. of original material, were used. Freshly prepared and standardised mercuric bromide strips were used in measuring the arsenic liberated. This test was conducted substantially as described by Sanger and Black (*Amer. Acad. Arts and Sciences*, 1907, **43**, 297–324). Freedom of all reagents used from contamination with arsenic was established by suitable blank determinations. Duplicate determinations, representing two separate portions of original material, gave 35μ of arsenic (as trioxide), or 1·75 grains calculated to the basis of the original weight of material submitted (2 lbs. 14 ozs.).

The Ramberg method, described by Cox (ANALYST, 1925, **50**, 3), has been found to be reliable for the determination of small amounts of arsenic in animal tissues, and was used in this laboratory in the investigation of experimental mixtures of arsenical spray materials (*Conn. Exp. Station Bull.*, 1926, **278**). Arsenic was distilled and titrated with standard potassium bromate solution, 1 c.c. of which was equivalent to 0·00037 grm. of As_2O_3. The quantity of arsenic found by this procedure, when calculated to the basis of the original weight of material submitted, was 1·69 and 1·80 grains of As_2O_3, obtained in duplicate determinations.

Finally, the Marsh test, as modified by Berzelius, was applied. A characteristic mirror was obtained, the weight of which, calculated to its equivalent in As_2O_3 and to the basis of the original material submitted, was 1·32 grains. This figure is, no doubt, less accurate than those obtained by the other two methods employed. The mirror is probably not of uniform composition; the darker portion of the mirror is metallic arsenic; but the lighter, brownish portion, according to Rettgers (Peterson, Haines and Webster, "*Legal Medicine and Toxicology*," **2**, 231), consists of suboxide, As_2O, and hydride, AsH. The spot test with silver nitrate and nitric acid, the characteristic yellow sulphide and the odour of arsine, confirmed the identity of arsenic.

To summarise the quantitative results, the amounts of arsenic found were as follows:

By Gutzeit method	1·75 and 1·75 grains
,, Ramberg method	1·69 and 1·80 grains
,, Marsh-Berzelius method	1·32 grains.

Vitamin Standards

REPORT OF THE PERMANENT COMMISSION ON BIOLOGICAL STANDARDISATION*

THE International Conference on vitamin standards was presided over by Professor E. Mellanby, and was attended by representatives of Denmark, France, Holland, Germany, Great Britain, Norway, Sweden, and the United States of America. The British participant was Professor J. C. Drummond.

It was the general opinion that, in the present state of our knowledge, only vitamins *A*, *D*, *B*, (also known as vitamin B_1), and *C* could be profitably discussed in connection with standardisation.

I. THE FAT-SOLUBLE VITAMIN *A*

(*a*) INTERNATIONAL STANDARD.—*The Conference recommends that carotene be accepted as an international provisional standard of reference for vitamin* A, *and that a selected sample of cod-liver oil be held in view as a possible secondary standard.*

(*b*) MODE OF PREPARATION.—It was decided that the information available does not yet justify the selection of one isomer of carotene as a standard. The similar biological activity of the two isomers that have recently been described is further justification for adopting as the provisional international standard a mixture prepared in an approved manner.

It was decided to employ a preparation of carotene made from carrots by Willstätter's method and purified by recrystallisation by the method described in the memorandum issued by the Department of Biological Standards, National Institute for Medical Research, London (Appendix, p. 76), until the melting-point determined is above 179° C. It was suggested that preparations should be made in various countries and despatched immediately and with all necessary precautions against decomposition to the National Institute for Medical Research, London, where they will be mixed to form a uniform preparation by the most suitable method. The details of this final purification are to be left to the discretion of the authorities of the National Institute. It was suggested that original preparations of, say, 4 to 5 grms., might be made in the following institutions:

Department of Physiological Chemistry, University of Amsterdam.
"Laboratoire de physiologie de la nutrition, École des Hautes Études," Paris.
"Tierphysiologisches Institut," Leipzig.
National Institute for Medical Research, Hampstead, London.
Department of Agricultural Chemistry, University of Wisconsin, U.S.A.
Biochemical Department, University of Stockholm, and "Institut für Organische Chemie der Universität," Zürich.
School of Hygiene and Public Health, Johns Hopkins University, Baltimore, U.S.A.

(*c*) PLACE OF PREPARATION.—It was decided that the National Institute for Medical Research, London, acting for this purpose as the *central laboratory on behalf of the Health Organisation of the League of Nations*, should be asked to undertake the final preparation of the sample of carotene to be used as the international standard for vitamin *A*.

(*d*) MODE OF DISTRIBUTION.—It was considered desirable that, as far as possible, the standard preparation should be distributed to workers through the appropriate official institution in each country, preferably that now responsible for the distribution of similar biological standards. The material should be sent out in tubes of 10 mgrms., as described in the Appendix, p. 76.

(*e*) DEFINITION OF UNIT.—The unit of vitamin *A* recommended for adoption is the vitamin *A* activity of 1γ (0·001 mg.) of the international standard.

Note.—Daily doses of about 3γ to 5γ of the international standard, when administered to young rats suitably prepared on a vitamin *A*-deficient diet, have been found adequate to restore growth and to cure xerophthalmia.

(*f*) PERMANENCE OF THE STANDARD.—The Conference recommends that this international standard and unit be accepted provisionally for two years.

(*g*) SUBJECTS RECOMMENDED FOR FUTURE INVESTIGATIONS.—It is highly desirable that, during the provisional period, further investigations of the standard should be made regarding

* League of Nations Health Organisation. Report of Conference on Vitamin Standards, held in London from June 17th to 20th, 1931. Official Number C.H. 1056(1). Annex IV, CH. 1055(1). London: George Allen & Unwin, Ltd. Price 1s. 6d.

the stability of the carotene preparation, both when sealed in the original tubes and after it has been removed and dissolved for biological testing. In the latter connection, emphasis is laid on the importance of minimising contact with air or oxygen of the solutions used for animal feeding. It is recommended that they be always stored in an inert gas and at low temperature.

It will be of great value if investigators submit to the League of Nations Health Organisation observations on the stability of the preparation, its behaviour in various solvents and under different conditions of storage, and any other information bearing on its use as a biological standard. The use of suitable "antioxidants" ("*antioxygènes*" in French) should, in particular, be studied.

(*h*) BIOLOGICAL METHODS FOR ESTIMATION OF VITAMIN *A*.—It was decided not to recommend any one particular method of conducting the biological assay, but to invite members of the Conference to submit to the League of Nations Health Organisation their observations on the value of the methods they have been using.

It is recommended that further attention be given to the methods based on the curative action of carotene for xerophthalmia and other lesions characteristic of vitamin *A* deficiency, as well as to those based on increase in weight.

(*i*) SELECTED SAMPLE OF COD-LIVER OIL FOR USE AS A POSSIBLE SECONDARY STANDARD.—It is recommended that a supply of an approved sample of cod-liver oil be obtained with the object of making a series of comparative tests to determine its suitability as an alternative standard. The Conference is informed that the United States Department of Agriculture is making arrangements for such a standard substance to be available in the United States during the coming year.

It was decided to ask the United States Department of Agriculture to obtain sufficient supplies of the oil for distribution in order to enable investigators of other countries to assay this oil in terms of the international unit of standard carotene. It is hoped thus to obtain evidence regarding the stability of vitamin *A* in cod-liver oil as affected by conditions and time of storage.

II. THE ANTIRACHITIC VITAMIN *D*

(*a*) INTERNATIONAL STANDARD TO BE ADOPTED AND ARRANGEMENTS FOR CONTROL.—*The Conference recommends that the standard solution of irradiated ergosterol at present issued from the National Institute of Medical Research, London, be adopted as international vitamin D standard for the next two years.*

If within this period it should become necessary, owing to threatened exhaustion of the present supply, to replace this solution by a fresh standard, the equivalence shall be determined by experts of different countries who have had the opportunity of comparing the proposed new standard with the one at present issued. It is suggested that the following Institutions, among others, be invited to co-operate in those tests:

"Allgemeines Chemisches Laboratorium," Göttingen.
"Tierphysiologisches Institut," Leipzig.
Food and Drugs Administration Laboratory, Department of Agriculture, Washington, D.C.
Biochemical Department, University of Stockholm.
Department of Agricultural Chemistry, University of Wisconsin.
School of Hygiene, Johns Hopkins University, Baltimore.
"Laboratoire de physiologie de la nutrition, École des Hautes Études," Paris.
Pharmaceutical Society, London.

(*b*) METHOD OF PREPARATION.—1. It is recommended that, in the preparation of the solutions of irradiated ergosterol, used as standards of reference for vitamin *D* (or as sub-standards), irradiation with ultra-violet light shall be done in ethereal solution in the absence of any appreciable traces of oxygen, and the solution should be kept meanwhile in rapid motion. The conditions of exposure should be such as to transform between 30 per cent. and 80 per cent. of the ergosterol. The solution of the product and further dilutions shall be made in a stable unsaturated natural vegetable oil, which has given a negative test for vitamin *D*.

2. The standard solution of irradiated ergosterol at present issued from the National Institute for Medical Research, London (Standard Solution III), was, however, prepared as follows, in January, 1929:

> A 0·1 per cent. solution of the ergosterol in absolute alcohol was exposed for half-an-hour in a silica cell, 1 cm. thick, to the unfiltered radiation from a K.B.B. (Kelvin, Bottomley and Baird) mercury vapour lamp, taking 2·5 amperes and 125 volts at atmospheric pressure, at 15 cm. distance from cell to lamp. The resulting solution was mixed with a little olive oil, and then evaporated at 45° C. at a low pressure to remove the alcohol. The concentrated oily solution thus obtained was diluted with pure olive oil to give a concentration corresponding with 1 mgrm. of the original ergosterol in 10 c.c. of olive oil at 18° C. The olive oil used was tested for stability and gave a negative test for vitamin *D*.

The additional larger quantity of standard (prepared January, 1931), which is available for distribution later if required, was prepared with observance of the general conditions indicated under 1.

(*c*) MODE OF DISTRIBUTION.—The National Institute for Medical Research, London, *acting for this purpose as the central laboratory on behalf of the League of Nations Health Organisation,* shall distribute to each country wishing to use the standard a sufficient quantity of the solution to enable the standard to be effectively applied according to the conditions in the particular country. Such quantity shall be supplied only to a central institution nominated for the purpose by the country concerned, which will be responsible for the distribution either of the portion of international standard solution received or, wherever possible, of an equivalent sub-standard prepared by comparison therewith.

(*d*) STABILITY OF THE INTERNATIONAL STANDARD.—The stability of the standard solution at present issued from the National Institute for Medical Research, London, has proved satisfactory on the results of tests over a period of two years, when preserved at or below 0° C., with exclusion of air.

(*e*) DEFINITION OF UNIT.—The unit of vitamin *D* recommended for adoption is defined as the vitamin *D* activity of 1 mgrm. of the international standard solution of irradiated ergosterol.

Note.—The international standard solution has been prepared to have such potency that approximately 1 mgrm. thereof given daily to a rachitic rat for eight successive days will produce a wide line of calcium deposits in the metaphysis of the proximal ends of the tibiae and of the distal ends of the radii.

(*f*) PERMANENCE OF THE STANDARD.—The international standard at present recommended shall be regarded as provisional for the next two years, in the hope that a more stable crystalline substance may in the meantime become available.

(*g*) SUBJECTS RECOMMENDED FOR FURTHER INVESTIGATION.—1. The influence of various oils as solvents upon the stability of solutions of irradiated ergosterol.

2. The crystalline antirachitic substances recently isolated from irradiated ergosterol. It was decided that Professor Windaus and Dr. Bourdillon shall be asked to investigate the constancy of the physical properties of the crystalline products recently isolated by them, respectively, in order to determine whether these may be regarded as pure substances. If so, the potency of these products should be accurately compared with that of the (present) international standard at intervals of three months during the next two years, in order to compare the stability in each case. If the results are satisfactory, it is hoped that one of these crystalline substances may eventually replace the solution of irradiated ergosterol as international vitamin *D* standard.

3. The toxicity of the present standard and of the crystalline products (see also Appendix, page 75).

(*h*) BIOLOGICAL METHODS FOR ESTIMATION OF VITAMIN *D*.—In using the international standard solution for the determination of the antirachitic potency of unknown preparations, it is recommended that not fewer than twenty rats (preferably more) be used for a determination, half of these to receive the standard and the remaining litter-mates the unknown substance. Provided this precaution is observed, it is considered permissible to use various biological methods of estimation, either prophylactic or therapeutic. For instance, the "line" test, *X*-ray examination, and determination of the bone ash, are all considered reliable methods.

III. THE ANTINEURITIC VITAMIN *B*

(*a*) INTERNATIONAL STANDARD.—*The Conference recommends the adoption, as international standard, of the adsorption product of the antineuritic vitamin* B *prepared in the Medical Laboratory, Batavia (Java), by the method of Seidell, as described by Jansen and Donath.*

(*b*) TERMINOLOGY.—The international standard preparation should be known as the "standard adsorption product of the antineuritic vitamin *B*."

(*c*) METHOD OF PREPARATION.—The international standard is prepared by extracting rice polishings with water, sufficient sulphuric acid being added to make the *p*H 4·5. Salicylic acid, to a concentration of 0·2 per cent., and toluene are then added to prevent bacterial decomposition. The process of extraction is continued for two days, after which the solution is filtered. For each 100 kilogrammes of the original rice polishings, 3 kilos. of fuller's earth (specially selected for its adsorptive powers) are added to the solution, which is then stirred for twenty-four hours. Subsequently, the solution is filtered off and the fuller's earth, after being washed with water and alcohol, is dried; 3 kilos. of the fuller's earth adsorbate represent the antineuritic vitamin *B* from 100 kilos. of rice polishings.

(*d*) PLACE OF PREPARATION.—It is recommended that the Medical Laboratory, Batavia, Java, should be asked, through Professor Jansen, of Amsterdam, to prepare a batch of 25 kilos. of the standard preparation. This should provide an adequate supply for many years.

(*e*) PLACE OF DISTRIBUTION.—It is suggested that this batch of standard adsorption product of antineuritic vitamin *B* should be kept at the National Institute for Medical Research, London, *acting for this purpose as central laboratory on behalf of the Health Organisation of the League of Nations.*

One hundred grms. would be an amount suitable for distribution to individual laboratories. No special precautions are necessary in keeping this preparation, except that it should be stored in a dry place. In the presence of moisture, bacterial decomposition readily takes place.

(*f*) DEFINITION OF UNIT.—The unit recommended for adoption is the antineuritic activity of 10 mgrms. of the international standard adsorption product.

Note.—A daily dose of 10 to 20 mgrms. of this preparation is required to maintain normal growth in a young rat on a diet deficient in the antineuritic vitamin *B*, but complete in all other respects, including the antidermatitis vitamin (B_2); the curative "day dose" for a pigeon (300 grms. weight) suffering from polyneuritis on a diet of polished rice is about 20 to 30 mgrms. (method of Kinnersley and Peters).

(*g*) PERMANENCE OF THE INTERNATIONAL STANDARD RECOMMENDED.—This standard adsorption product should serve as a provisional international standard for five years, or until advances in the knowledge of this vitamin make a revision desirable.

(*h*) RECOMMENDATIONS FOR FURTHER INVESTIGATIONS.—1. The standard adsorption product should be investigated for its content of other vitamins *B*.

2. Although there is no evidence that loss of potency is liable to occur in the standard adsorption product, the Conference suggests that the following laboratories be asked to undertake a further investigation of its stability:

Department of Physiological Chemistry, University of Amsterdam.
National Institute of Health, United States Public Health Service, Washington, D.C.
Institute of Hygiene, University of Copenhagen.
School of Biochemistry, University of Oxford.
"Tierphysiologisches Institut," Leipzig.
Biochemical Institute, University of Stockholm.
"Laboratoire de physiologie au Centre de recherches sur l'alimentation (Institut des recherches agronomiques)," Paris.
Lister Institute for Preventive Medicine, London.

(*i*) BIOLOGICAL METHODS FOR ESTIMATION OF THE ANTINEURITIC VITAMIN *B*.—The Conference expresses no opinion on the relative merits of current biological methods for estimation of the antineuritic vitamin *B* (as recorded in the report on this vitamin, presented to this Conference and in the literature generally). It considers that good evidence is provided by that report that the different methods described, either prophylactic or curative in type, and employing either the rat or the pigeon as experimental animal, may yield equally valid results.

IV. THE ANTISCORBUTIC VITAMIN *C*

(*a*) INTERNATIONAL STANDARD.—*The Conference recommends the adoption as international standard of the fresh juice of the lemon,* Citrus limonum.

(*b*) DEFINITION OF UNIT.—The unit of the antiscorbutic vitamin *C* recommended for adoption is the vitamin *C* activity of 0·1 c.c. of fresh juice of the lemon, *Citrus limonum.*

Note.—This is about 1/10th of the daily dose necessary to prevent development of macroscopic scorbutic lesions in a young guinea-pig maintained on a scurvy-producing diet.

(*c*) METHOD OF USE.—The fresh lemon juice used as standard may be decitrated as follows: to the expressed juice, after filtration through muslin, an excess of calcium carbonate is added until effervescence stops. After standing for one hour, the mixture is filtered through a Buchner funnel. The decitrated juice should have a reaction of *p*H about 6 and should be administered to the experimental animal within two hours after filtration.

(*d*) PERMANENCE OF STANDARD.—This international standard shall be regarded as provisional for the next two years.

NOTE ON THE TOXICITY OF IRRADIATED ERGOSTEROL

In view of the toxic effects which have been reported after administration of certain specimens of irradiated ergosterol, this Conference suggests the advisability of testing all preparations of irradiated ergosterol, destined for medicinal use, for toxicity as well as for antirachitic potency.

APPENDIX

MEMORANDUM ON CAROTENE SUPPLIED FOR TESTING ITS SUITABILITY AS A POSSIBLE STANDARD FOR VITAMIN *A*

PREPARATION.—The material provided has been prepared as follows:—Commercial carotene (B.D.H.) was dissolved in benzene and filtered, and the clear solution was poured into a large volume of warm absolute alcohol. The crystallisation was allowed to proceed at 37° C., and the crystals were filtered off at the same temperature. All these operations were carried out in an atmosphere of carbon dioxide. The crystalline material was dried *in vacuo*. Melting point 179° to 180° C. (taken in electrically heated "Berl block").

For distribution into tubes the material was dissolved in benzene at 37° C. to make a 2 per cent. solution; 0·5 c.c. of this solution was run into each of the brown glass tubes in which the material is distributed, the whole process of solution and filling out being again carried out in an atmosphere of carbon dioxide. The filled tubes were transferred to a desiccator containing paraffin shavings and calcium chloride. The desiccator was evacuated and left attached to the pump until the benzene had completely evaporated, and the carotene had been deposited, mainly as a crystalline residue, at the bottom of each tube. When drying was complete, the desiccator and contained tubes were again filled with carbon dioxide, evacuated, and refilled with carbon dioxide. The tubes, before filling, had been drawn out into narrow constrictions to facilitate sealing, which was thus rapidly effected with minimal contamination of the carbon dioxide by air.

Suggestions for Use.—It is assumed that less than the whole contents of one tube (10 mgrms.) will be required for a test. The tube having been opened, the necessary quantity of carotene can be removed with the aid of a fine glass rod or narrow platinum spatula, and immediately dissolved in the chosen solvent. The partly used tube should be enclosed in a test tube of suitable size, which should then be drawn out in preparation for sealing. The test tube and contained specimen tube can then be refilled with carbon dioxide in the vacuum desiccator, as above described, removed, quickly sealed, and preserved in a cold, dark place until again required. The prepared solution, if it is to be used for several tests, should be preserved from light and oxygen by similar or equivalent precautions.

Department of Biological Standards,
National Institute for Medical Research, London.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs Analysis

Detection of (1) Rye Flour in Wheat Flour and (2) Barley Flour in Rye and Wheat Flours. P. Rudolph and H. Barsch. (*Chem. Ztg.*, 1931, **55**, 995–996.)—Rye and wheat flours may be distinguished from one another by stirring about 5 grms. of the material with sufficient hydrochloric acid (sp. gr. 1·124) to make a tough, pasty mass. When treated thus, rye meal soon assumes a red colour, which gradually increases in depth, whereas wheat flour exhibits no change. The presence of 10 per cent. of rye flour in wheat flour is detectable in this way.

The presence of barley meal in rye or wheat flour may be recognised as follows: 5 grms. of the flour are digested, with frequent swirling, with 50 c.c. of cold water. The mixture is filtered bright through a moist paper, and 10 c.c. of the filtrate are treated with 2 c.c. of hydrochloric acid (sp. gr. 1·124), which gives a pronounced turbidity and, on heating, a flocculent precipitate with either rye or wheat flour extract; with barley meal, no turbidity and, at most, a slight film is produced. Five c.c. of the acid wheat or rye extract, after cooling and filtering, or of the unfiltered acid barley meal extract, are treated with 10 c.c. of 96 per cent. alcohol. The barley extract immediately turns turbid, and, later, gives a flocculent deposit, whilst rye or wheat extract develops, at most, a very slight opalescence. An addition of 20 per cent. of barley meal to rye or wheat flour is readily detectable in this way.

Further, aqueous barley meal extract is coloured intensely yellow by 25 per cent. ammonia solution. Barley meal itself is coloured brown by 25 per cent. ammonia solution, whereas wheat flour is not changed in colour, and rye meal is turned a greenish yellow-grey colour. The oxygen of the air seems to play a part

in the colour-formation, as the colour gradually increases in depth on the exposed surface of the flour. If the flours are stirred with ethyl acetate, instead of ammonia solution, barley meal becomes brown or brownish-yellow, and wheat or rye flour pale green. T. H. P.

Normal Proportion of Bromine in Edible Seeds, Wheat, and Bread. A. Damiens and S. Blaignan. (*Compt. rend.*, 1931, **193**, 1460–1462.)—In order to determine whether bromine is normally present in plants, as it has already been shown to be in animals, the proportions of bromine, and of chlorine, and the ratio 1000 Br/Cl have been found for a number of samples of various cereal grains, beans, peas, bread, etc., and in every case, with the exception of rice and haricot beans, bromine was present. Wheat grains (3 samples) and bread (31 samples) contained, respectively, 0·21 and 0·09 to 0·61 mgrm. of bromine per 100 grms. of dry matter, and 0·07 and 0·82 to 1·8 of chlorine, the ratio 1000 Br/Cl being 2·8 and 0·07 to 0·46. The 1000 Br/Cl ratio for brewers' yeast was relatively high (5·2). Most of the other products examined showed a higher ratio than wheat, attaining 85 for lentils. D. G. H.

Indican in the Milk of the Cow and Goat. C. Hervieux. (*Compt. rend.*, 1931, **193**, 1480–1482.)—A modification of Jolles' reaction for urine (*C.R. Soc. Biol.*, 1907, **63**, 469) was used to detect indican in milk. One hundred c.c. of milk and 100 c.c. of dilute (1:5) trichloroacetic acid are mixed, and, after standing, 100 c.c. of the clear filtrate are collected. To this are added 10 c.c. of a 5 per cent. alcoholic solution of thymol, and to the cloudy liquid an equal volume of fuming hydrochloric acid containing 5 grms. of ferric chloride per litre. After standing for 1 hour, 10 drops of chloroform are added, and the mixture is shaken cautiously from time to time to get rid of emulsions. The chloroform gradually assumes the mauve or violet tint characteristic of indican in milk. Twenty-five individual and mixed samples of normal cow's and goat's milk were thus examined, and in every case the coloration was shown, and it is assumed that potassium indoxyl sulphate passes into the milk from the blood stream in the mammary glands. D. G. H.

New "Sorbite Process" for the Detection of Fruit Wine in Grape Wine. F. M. Litterscheid. (*Z. Unters. Lebensm.*, 1931, **62**, 653–657.)—The wine is pretreated according to Werder's method (ANALYST, 1929, **54**, 476), and 100 c.c. of the decolorised liquid are evaporated on the water-bath to 4 to 5 c.c., and, after cooling for 10 minutes, the liquid portion is separated from the tartar by filtration through a plug of glass-wool into a 25 c.c., graduated, glass-stoppered cylinder. The residue is washed 5 times with 5 drops of water, and the total filtrate (not more than 6 c.c.) is shaken with twice the volume of hydrochloric acid (sp. gr. 1·18 to 1·19) and 0·2 c.c. of *o*-chlorobenzaldehyde for 1 minute, and then at intervals over 30 minutes. After 7 hours, the condensation-product of sorbitol and the reagent, which separates in voluminous white flocks, is removed by filtration (but not washed), and the filtrate is shaken with 5 drops more of reagent, any further precipitate being collected, after 5 hours, on the same filter. The combined precipitates are then washed with 50 to 60 c.c. of cold water, followed by 50 to

75 c.c. of cold methyl alcohol, the washings being collected separately from the filtrate, and the last traces being removed with the aid of a suction pump. Finally, the compound is dried for 30 minutes at 105° C.; it is readily soluble in chloroform and sparingly soluble in hot alcohol, and may be further purified (though this is not usually necessary) by precipitation with alcohol from a hot solution in chloroform, followed by crystallisation; it melts indefinitely at 175° to 210° C. (usually at about 200° C.). The filtrate from the second precipitation (excluding washings) is treated again with reagent, and any corresponding mannitol compound (m.pt. above 260° C.) will thus be obtained after prolonged standing. The sorbitol derivative is decomposed into its components by the action of 2 c.c. of warm hydrochloric acid (sp. gr. 1·18 to 1·19) per 1 grm. for 15 minutes, the excess of acid, together with the regenerated reagent, being expelled on the water-bath. The residue is then converted into hexa-acetyl sorbitol by the Lutin-Zäch method, in which it is warmed with 1 drop of pyridine and 1 c.c. of acetic anhydride (b.pt. 137° C.) per 0·1 grm. for 1 hour on the water-bath, the solution then being poured into hot water (2 c.c. per 0·1 grm.). Methyl alcohol is added to the mixture until there is no further disappearance of the solid on shaking, the solution is filtered while hot, and the acetyl derivative is allowed to crystallise (for 12 hours for small amounts). Satisfactory results were obtained for 0·1 per cent. solutions of mannitol or sorbitol, and for 2·5 to 10 per cent. by volume of decolorised apple wine in genuine white wine. J. G.

Detection of Cocaine in the Presence of Novocaine by means of Cobalt Thiocyanate. J. L. Young. (*Amer. J. Pharm.*, 1931, **103**, 709–710.)—Since the blue precipitate formed by the interaction of cobalt thiocyanate and the hydrochlorides of cocaine and novocaine is only soluble in a solution of stannous chloride in the case of novocaine, the two substances may be detected when present in combination. A few drops (4 to 5) of a 2 per cent. solution of cobalt thiocyanate are added to a small quantity of the salt under examination. If cocaine only is present, flakes of a Prussian-blue colour will form, whereas, with novocaine, or a mixture of novocaine and cocaine, the entire solution will become blue. On addition of 4 to 5 drops of freshly-prepared stannous chloride solution (5 grms. of tin added to 10 grms. of stannous chloride in 100 c.c. of 1:1 hydrochloric acid), the novocaine precipitate dissolves on stirring, leaving a pink solution; no change is apparent if only cocaine is present, and with both novocaine and cocaine, the precipitate of the former dissolves and the blue flakes or streaks of cocaine remain. D. G. H.

Quantitative Colorimetric Determination of *Digitalis* Glucosides by means of Baljet's Reagent. J. A. C. Van Pinxteren. (*Pharm. Weekblad*, 1932, **69**, 4–8.)—Baljet's colorimetric method (*id.*, 1918, **55**, 602) and modifications of the method proposed by Knudson and Dresbach (ANALYST, 1931, **56**, 675) are criticised. In particular, in the latter case it is difficult to match the digitalis picrate with potassium dichromate, as the solutions do not follow Beer's Law, and the intensity of the former colour doubles itself between 30 minutes and 2 hours after it is generated. Ouabain gives better results as a comparison colour standard, especially if 0·5 mgrm. of ouabain is added to the unknown solution before

production of the colour, and due allowance is made for its presence in calculating the result. A Focke physiological value of 4 for Digitalis leaves was found to be equivalent to 1·7 mgrms. of ouabain per 50 mgrms. of leaf. A table of data relating the cat-physiological value (limiting dose per kilo.) and the ouabain value is given, but calculation of the former from the latter, based on an experimentally-found relationship for one sample of *Liquor digitalis* (cat-value 2 c.c. ≡ 0·78 mgrm. of ouabain) gives results in poor agreement with those found experimentally for other samples. The colorimetric value represents the resultant of those values due to all the glucosides present, *viz.* in an infusion, digitoxin 12·4, gitalin 72·2, and digitalin 15·4 per cent. (Hoekstra, *Diss. Utrecht*, 1931, p. 67). Colorimetric methods, therefore, cannot replace physiological methods with complete success. J. G.

Distribution of Saponin in *Agrostemma Githago* and in *Saponaria Officinalis*. F. G. de Wilde. (*Pharm. Weekblad*, 1932, **69**, 65–78.)—*Determination of Saponin.*—A physiological method is considered preferable to chemical methods (*cf.* Kofler, ANALYST, 1922, **47**, 403; 1924, **49**, 239). A 1:50 to 1:400 decoction of the powdered drug is prepared (according to *Ned. Pharm.*, Ed. V) in a physiological salt solution adjusted to *p*H 7·4 with a phosphate buffer, and is filtered when cool, and 0·95, 0·90, 0·85, etc., down to 0·50 c.c., are diluted in separate test-tubes to 1 c.c. with the buffered saline. Each solution is then shaken with 1 c.c. of a 4 per cent. suspension of washed blood corpuscles in buffered saline, and, after 12 to 20 hours, the haemolytic index is given by the greatest dilution of drug producing complete haemolysis. A parallel experiment with "Saponin pur. albiss. Merck" (index 25,000) under the same conditions, provides a quantitative means of standardisation. *Determination of Sugars* (*cf.* MacLean, *id.*, 1919, **44**, 344).—A decoction of 1 grm. in 50 c.c. of water is prepared by the official method and, after replacement of any water lost by evaporation, 40 c.c. of the cool, filtered solution are treated with basic lead acetate solution until no further precipitate results, when the mixture is diluted to 50 c.c. and filtered. Excess of lead is removed by addition of excess of sodium sulphate solution to 40 c.c. of the filtrate, which are then diluted to 50 c.c., filtered again, 1 to 5 c.c. being diluted to 20 c.c. with a solution containing 15 grms. of sodium sulphate and 0·1 c.c. of acetic acid per 100 c.c.; 2 c.c. of a solution of 12 grms. of potassium hydrogen carbonate, 8 grms. of potassium carbonate, 0·350 grm. of copper sulphate, 0·050 grm. of potassium iodate and 0·500 grm. of potassium iodide (presumably in 50 c.c., *cf. loc. cit.*) are then added. The mixture is heated to boiling in 1 minute 40 seconds, and, after a further 6 minutes, it is cooled and the iodine liberated by addition of 2 c.c. of 25 per cent. sulphuric acid is titrated after 1 minute with 0·0025 *N* sodium thiosulphate solution in the presence of 3 drops of a 1 per cent. solution of starch. Allowance is made for the blank on the reagents, and a table is given relating the titration to the dextrose value. For an inversion, 10 c.c. of cleared decoction are heated with 1 c.c. of 25 per cent. hydrochloric acid for 5 minutes at 70° C., and the cooled solution is neutralised to methyl red, and then diluted to 20 to 40 c.c., and treated as described.

Distribution of Saponins and Sugars during the First Year of Growth of Agrostemma Githago.—Three periods were studied, *viz.* (1) the appearance of the first

stem leaves; (2) growing period; (3) ripening period. No data are given, but, in general, an increase in saponin, especially during the early stages of germination of the seed, corresponding with a decrease in sugar, was observed. It is suggested, therefore, that the saponin results as an intermediate product in the breakdown of more complicated reserve material, and can itself serve as a reserve material. Analyses of other parts of the plant confirm this theory. The total saponin content of the bud is the same as that of the flower after complete development, but decreases when fading sets in.

Saponaria Officinalis gave results in confirmation of the theory, though these are somewhat complicated by the fact that the plant was over a year old. J. G.

Denicotinisation of Tobacco Smoke during Smoking. R. Kissling. (*Chem. Ztg.*, 1932, **56**, 31.)—Methods hitherto employed for removing nicotine have usually involved the passage of the smoke through an asbestos wad impregnated with gallotannin or ferric chloride, but have seldom proved very effective. "Bonicot's fluid," 3 drops of which are placed in the "head" of the cigar, was found to contain 96·60 per cent. of water, 2·73 per cent. of alcohol, and 0·67 per cent. of solids (mainly ferrous ammonium sulphate, with small amounts of citric and tartaric acids and traces of sodium chloride; *cf. Eng. Pat.*, 1931, 348,974). Trials were made with a number of varieties of Brazilian cigars (about 5 grms. in weight), smoked in batches of 10, at the rate of 30 minutes each, 24 per cent. of the original weight being left unsmoked. The original nicotine content was about 1 grm. per 10 cigars, of which 0·240, 0·233 and 0·197 grm. were found in the unsmoked ends, and 0·450, 0·480 and 0·493 grm. in the smoke, in experiments with Bonicot's liquid, distilled water and untreated cigars, respectively. Bonicot's liquid, therefore, has relatively little effect. J. G.

Determination of Phenol and its Homologues in Disinfecting Fluids. A. F. McCarley. (*J. Soc. Chem. Ind.*, 1932, **51**, 38T.)—Phenol may be satisfactorily and conveniently determined in disinfecting fluids (containing up to 5 per cent. of phenols) by treating 50 grms. of the sample with 100 c.c. of saturated barium hydroxide solution and 25 c.c. of barium chloride in a 750 c.c.-flask immersed in boiling water for 15 minutes, with constant shaking. The liquid is filtered through a coarse paper on a fluted funnel, and the contents of the paper are scraped back into the flask, and treated as before with 50 c.c. of barium hydroxide solution and 20 c.c. of barium chloride solution. Vigorous shaking will cause an agglomeration, and the clear liquid can be readily filtered. The conglomerate is again treated with barium hydroxide. Any uncombined oil is removed from the combined filtrates by shaking with petroleum spirit, and the alkaline solution is treated with excess of hydrochloric acid. The acid solution is then extracted 3 times with methylated ether, and the ethereal solution is washed with sodium carbonate solution. Three extractions with sodium hydroxide solution are then made, and the alkaline solution is evaporated to 15 c.c. and treated with sulphuric acid in a burette. The volume, multiplied by 2·1, gives the percentage of phenols. Results were in agreement with those obtained by the method involving steam distillation and subsequent titration with soda. D. G. H.

Determination of the Total Nitrogen and Solid Matter in Yeast. R. S. W. Thorne. (*J. Inst. Brewing*, 1932, **38**, 23–29.)—Neither the original Kjeldahl method nor the Gunning modification thereof (with or without added potassium permanganate) determines the whole of the nitrogen in yeasts of all kinds, the results being low, sometimes to the extent of 10 per cent. Values corresponding with 99 to 100 per cent. of those furnished by the Dumas method are, however, obtained in all cases by the following modified procedure, suggested by Fulmer and Christensen (*J. Phys. Chem.*, 1925, **29**, 1415): A mixture of about 0·5 grm. of the yeast with 0·4 grm. of copper sulphate, 10 grms. of potassium sulphate, 1 c.c. of concentrated sulphuric acid, and 30 c.c. of 50-volume hydrogen peroxide solution (15 c.c. of 100-volume peroxide diluted to 30 c.c. with water) is evaporated slowly almost to dryness on a steam-bath; 20 c.c. of concentrated sulphuric acid are then added, and the digestion is continued in the usual way. The use of too great an excess of hydrogen peroxide causes low results, but the proportions stated above prove satisfactory, even when the strength of the peroxide solution employed has fallen to 90 per cent. of its original value.

With wort, also, the Kjeldahl-Gunning method yields low results, and determinations made by this method on a fermenting wort 0, 2, 3, 4, and 7 days after the commencement of fermentation, gave proportions of nitrogen lower by 1·5, 3·7, 5·5, 5·1, and 4·3 per cent., respectively, than those yielded by the Fulmer and Christensen method.

For the routine determination of the total solid matter in yeast, the following rapid method gives figures in almost exact agreement with those obtained by Fletcher's method (*J. Inst. Brewing*, 1931, **37**, 506), according to which the yeast is treated with a little absolute alcohol—to arrest enzyme action at once—and is then dried to constant weight in a steam-oven: The yeast (1 to 2 grms.) is weighed into a flat-bottomed nickel dish and moistened with a few drops of absolute alcohol. About 1 c.c. of water is then added, and the whole stirred into a paste. This is dried as completely as possible on a steam-bath (in about 5 minutes) and placed in a Mojonnier oven for 1 hour, after which time it will have reached constant weight. The object of making the yeast into a paste with water is to obtain a uniform layer at the bottom of the dish, and so to avoid discrepancies which might arise if the yeast were dried in irregular lumps. T. H. P.

Biochemical

Determination of the Iodine Value of Oils and Lipids. M. Yasuda. (*J. Biol. Chem.*, 1931, **94**, 401–409.)—A method published by Rosenmund and Kuhnhenn (*Z. Unters. Nahr. Genussm.*, 1923, **46**, 154; ANALYST, 1924, **49**, 105) for the determination of the iodine value of fats and oils, in which pyridine sulphate dibromide was used as a halogenising agent, was used later for the micro-determination of the iodine value by Dam (*Biochem. Z.*, 1924, **152**, 101; 1930, **220**, 158) and by Page (*Biochem. Z.*, 1930, **223**, 445). A comparison was made between the Hanus method and the Rosenmund–Kuhnhenn method, with special reference to the time required for the reaction, and the amount of halogen used in the two methods. Determinations of the iodine value were made by both the Hanus and Rosenmund–Kuhnhenn methods on oleic, ricinoleic and linolic acids, cholesterol, cholesteryl

palmitate and cod-liver oil. Ricinoleic acid was taken as an example of unsaturated hydroxy fatty acid; cod-liver oil served as an example of a mixture of various lipids. Tables and a figure show the results, from which the following conclusions are drawn:—(1) For the determination of the iodine value of oleic and ricinoleic acids the Hanus and the Rosenmund methods give the same value, which is very near the theoretical. (2) For linolic acid and cod-liver oil the Hanus method gives values 2 to 4 per cent. higher than the other. (3) With linolic acid the Rosenmund method is much less influenced by the amount of halogen than the Hanus method. (4) The Hanus method is not suitable for the determination of the iodine value of cholesterol and its esters; the values obtained by this method deviate greatly from the theoretical value. Therefore, the Rosenmund–Kuhnhenn method was applied to the determination of the iodine value of small amounts of various lipids in the tissues of the animal body; *i.e.* to the total fatty acids, phospholipid, and its fatty acids. The micro method, which is described, is simple and easily carried out, and was found to give exactly the same iodine values as the macro method for standard lipid solutions, namely, 90 for oleic, 85 for ricinoleic, and 160 for linolic acid. The excess of halogen should be at least 25 per cent. in the case of oleic acid, and about 260 per cent. in the case of linolic acid. Procedure is described by which the iodine value of lipids in small amounts of tissues can be determined by the use of the pyridine dibromide solution of Rosenmund and Kuhnhenn and the oxidative micro method for the determination of lipids of Bloor (*J. Biol. Chem.*, 1929, **82**, 273; 1928, **77**, 53). Some results obtained with rat tissues are given. P. H. P.

Determination of Galactose in Blood and Urine. V. J. Harding and G. A. Grant. (*J. Biol. Chem.*, 1931, **94**, 529–539.)—Knowledge of the metabolism of galactose is hampered by lack of a suitable analytical method. Ordinary bakers' yeast used in comparatively large quantities for a short time has proved a very useful reagent for the removal of glucose from biological fluids, such as blood and urine. Many yeasts have the power of fermenting galactose, especially if they are first grown on a nutrient medium of hydrolysed lactose, or on galactose itself (*i.e.* if they are acclimatised). Both Abderhalden (*Fermentforschung*, 1924, **8**, 42) and von Euler and Nilsson (*Z. physiol. Chem.*, 1925, **143**, 89) showed that galactose-acclimatised yeast could be dried and sterilised, and yet retain its power of fermenting galactose. There thus seemed no reason why such an acclimatised yeast should not be used as an analytical reagent for galactose, in a manner analogous to ordinary yeast for glucose. It would first be necessary to remove the ordinary fermentable sugars, and then to treat the fluid with the acclimatised yeast. Ordinary bakers' yeast was acclimatised to galactose for three 3-day incubation periods; the preparation obtained was called "galac" yeast. Under the analytical conditions described, galac yeast does not remove maltose, lactose, arabinose, xylose, or glutathione; it removes glucose, fructose, mannose and sucrose, as well as galactose. The method of determination takes a larger amount of yeast and a slightly longer time than the usual process for removing glucose, and attempts were made to reduce both these factors. For determinations of galactose in blood or urine the fermentable sugars are removed from a sample by means of ordinary

yeast, the fermentable sugars, plus galactose, are removed from a second sample by means of galac yeast, and the difference represents galactose. The results show that ordinary bakers' yeast, grown in a medium containing galactose, can be utilised to remove galactose quantitatively from aqueous solutions, from Folin–Wu blood filtrates, and from urine treated with sulphuric acid and Lloyd's reagent. No galactose can be found in normal blood (either plasma or corpuscles) or in normal urine during fasting. No galactose can be found in blood after fasting or in urine, in late pregnancy or 3 days' *post partum* at the beginning of lactation. The non-fermentable "sugar" found in urine after fasting, in late pregnancy, and the *puerperium*, on hydrolysis, gives "fermentable sugar" and "galactose sugar." The amounts of "fermentable sugar" and "galactose sugar" on hydrolysis are inconsistent with the idea that lactosurias are produced by the simple addition of lactose to the normally occurring non-fermentable urinary reducing substances.

P. H. P.

Colorimetric Method for Determination of Allantoin. H. W. Larson. (*J. Biol. Chem.*, 1932, **94**, 727–738.)—The method of Wiechowski (*Beitr. chem. Physiol. u. Path.*, 1908, **11**, 109) for the determination of allantoin, as modified by Handovsky (*Z. physiol. Chem.*, 1914, **90**, 211), is the one now in general use. However, the published methods for allantoin determination occupy from 6 to 24 hours, and most of them are probably inaccurate, giving, at best, only approximate values. The need for a more accurate and rapid method has become urgent, owing to the importance of animal purine metabolism. Folin and Svedberg (*J. Biol. Chem.*, 1926, **70**, 418), in working with various copper reagents for carbohydrate determination, developed an ammoniacal copper reagent which is practically unaffected by urinary sugar, but which is reduced by nitrogenous compounds such as creatine, creatinine and allantoin. With the use of this reagent a rapid and accurate colorimetric method for the determination of allantoin has now been devised; the general outline of the method is as follows:— Five c.c. of animal urine are treated with an excess of 30 per cent. phosphotungstic acid, followed by an excess of saturated basic lead acetate solution and 5 per cent. sulphuric acid. This treatment removes interfering substances. The procedure is carried out in the same 50 c.c. centrifuge tube. After the addition of each reagent the tube is gently rotated to insure proper mixing, and the mixture is centrifuged until perfectly clear, when 2 c.c. of this liquid are pipetted into a Folin-Wu sugar tube together with 2 c.c. of Folin's ammoniacal copper reagent, which is reduced by allantoin. This tube is then heated in a boiling water-bath for 10 minutes, cooled, and 2 c.c. of acid molybdate reagent are added. The colour obtained is compared with an allantoin (1 mgrm.) standard. Recoveries of allantoin added to rat urine range from 90 to 100 per cent. Two hours are sufficient for the complete determination, whereas the Wiechowski–Handovsky method requires 10 or 12 hours. The method was designed primarily for use with rat urines, but has been used successfully with other urines. It depends upon a proper balancing of reagents involved. A table gives comparative allantoin values obtained by the Wiechowski–Handovsky method and by the colorimetric method.

The colorimetric values differ from the others by −24·0 per cent. to +31·7 per cent. The Wiechowski–Handovsky method shows low recoveries of added allantoin. In general, the colorimetric method gave higher allantoin values than the Wiechowski method, and experiments were devised to prove that the reduction of the copper reagent came from allantoin only, and not from other substances which might be present. It is suggested that one source of error in the Wiechowski–Handovsky method is the considerable loss of allantoin due to adsorption on the bulky precipitates of lead and silver sulphides. By a modification of the colorimetric procedure 25 mgrms. of allantoin were obtained from 1205 c.c. of human urine excreted during 24 hours. Specimens of mixed human urines yielded 25 and 30 mgrms. of allantoin per litre. Mercury-allantoin precipitates were decomposed with hydrogen sulphide; after being recrystallised twice the allantoin obtained melted at 236–237° C. P. H. P.

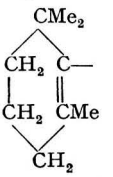
Vitamin *A* from Fish Oils. II. P. Karrer, R. Morf and K. Schöpp. (*Helv. Chim. Acta*, 1931, **14**, 1431–1436.)—In continuation of their work on *Hippoglossus hippoglossus* (ANALYST, 1931, **56**, 824) the authors have found that high Lovibond (vitamin *A*) values are obtainable from the unsaponifiable matter of the oil from *Scombresox saurus* (mackerel species). Sterols were, therefore, frozen out from a solution in methyl alcohol by means of a mixture of carbon dioxide and acetone, and an extract in petroleum spirit of the residual solution (containing the vitamin *A*) was passed through a filter-column containing fibrous adsorbent alumina (*cf. loc. cit.*). The top layers were then found to contain traces of sterols, whilst the relatively low Lovibond values of the bottom layers indicated, probably, the presence of compounds of lower molecular weights. The middle portion, which gave the highest Lovibond value, was further purified by fractional adsorption twice on argillaceous earth, and each adsorbate was divided into 3 fractions, the middle fraction of which was dried in a vacuum and analysed ($C_{20}H_{30}O$ or $C_{22}H_{32}O$); a third adsorption produced no appreciable change in composition. The resulting compound had the properties of an alcohol capable of forming esters (*cf.* Vogt, *Merck's Jahresber.*, 1930, p. 34), which often differed in solubility from the original substance, notably in being soluble in cold methyl alcohol. This provides a means of separation and purification (by fractional solution 5 times) of the *p*-nitrobenzoic acid ester, which was shown to have the formula $C_{20}H_{29}O.CO.C_6H_4NO_2$. The acetic ester ($C_{20}H_{29}O.COCH_3$), which is produced by the action of acetic anhydride in pyridine solution, is also sparingly soluble in methyl alcohol, and may be saponified by an alcoholic alkaline solution at 60° C., and the vitamin substance thus regenerated may be shown to have the same composition and Lovibond value as the original. The vitamin compound (*cf. loc. cit.*) is a clear, pale-yellow viscous oil, fluid when warm; its optical inactivity indicates the absence of active sterols, whilst animal experiments confirm the absence of vitamin *D*. Geronic acid results from the action of ozone, and acetic acid is produced by decomposition with potassium permanganate or chromic acid, whilst catalytic reduction and distillation in a vacuum yield a compound having the formula $C_{20}H_{40}O$ or $C_{22}H_{44}O$. It appears to resemble carotene in

structure, having a similar carbon ring (*vide infra*), and an aliphatic side-chain (on the C atom indicated), which is given by the authors as

$$-CH{=}CH{-}C(Me){=}CH{-}CH{=}CH{-}C(Me){=}CH{-}CH{=}CH{-}CH_2OH,$$

or $$-CH{=}CH{-}C(Me){=}CH{-}CH{=}CH{-}C(Me){=}CH{-}CH_2OH.$$

Its Lovibond value is not greater than that of the *Hippoglossus* product (*loc. cit.*), and further work on this point is in progress. J. G.



Assay of Antineuritic Vitamin and its Concentration with Silver. R. J. Block, G. R. Cowgill and B. H. Klotz. (*J. Biol. Chem.*, 1932, **94**, 765–782.)—The method of assay for antineuritic vitamin *B* used in the investigation is described. It is a combination of weight-maintenance and curative techniques carried out on pigeons given a diet of polished rice *ad libitum*, supplemented daily with meat residue, cod-liver oil, and the Osborne–Mendel salt mixture. The plan of assay differs from that of many other workers, in that polished rice does not constitute the *sole* diet. Since it is not a complete food, the polished rice, if it is to be used, should be supplemented, so as to supply a food mixture deficient only in respect of the one variable of interest, namely, antineuritic vitamin *B*. In this case the polished rice serves as the chief source of calories, the meat residue as a source of good protein and heat-stable vitamin *G* (B_2) factor, relatively free from antineuritic vitamin, the Osborne–Mendel salt mixture furnishes the mineral nutrients, and the cod-liver oil supplies the fat-soluble vitamins. The supplements are given (in sufficient quantities to meet the requirements) in a gelatin capsule; one capsule is given daily to each bird. Ten experiments were carried out on the concentration of the antineuritic vitamin by means of silver. As sources of the silver ion, the nitrate and lactate were used; nitric, sulphuric or lactic acid was employed as source of the hydrogen ion; and barium or sodium hydroxide was used for alkalising (to bring the reaction from *p*H 4 to *p*H 7·0). Silver nitrate, lactic acid and barium hydroxide gave the most favourable purification with the least loss of activity; treatment with silver lactate, lactic acid and sodium hydroxide resulted in the greatest increase of potency, but in a poor yield of the vitamin. The behaviour of antineuritic vitamin in the ternary mixture of water, ethyl alcohol and carbon tetrachloride was studied. Whereas the inorganic salts are precipitated as the water is removed and the concentration of the alcohol approaches 100 per cent., it was found that the vitamin remains in the liquid phase, from which it may be recovered quantitatively. Solubility of the vitamin in other binary mixtures was studied. Six other alcohols were substituted for ethyl alcohol in the carbon tetrachloride procedure; of these, only *n*-butyl and allyl proved to be as good as ethyl alcohol for concentrating the vitamin. The solubility of the antineuritic vitamin in mixtures of methyl alcohol with acetone, allyl and amyl alcohols was studied. None of these mixtures can be used profitably to concentrate the vitamin. P. H. P.

Preparation of Vitamin *C* Concentrates from Lemon Juice. J. S. Svirbely and C. G. King. (*J. Biol. Chem.*, 1931, **94**, 483–490.)—In order to investigate the preparation of concentrated antiscorbutic fractions from lemon juice, a study was first made of the solubility of the active material in organic solvents. It has been shown that vitamin *C* is soluble in mixtures of petroleum spirit and acetone (1:1), petroleum spirit and butyl alcohol (2:1 and 4:1) and petroleum spirit and propyl alcohol (1:1 and 3:1) in ethyl acetate, butyl alcohol and propyl alcohol, but is insoluble in absolute ethyl ether. Ammonia gas destroys the active material when passed into solutions containing the vitamin dissolved in organic solvents. The general procedure used in testing the vitamin *C* content of the various fractions was that recommended by Sherman, La Mer and Campbell (*J. Amer. Chem. Soc.*, 1922, **44**, 165; ANALYST, 1922, **47**, 216), with shorter test periods. Details have been worked out for the preparation of active fractions from volumes of lemon juice up to 2·5 litres. The method is approximately the same as that previously reported by Grettie and King (*J. Biol. Chem.*, 1929, **84**, 771), and by Sipple and King (*J. Amer. Chem. Soc.*, 1930, **52**, 420), with certain modifications and precautions found necessary when dealing with larger quantities of juice. The most concentrated preparations yet obtained (0·03 to 0·5 mgrm. of total solids per c.c. of lemon juice) are sufficiently stable to be kept for weekly feeding periods, and have given no indication of more than one factor being involved. Evidence was given previously by McKinnis and King (*J. Biol. Chem.*, 1930, **87**, 615; ANALYST, 1930, **55**, 592) that vitamin *C* is probably acidic in nature. In the present investigation the active material obtained was consistently characterised by being distinctly acidic, and exerted a strong reducing action. Tests for carbylamine and phenols were negative. Strongly positive tests were obtained with orcinol (blue-green) and resorcinol (pink). P. H. P.

Chemical Investigations on the Antiscorbutic Vitamin. I. O. Rygh, A. Rygh and P. Laland. (*Z. physiol. Chem.*, 1932, **204**, 105–111.)—Previous chemical investigations on vitamin *C* are outlined (*cf.* Zilva, ANALYST, 1927, **52**, 425, 552; 1928, **53**, 552; 1930, **55**, 289; Veeder and Lawson, *id.*, 1927, **52**, 424), and the resulting conclusions regarding the chemical properties of the pure vitamin are used as a basis of isolation. *Isolation.*—Freshly-pressed apple juice or lemon juice was evaporated to one-fifth of its volume under reduced pressure (below 25° C.), and oils and phytosterols were removed by extraction several times with ether (free from peroxides). The solution was then neutralised to litmus and was again extracted repeatedly with an equal volume of ether, the extract being dried by means of sodium sulphate and distilled. These operations were carried out, as far as possible, in an atmosphere of carbon dioxide or nitrogen. In some cases hydrogen chloride gas was bubbled into the concentrated ethereal solution and the resulting precipitate was collected. The yields were 600 and 50 mgrms. from 10 litres of unripe orange juice and 40 litres of apple juice, respectively (*cf.* following abstracts). In all cases mixtures containing variable proportions of crystals and a heavy oil were obtained which, on recrystallisation from alcohol, gave needles only, m.pt. 172° C. (176° C. after further treatment with animal charcoal). Their physical properties (including solubility, mixed m.pt., ultra-violet spectrum, $[\alpha]_D$),

molecular weight (determined by titration with alkali of a solution in acid), and chemical tests (Mayer's reagent, the sodium acetate and sulphuric acid tests, elementary analysis, salt formation) showed them to be identical with narcotine. Pure narcotine, however, has no antiscorbutic properties, but experiments with 15,000 apples of various degrees of ripeness showed that the narcotine content increases with the degree of ripening, a negligible amount being found in the fully-ripe fruit. These, and experiments on animals, indicate that narcotine is the precursor of vitamin *C*, which is formed from it during the ripening process.

Animal and Irradiation Experiments.—Batches of six guinea-pigs were fed on a basic diet containing 340 grms. of casein, 1280 grms. of maize starch, 40 grms. of physiological salt mixture, 30 grms. of sodium chloride, 100 grms. of calcium lactate and 50 grms. of filter paper, which were cooked with 3 litres of water, and the resulting pulp was mixed with 200 grms. of yeast extract, 125 grms. of butter, and 10 c.c. of cod-liver oil. A Uviol tube containing 2 grms. of commercial narcotine and 25 grms. of ethyl acetate was exhausted at a pressure of 2 mm. and sealed, and was then exposed for 2 hours at 25 cm. from a mercury lamp. The temperature rose to 40° C., and the narcotine dissolved, but was deposited on cooling, and was then recrystallised from alcohol, yielding pale yellow needles, m.pt. 172° C. Fivefold recrystallisation produced no change in m.pt., but treatment with animal charcoal raised it to 176° C. Further irradiation produced a brown resinous mass which was insoluble in acids and in the ordinary solvents; $[\alpha]_D$ was unchanged. All guinea-pigs fed on the above diets, with or without addition of 0·5 mgrm. per day of ordinary or irradiated narcotine dissolved in dilute tartaric acid solution, died after 4 to 5 weeks, showing symptoms of scurvy, except in the last instance. These experiments are being continued (*cf.* Bezssonoff, *Bull. Soc. Chim. Biol.*, 1927, **9**, 555; Zilva, *Biochem. J.*, 1924, **18**, 632, 641; 1927, **21**, 354; 1928, **22**, 779). J. G.

Experiments on the Isolation of Narcotine from Different Vegetables and from Milk. P. Laland. (*Z. physiol. Chem.*, 1932, **204**, 112–114.)—Narcotine was isolated from the undermentioned young, unripe vegetables by appropriate modifications of the method already described (*cf.* preceding abstract), the mixture of oil and crystals being then extracted in dilute tartaric acid, the former separated and the latter precipitated with sodium hydroxide solution and extracted with ether. The final product was dried in a vacuum at 60° C., and was then obtained as needles (m.pt. 172° C.), which, after treatment with animal charcoal, melted at 176° C., and had all the properties of narcotine (*cf. loc. cit.*). *Tomatoes.*—Twenty kilos. yielded a juice of *p*H 4·2 and 20 mgrms. of narcotine. *Cabbages.*—One hundred kilos. yielded 25 litres of juice; *p*H, 6·3, yield 40 mgrms. *Potatoes.*—Twenty kilos. yielded 10·4 kilogrms. of juice; *p*H, 5·6, yield 12 mgrms. *Mountain cranberries.*—Seventy-five kilos. taken; juice weakly antiscorbutic; *p*H 2·87; yield none. *Milk.*—The casein was precipitated by acid from 50 litres and the filtrate was treated as described; the yield was very small (*cf.* following abstract). J. G.

Chemical Investigations on the Antiscorbutic Vitamin. II. Narcotine and its Derivatives as Antiscorbutics. O. Rygh and A. Rygh. (*Z. physiol. Chem.*, 1932, **204**, 114–122.)—Guinea-pigs were fed on a basic diet containing

oats-grits 50 parts, wheat bran 20, skim-milk powder (autoclaved for 2 hours at 110° C.) 15, fresh butter containing 1 per cent. of cod-liver oil 10, common salt 1, and Osborne's physiological salt mixture 0·5 part, to which was added a solution in dilute tartaric acid of the substance to be tested. The animals, including the controls, were killed after 30 days and dissected, and 0 to 10 points were allotted according to the degree of scorbutic attack observed in the various parts of the body (*i.e.* 10 corresponds with the highest degree of attack), a final value being obtained from the mean of the observations for the individual organs. Irradiated narcotine (*cf.* preceding abstracts) gave values as follows:—A daily dose of 10 mgrms., 4; 5 mgrms., 2; 2 mgrms. to 100γ, 0; 10 to 1γ, 5 to 6; 0·1γ, 7; control, 9. The intestine and suprarenal showed no scorbutic effect for doses of 10 mgrms. to 10γ, and no *X*-ray evidence of scurvy was obtained for 2 mgrms. to 100γ (*i.e.* 0 points). The leg-bone and rib values fell to 0 at 1 mgrm., and at 2 mgrms. to 100γ, respectively, and then increased. Antiscorbutic value was absent from cotarnine, meconine, cotarnaminic acid and normeconine, and feeble in anhydrocotarnine phthalide and narcotine-*n*-oxide; dimethyl nornarcotine, nornarcotine, and especially, methyl nornarcotine were active, and the last was selected for further tests. It is an *o*-dihydroxy derivative obtained after removal of the 2 methyl groups from the pair of adjacent methoxy groups in the narcotine by heating it for 8 days at 100° C. with an 8-fold volume of concentrated hydrochloric acid, followed by recrystallisation and treatment with hydriodic acid. Its hydrochloride is red, but it is bleached by sulphur dioxide, though not by animal charcoal, and it is soluble in dilute alcohol or in water to give a frothy solution, which gives a precipitate with acids if the solution is concentrated. It gives a positive phenol reaction and has strong reducing properties, being readily oxidised to a brown substance (*cf.* Matthiessen, Foster and Wright, *Proc. Roy. Soc.*, 1860 to 1870). *Animal experiments.*—Five mgrms. to 100γ, 3 to 1; 100γ to 10γ, 0; 1γ, 4; 0·1γ, 6; control, 9 to 10. Thus, a dose of 20γ gave the optimum antiscorbutic value (*i.e.* lowest average of points). The suprarenal and intestine were unaffected throughout. It is stated that the action of germinating seeds also converts narcotine into an antiscorbutic substance having the reactions of a phenol. The relation of these data to the effects of other vitamins and of other constituents of the basic diet is discussed (*cf.* Rygh, Rygh and Laland, preceding abstracts, and *Z. physiol. Chem.*, 1931, **200**, 261).* J. G.

* S. Smith and S. S. Zilva, at a meeting of the Biochemical Society (*Chem. and Ind.*, 1932, **51**, 166), described experiments which do not confirm the conclusions of O. and A. Rygh.—EDITOR.

Bacteriological

Distribution of Agar-liquefying Bacteria. H. Nicol. (*Nature*, 1931, **128**, 1041–1042.)—Several strains of an organism which attacks and softens agar-agar have been isolated from a swampy garden soil from Palmer's Green and from the clover side of Agdell Field, Rothamsted, both sampled in wet weather in spring. The organisms form small, slowly-growing, yellow colonies, which develop well in the usual agar media, and are invariably situated in the centres of circular areas of softening. A characteristic "hammered copper" appearance is thus given

to the plate. No strain liquefies the agar, but, in stroke cultures, the whole of the agar is, in time, softened, although growth proceeds only on the surface. Optimum growth occurs mostly in a medium containing about 0·3 per cent. of sodium chloride, but one strain (Palmer's Green) grows on a medium similar to Gran's, with 3 per cent. of salt. The presence of dextrose depresses the development of most of the strains. The organisms were isolated from filter-paper strips during a search for cellulose-decomposing bacteria, with which the agar-liquefiers are frequently associated. T. H. P.

Effect of Rhizopin on the Growth of Aspergillus Niger. N. Nielsen. (*Compt. rend. Lab. Carlsberg*, 1931, **19**, No. 5, 1–10.)—It was shown earlier that growth of *Rhizopus suinus* under certain special conditions is accompanied by the separation of a substance, termed rhizopin, which is able to promote the development of the *Avena coleoptiles*. It was found, also, that rhizopin solutions further the growth of yeast, but whether the same substance is the active component in the two cases remains undecided. Further work shows that rhizopin solutions increase the growth of *Aspergillus niger* to as much as nine times the normal yield, and also enhance conidia-formation by this organism. T. H. P.

Organic Analysis

Industrial Analysis of Glacial Acetic Acid. E. Charles. (*Ann. Chim. anal.*, 1932, **14**, 5–13.)—The formic acid, water, and higher acids present in glacial acetic acid may be determined by the following procedure: (1) The melting-point of the acid is measured, conveniently in a test-tube with a 15 c.c. bulb blown at the bottom. About three-fourths of the bulb is filled with the acid, into which a thermometer, reading to 0·05° C., and allowing the temperature to be estimated to within 0·02° C., is dipped. The tube is cooled in either ice-water or a mixture of crystalline sodium sulphate and hydrochloric acid, the acid being stirred with the thermometer. When solidification occurs—usually at a temperature about 10° C. below the melting point—the tube is transferred to water at 20° C. and stirred. When only a small quantity of crystals remains, the tube is withdrawn from the water and the first reading of the temperature is made. The tube is then placed again in the cooling mixture and the operations are repeated until a concordant melting point (A_1) is obtained. The difference of this value from 16·70° C. is due to the various impurities.

(2) The formic acid may be determined in various ways: (*a*) In conjunction with the water, by measuring the carbon monoxide evolved during the reaction with acetic anhydride; a special apparatus for this is described. (*b*) By Daniel's method (ANALYST, 1927, **52**, 549). (*c*) By utilising the reaction with bromine, $H.CO_2H+Br_2=2HBr+CO_2$; use is made of aqueous bromine solution saturated at 18° to 20° C., this containing 31·5 grms. of bromine per litre (≡9 grms. of formic acid). Ten c.c. (or about 10·5 grms.) of the acid are shaken with 25 c.c. of 20 per cent. sodium carbonate solution to expel the carbon dioxide, and the bromine water is then run in from a burette until a yellow coloration persists for some minutes. The volume V of bromine solution used is diminished by 0·35 c.c., this

quantity in excess being required to give the coloration. The percentage F of formic acid is given by $F = 0{\cdot}086\ (V-0{\cdot}35)$.

(3) A rapid determination of the water may be made as follows: 95 c.c. (100 grms.) of the acid, 3 drops (0·1 grm.) of sulphuric acid (66° Bé.) and 5 c.c. of acetic anhydride of known titre (to obtain more exact results, the materials should be weighed) are boiled together gently for 5 minutes in a 200 c.c. flask fitted with an air-cooled reflux tube. The flask is then cooled and the melting point of the contents measured; this temperature, increased by 0·04° C. (lowering due to the sulphuric acid), gives A_2. If the subsequent calculation shows that all the acetic anhydride taken has reacted, the test must be repeated with a greater amount of the anhydride; for great accuracy, the excess of the anhydride over the amount which reacts with the water and with the formic acid should be as small as possible. All reagents must, of course, be rigorously dry. Since 1 grm. of formic acid reacts with 2·22 grms. of acetic anhydride $[(CH_3CO)_2O+H.CO_2H=2CH_3.CO_2H+CO]$, while the melting point of the acetic acid is lowered by 0·38° C., if 1 per cent. of the anhydride is present, and by 0·8° C. if 1 per cent. of formic acid is present, it can be shown that the percentage of water E is given by:

$$\frac{0{\cdot}38(P-2{\cdot}22F) \pm [A_1-(A_2-0{\cdot}8F)]}{4{\cdot}15},$$

P being the weight of acetic anhydride taken per 100 grms. of the acid.

(4) The percentage of higher acids is given by the expression

$$2[16{\cdot}70-(A_1+2E+0{\cdot}8F)],$$

since 2 per cent. of these higher acids depresses the melting point of acetic acid by 1° C.

Application of these methods to a number of test samples of acetic acid containing various proportions of the different impurities has apparently given surprisingly exact results. Thus, a mixture with the percentage composition: formic acid 1·168, water 0·111, higher acids (70 per cent. of propionic and 30 per cent. of butyric) 2·60, and acetic acid 96·12, gave 1·169, 0·126, 2·66, and 96·05 per cent. of the respective constituents.

Other impurities may be tested for as follows: Mineral matter in the usual way; aldehydes by Schiff's reagent, which gives approximately quantitative results; furfural by means of aniline; empyreumatic substances by the brown coloration given with concentrated sulphuric acid or, better, by treating 5 c.c. of the acid with 15 c.c. of water and 3 c.c. of 0·1 per cent. potassium permanganate solution: the pink colour should persist for at least 15 minutes for the acid to be satisfactorily free from empyreumatic matter. T. H. P.

Oxidation of Dihydroxystearic Acid by Potassium Permanganate in Acetone. J. Bougault and G. Schuster. (*J. Pharm. Chim.*, 1932, **124**, 5–7.)—The only product of the oxidation of castor oil by means of Hilditch's method with potassium permanganate in boiling acetone solution, was found to be triazelain. Three per cent. of stearic acid, added to the castor oil before oxidation, was recovered almost quantitatively, but dihydroxystearic acid is oxidised in the same

way as oleic acid, and 1·17 grm. of azelaic acid (theory, 1·18 grm.) was obtained by oxidising 2 grms. of dihydroxystearic acid (dissolved in 20 c.c. of acetone) by means of 4 grms. of permanganate. Therefore, the fact that only azelaic acid results from the oxidation of castor oil affords no proof of the presence or absence of dihydroxystearic acid in the original oil. Further, the iodine value will not necessarily afford any indication of the measure of oxidation; thus, a triglyceride of dihydroxystearic acid will give triazelain, and will behave like triolein, but the iodine value of the triolein will be 86, and that of dihydroxystearic triglyceride will be 0. D. G. H.

Iodimetric Method for the Determination of Citric Acid. P. A. Kometiani. (*Z. anal. Chem.*, 1931, **86**, 359–366.)—On the basis of the work of others (which is described) a method has been evolved in which the citric acid is oxidised to acetone dicarboxylic acid, and this is then brominated to give pentabromoacetone ($CHBr_2.CO.CBr_3$; m.pt., 73° C.; insoluble in water, but soluble in organic solvents). This reacts quantitatively with hydriodic acid in alcoholic solution, liberating 6 atoms of iodine per molecule, which may then be titrated. To a clear solution of 5 to 40 mgrms. of citric acid are added 1 c.c. of 1:1 sulphuric acid and 0·3 c.c. of 22·5 per cent. potassium bromide solution for each 10 c.c. present, though the final volume should not exceed 100 c.c. The mixture is heated at 40° to 45° C., and the citric acid is oxidised by addition, drop by drop, of a saturated solution of potassium permanganate until there is no further decolorisation, an excess being avoided to obviate oxidation of the acetone dicarboxylic acid to formic and oxalic acids. The bromine liberated at this stage also reacts, and the liquid turns yellow and then brown; the appearance of drops of oil on the surface of the liquid indicates the presence of bromination products due to an excessive temperature. After 5 minutes on the water-bath the solution is cooled and any free bromine or hydrated manganese dioxide is removed by addition of a saturated solution of ferrous sulphate containing 1 drop of sulphuric acid. The pentabromoacetone is allowed to settle for 1 hour, and is then filtered off on a Gooch crucible, washed with cold water, and finally dissolved in 25 to 50 c.c. of 96 per cent. alcohol, after which 5 c.c. of acetic acid are added. The mixture is heated almost to boiling, 5 c.c. of a 20 per cent. solution of sodium iodide in 96 per cent. alcohol are added, and, after 5 minutes on the water-bath and 10 to 15 minutes' cooling, the solution is diluted 10-fold with water, and the iodine is titrated with 0·1 *N* sodium thiosulphate solution, with 1 per cent. starch solution as indicator; then 1 c.c. $\equiv$ 3·501 mgrms. of citric acid. Dilution sensitises the end-point by rendering the reaction between unsaturated organic compounds and hydriodic acid irreversible. The formation of pentabromoacetone may be used as a qualitative micro-reaction which has a sensitiveness of 1:100,000. Tests of the method on solutions containing 4 to 40 mgrms. of pure citric acid gave results 0·1 to 1·0 mgrm. low, the most accurate results being obtained with the largest quantities. The errors are slightly greater if acetone or methyl alcohol is used as solvent in place of ethyl alcohol.

Milk.—The sample (10 c.c.), diluted with an equal volume of water, is shaken with 2 c.c. of 1:1 sulphuric acid and 4 c.c. of a solution prepared by dilution to

2 litres of a mixture of 100 c.c. of 30 per cent. sulphuric acid and a solution in water of 120 grms. of disodium hydrogen phosphate and 200 grms. of sodium tungstate. The precipitate is removed by filtration after 1 minute, and is washed with a little cold water, and the method described above is applied to the filtrate. Cows' milk contained 1·8 to 2·0 grms. of citric acid per litre, the somewhat higher values (up to 4 grms. per litre) found by Schwaibold and others (*Milch Forsch.*, 1925, **2**, 306, 312) being explained by the inaccuracy of their methods; buffaloes' milk contained 3·0 to 3·5 grms. per litre. J. G.

Detection of Nitrocellulose in Coats of Paint or Lacquer. P. Slansky. (*Chem. Ztg.*, 1932, **56**, 20).—About 50 sq. cm. of the coating, cut into pieces, are extracted with acetone for about an hour in a Soxhlet apparatus. The extract is evaporated to 2 to 3 c.c. and diluted, in a test-tube, with 15 to 20 c.c. of chloroform. If the liquid remains clear, nitrocellulose is absent. Any nitrocellulose present separates as a jelly, which is filtered off, washed thoroughly with chloroform, and saponified with about 20 c.c. of 0·1 *N* sodium hydroxide solution. The alkaline solution is then tested for nitric acid with either diphenylamine and sulphuric acid or, better, ferrous sulphate and sulphuric acid.

If the coating consists of several layers, and it is desired to know which of these contains nitrocellulose, the separate layers are rubbed off with small pieces of filter-paper wetted with acetone, the papers being then extracted with acetone and the extracts treated as described above. In order to ascertain of how many layers the coating consists, a sample is treated gradually with either 0·1 *N* sodium hydroxide solution or acetone, which saponifies or dissolves the layers in turn. Even with pure oil paint or oil lacquer, the layers are removed separately, since they exhibit different velocities of saponification. Care must be taken that each layer is steeped with the alkali only until it is removable by moistening it with water and cautiously rubbing it. The above procedure naturally serves also for the detection of nitrocellulose in liquid paint—by extraction with acetone and dilution of the extract with chloroform. T. H. P.

Inorganic Analysis

Determination of Bismuth as Metal. E. Rupp and G. Hamann. (*Z. anal. Chem.*, 1932, **87**, 32–35.)—The high results experienced in the reduction with alkaline formaldehyde are shown to be due, not to alkali adsorption, but to presence of suboxide. The following procedure gives good results: The solution (not more than 0·3 grm. Bi) is heated on the water-bath with formaldehyde and excess of sodium hydroxide until the supernatant liquid is quite clear. It is then boiled after addition of more aldehyde, filtered through a tared filtering tube provided with an asbestos pad, the precipitate repeatedly washed by decantation with hot water, collected, washed with alcohol and ether, and dried. A current of dry hydrogen is passed through the tube, which is heated to between 200° and 250° C. for 5 minutes. After cooling in hydrogen, the tube is again weighed.

In Strecker and Herrmann's process (precipitation of metal by magnesium from weakly acid chloride solution) it was found best to proceed as follows: Neutralisation with sodium hydroxide until slightly turbid, addition of a minimum

of acid to remove the turbidity, treatment with 3 grms. of Rochelle salt, and addition of 0·5 to 0·6 grm. of magnesium in small portions. After half-an-hour's heating, the turbid solution (basic magnesium salt) is cleared with ammonium sulphate solution, the free ammonia being expelled by boiling. The metallic bismuth is collected as before, washed, dried, and weighed. W. R. S.

Separation of Germanium and Arsenic. H. J. Abrahams and J. H. Müller. (*J. Amer. Chem. Soc.*, 1932, **54**, 86–94.)—A study has been made of the use of hydrogen sulphide for the separation of germanium and arsenic, which indicated that germanium does not precipitate as sulphide until the acid concentration of the solution exceeds 0·09 *N*. The following method was adopted in the test experiments: To the neutral solution (60 to 70 c.c.) containing germanium and (arsenious) arsenic, 3 c.c. of approx. 0·1 *N* sulphuric acid and 1 grm. of ammonium sulphate were added, and the mixture was saturated with hydrogen sulphide under pressure (presumably at the ordinary temperature). The arsenic sulphide was filtered off, washed with dilute ammonium sulphate solution containing hydrogen sulphide, and the arsenic was determined as magnesium pyroarsenate according to the directions of McNabb (*J. Amer. Chem. Soc.*, 1927, **49**, 1451). The filtrate containing the germanium was made 6 *N* in acidity with sulphuric acid, and the precipitate of germanium sulphide was filtered off on a sintered glass crucible and washed with a few c.c. of 2 to 3 *N* sulphuric acid saturated with hydrogen sulphide. The crucible was placed in a quartz beaker and covered with water, which was kept actively boiling until the germanium sulphide had dissolved, a cover glass being kept on the beaker to exclude air as far as possible, and thus prevent the formation of free sulphur. The crucible was then removed and the liquid was evaporated almost to dryness; a small quantity of nitric acid was added, the evaporation completed, and the residue ignited to germanium dioxide, in which form the germanium was weighed. There is some tendency for the arsenic sulphide precipitate to occlude germanium sulphide; the effect is negligible when the proportion of arsenic to germanium is small, but when it exceeds 15 to 20 per cent. the arsenic sulphide should be re-precipitated—the authors dissolved the arsenic sulphide in the minimum quantity of dilute ammonia (1 : 2), diluted the solution to 150 c.c., and added *N* sulphuric acid until the acidity of the solution was 0·05 to 0·1 *N*; the liquid was saturated with hydrogen sulphide, filtered, and the germanium in the filtrate was recovered by further acidification, etc., as described above. The method was used for the analysis of germanite. The germanium and arsenic in this material were first separated from the other constituents of the ore by distillation of the chlorides in hydrochloric acid; the arsenic and germanium in the distillate were separated as described. The results of a complete analysis of germanite were as follows: Ge, 7·37; As, 3·92; Cu, 45·38; Fe, 5·89; Ga_2O_3, 0·68; Al_2O_3, 0·13; Zn, 2·87; Pb, 0·71; S, 30·96; W, 0·10; gangue, 1·47 per cent. S. G. C.

Separation and Determination of Iridium. L. Moser and H. Hackhofer. (*Monatsh. Chem.*, 1932, **59**, 44–60.)—In the following processes of separation, iridium is precipitated by hydrolysis under oxidising conditions as greenish-black hydrated dioxide; precipitation is brought about by sodium bromide

and bromate. *From platinum.*—The faintly acid chloride solution (maximum 0·3 grm. Pt, 0·1 grm. Ir; 200 to 400 c.c.) is treated with 1·5 to 2 grms. of sodium bromate at 60° C., and a slight excess of 10 per cent. sodium bromide solution, and boiled until the smell of bromine is no longer perceptible (about 45 minutes). Precipitation is complete if fresh addition of the two salts does not cause a cloudiness and an odour of bromine. The precipitate is left to settle on the water-bath, filtered on close-textured paper, and carefully washed with hot ammonium nitrate solution. The filtrate is tested for complete precipitation by concentration and renewed addition of reagents. Filter and precipitate are dried in a porcelain crucible covered with a perforated mica plate; a stream of hydrogen is introduced, and a temperature of 160° to 180° C. maintained for half-an-hour. This prevents decrepitation. The precipitate is cooled under hydrogen, heated in air for the destruction of the paper, ignited in hydrogen, and cooled in carbon dioxide. It is then leached several times with hot water acidified with nitric acid for the removal of adsorbed soda; after another ignition with the above precautions, it is weighed as iridium. The platinum in the filtrate is determined, after boiling with nitric acid for the destruction of the bromate, by precipitation with ammonium acetate and hydrazine hydrochloride. The ignited metal should be leached and re-ignited before the final weighing. *From gold.*—The above directions apply also to the separation of iridium from gold; the solution should be diluted to 600 c.c. or more, because filter paper adsorbs the gold salt unless the concentration is very low. *From palladium.*—The hydrolysis method is applied as before, but the ignited and reduced iridium must be subjected to further treatment, as it contains palladium. It is heated on the water-bath with *aqua regia* diluted with its own volume of water; after 3 to 4 extractions, it is collected, washed, ignited, reduced, and weighed. The palladium in the filtrate is precipitated with hydrazine after destruction of the bromate (*vide supra*). The weighed metal should be leached with hot water and re-ignited, as a precaution against adsorbed alkali chloride. *From copper.*—The hydrolysis precipitate obtained as before contains nearly the whole of the copper. It is dried and then heated for half-an-hour at 170° C. in a current of hydrogen. Filter and precipitate are then digested on the water-bath with nitric acid (100 c.c. water to 7 c.c. of strong acid) for half-an-hour; the solution is decanted through a filter and the extraction repeated. The iridium is collected, again treated with hydrogen at 170° C., cooled, ignited, reduced, and weighed. Freedom from alkali is ensured by leaching and renewed ignition. W. R. S.

Separation and Determination of Rhodium. L. Moser and H. Graber. (*Monatsh. Chemie*, 1932, **59**, 61–72) (*cf.* preceding abstract).—Hydrolytic precipitation of greenish-black rhodium hydroxide by means of bromide and bromate is applied to the separation from platinum and gold. The weakly acid solution (neutralised, if necessary, with sodium carbonate) of the chlorides (0·003 to 0·1 grm. Rh, 0·003 to 0·3 grm. Pt; 300 to 400 c.c.) is treated with 30 c.c. of half-molar sodium bromate solution, then at 70° C. with molar sodium bromide solution. The liquid is boiled for an hour in the covered beaker; on being tested with more bromide and bromate, it should not evolve bromine on boiling. The precipitate is left to settle on the water-bath, collected, washed with hot ammonium

nitrate solution, dried at 110° C., ignited in a porcelain crucible, reduced in hydrogen, cooled in carbon dioxide, and weighed. It is then leached, at boiling heat, with water acidified with hydrochloric acid, filtered off, washed free from chloride, again ignited with the same precautions, and weighed. A second test for adsorbed alkali is recommended. For the recovery of platinum in the filtrate, and the necessary dilution of the solution in the separation of gold from rhodium, the directions are the same as in the preceding abstract.

The method does not effect a separation of rhodium from copper or iron. *Copper.*—In the separation from copper the 0·1 *N* sulphuric acid solution, containing 15 c.c. of 0·1 molar magnesium chloride solution per 100 c.c., is treated with hydrogen sulphide, first at boiling heat, then at 90° C., and, finally, left to cool while the gas is passing. The precipitate is collected and washed free from sulphate with hydrogen sulphide water, ignited wet, transferred to a beaker, and extracted with 100 to 200 c.c. of nitric acid (1:1). After decantation, the extraction is repeated. The residue is collected, ignited in hydrogen, and cooled in carbon dioxide; the reduced metal is again extracted twice with strong nitric acid on the water-bath. The final residue is collected, ignited and reduced with the usual precautions, leached, ignited, reduced, and weighed as rhodium. *Iron.*—The separation from iron is based on the precipitation of rhodium as sulphide. The chloride solution (0·1 *N* concentration of hydrochloric acid; 200 to 400 c.c.) is treated with magnesium chloride and hydrogen sulphide exactly as in the separation from copper. The sulphide precipitate is ignited, reduced, etc., as before, and weighed as rhodium. W. R. S.

Determination of Tungsten in Steel as Hydrated Tungstic Acid. H. Wdowiszewski. (*Z. anal. Chem.*, 1932, **87**, 36–38.)—The yellow precipitate, obtained by solution of the steel in hydrochloric acid and subsequent oxidation with nitric acid, is usually ignited in platinum and weighed as WO_3 (factor, 0·7931). The precipitate may, however, be collected on asbestos in a Gooch crucible, washed with 2 per cent. hydrochloric acid, then twice with alcohol, dried at 100° to 110° C., and weighed as H_2WO_4 (factor, 0·736). The results are close, and the method is advantageous in saving wear of the platinum crucible. In the subsequent analysis of the precipitate (always necessary in accurate work), fusion is obviated; the weighed precipitate is washed with hot dilute ammonia, in which it is soluble, leaving silica and ferric (chromic) oxide insoluble. The filtrate is treated with a small excess of sulphuric acid and benzidine solution in the usual manner.

W. R. S.

An Overlooked Source of Error in the Ferrocyanide Titration of Zinc. B. Park. (*J. Amer. Chem. Soc.*, 1932, **54**, 180–181.)—When a ferrocyanide solution is kept a small proportion of ferricyanide is formed. Whereas in the ferrocyanide method for determining zinc, as described by, *inter alios*, Low, *Technical Methods of Ore Analysis*, hydrogen sulphide is added to the zinc solution before the titration with ferrocyanide, the standardisation of the ferrocyanide solution is carried out by titrating a standard solution of zinc in the absence of hydrogen sulphide. A hitherto unsuspected error is liable to arise owing to the reduction of any ferricyanide in the ferrocyanide solution by the hydrogen sulphide, thus

increasing the ferrocyanide content of this solution as determined in the standardisation. Thus, the author has found (1) that the zinc equivalent of a ferrocyanide solution altered from 0·00532 to 0·00524 grm. of zinc per c.c. on keeping for seven years; (2) that this old ferrocyanide solution had a greater zinc equivalent in the presence than in the absence of hydrogen sulphide in the zinc solution titrated (38·18 c.c. and 39·65 c.c. of ferrocyanide solution, respectively, for 0·2490 grm. of zinc). Only a slight difference was detected in the zinc equivalent of a fresh ferrocyanide solution in the presence and absence of hydrogen sulphide (39·15 c.c. and 39·33 c.c. of ferrocyanide solution, respectively, for 0·2490 grm. of zinc). It is, therefore, recommended that hydrogen sulphide be also added to the zinc solution used for the standardisation. S. G. C.

Determination of Manganese by Means of Persulphate. R. G. Harry. (*J. Soc. Chem. Ind.*, 1932, **51**, 24T.)—The precipitation of manganese by ammonium persulphate was carried out in presence of about 4 per cent. of various other metals, the sulphate solution, containing 10 c.c. of 20 per cent. sulphuric acid in 500 c.c. total bulk, being boiled and treated with 50 c.c. of fresh 12·5 per cent. persulphate solution; after 20 minutes' boiling, the same quantity of persulphate was again added. The washed manganese peroxide was dissolved in a known excess of acid ferrous sulphate solution; the excess of ferrous iron was ascertained by dichromate titration. It was found that copper, lead, zinc and calcium were without influence in this procedure. High results were obtained in presence of titanium, bismuth, tin, vanadium and cobalt; whilst antimony, arsenic, nickel, and tungsten caused the results to be low (*cf.* ANALYST, 1932, 125). W. R. S.

Determination of Manganese as Manganese Ammonium Phosphate. P. Nuka. (*Z. anal. Chem.*, 1932, **87**, 7–26.)—The most favourable conditions for the formation of a precipitate of correct composition and the choice of the most suitable wash-liquor for the precipitate were re-investigated. The conclusion is, that hot one per cent. diammonium phosphate solution, followed by 60 to 65 per cent. alcohol, is the best wash-liquor; if the phosphate is produced as a flocculent precipitate which becomes crystalline only on warming, variable results and positive errors ensue. More constant results, in closer agreement with calculated values, are obtained by gradual precipitation of the crystalline modification. The boiling, neutral solution, free from sulphates and containing 15 to 20 grms. of ammonium chloride, is stirred and treated, drop by drop, with 50 c.c. of 2 per cent. diammonium phosphate solution; 25 c.c. of 6 per cent. solution of the same salt are then added more quickly. Acetates do not interfere. W. R. S.

Determination of Aluminium. Formation of Lithium Aluminate. J. T. Dobbins and J. P. Sanders. (*J. Amer. Chem. Soc.*, 1932, **54**, 178–180.)—To a solution of aluminium nitrate (100 c.c.) were added a few drops of phenolphthalein indicator, and lithium chloride solution (10 per cent.) "in excess of that required to precipitate the estimated amount of aluminium in the sample." Ammonia was added, drop by drop, with stirring, until the solution became a faint pink. The voluminous precipitate was allowed to settle for 5 minutes, filtered off (it is stated to filter readily), washed with water until free from chloride, and, after

preliminary drying, ignited, at a high temperature, to constant weight; the residue was weighed, and the aluminium was calculated from the formula $2Li_2O,5Al_2O_3$. The process, tested on amounts of aluminium of the order of 0·1 grm., gave good results. No independent verification of the formula of the lithium aluminate produced in this method appears to have been carried out. The statement made in the summary of the work, that the process yields more concordant results than the ammonium hydroxide method, is not supported by experimental data.

S. G. C.

Microchemical

Microchemical Examination of Pictures. H. Hetterich. (*Mikrochem.*, 1932, **10**, 27–44.)—*Examination of Media.*—The protein group of media, such as protein, egg yolk, glue (size) and casein, contain nitrogen, whilst the dry oils (such as linseed oil), the waxes, starch, dextrin, cherry gum, gum arabic, and others contain no nitrogen. Emich's (Emich, *Mikrochemisches Praktikum*, 1931, p. 106) test for nitrogen is used to distinguish the two groups; the organic compound is heated with precipitated lime in a thin-walled capillary tube, sealed at one end. The ammonia developed on heating changes the colour of a litmus paper (soaked in litmus containing $N/20$ acid) at the open end. The test is sensitive to 0·1γ of nitrogen. Small amounts of pigment, with the exception of indigo and Paris blue, do not interfere with the test. The proteins of the nitrogen-containing group of media also contain sulphur, for which the Grünsteidl test (*Z. anal. Chem.*, 1929, **77**, 283) is used, in which the sulphur is converted into sulphide by heating with sodium hydroxide in a micro porcelain crucible. By further evaporation with 0·1 per cent. potassium cyanide solution the thiocyanate is formed, and this gives the usual red colour with ferric salts. When much phosphorus is present, as in casein, Feigl's test (Feigl, *Qualitative Analyse mit Hilfe von Tüpfelreaktionen*, 1931, p. 282) for sulphides is preferable, in which bubbles of nitrogen are formed when a drop of iodine nitride reagent (100 c.c. of 0·1 N iodine containing 3 grms. of sodium nitride) is added to a drop of a sulphide solution. The presence of phosphorus is used as a test for casein. The compound is oxidised with concentrated sulphuric acid and perhydrol to phosphoric acid, which is identified by the blue colour produced on adding ammonium molybdate solution in nitric acid, and then benzidine. Silicic acid (which may come from the walls in wall paintings) interferes with this test, but can be rendered inert by carrying out the test in the presence of ethyl alcohol. The "fatty oils" of the nitrogen-free media are identified by the conversion of the glycerol grouping into acrolein, by heating with solid alkali and sodium bisulphate in a thin-walled capillary tube. The acrolein vapour is identified by the red coloration of acid Schiff's reagent (0·1 grm. of fuchsin and 0·7 grm. of sodium bisulphite in 88 c.c. of water, treated, after an hour's standing, with 25 drops of concentrated sulphuric acid). *p*-Nitro-phenylhydrazine hydrochloride in aqueous solution, containing a little dilute acetic acid, is also used for the test; a small drop is drawn into a fine capillary, which is placed inside the capillary in which the acrolein is developed, so that the vapour passes through the solution, when orange-coloured crystals of the osazone, which can be seen under the microscope, are formed. The "fatty oils" may also be saponified

on the microscope slide with alcoholic potash, but the crystalline products are all similar.

Examination of Pigments.—Feigl's "spot" test methods (vide *supra*) and the usual microscopic methods are used for the identification of the pigments. An example is given of a detailed analysis of the painting on a wooden Madonna statue of 1620 A.D. Six different layers, of total thickness 0·6 mm., of which 0·1 mm. was pigmented, were distinguished in a cross section under the microscope. These were separated and analysed. The bottom layer was calcium carbonate, calcium sulphate and glue or size; the second layer, calcium carbonate and glue; the third, azurite, white lead and gum arabic; the fourth, ultramarine and white of egg; the fifth, azurite and oil (or tempera rich in oil), and an oil varnish (*cf.* Laurie, ANALYST, 1930, **55**, 162). J. W. B.

A New Cymene Bath for Pregl's Micro-Combustion. A. Verdino. (*Mikrochem.*, 1931, **9**, 123–125.)—A new cymene bath ("Hohlgranate") for maintaining the lead peroxide of the combustion tube at constant temperature is described. A safety device renders it less likely to boil over or catch fire than with the old model. The outer dimensions are the same (obtainable from P. Haack, Vienna). J. W. B.

Electric Heater for Pregl's Micro-Combustion. W. Füner. (*Mikrochem.*, 1932, **10**, 66–69.)—A simple electric heater for micro-combustion consists of a heater, 19 cm. long (to replace the long burner), made of nickel-chromium wire covered with kieselguhr, taking 1·3 to 2 amp. of current, which will heat the oxidising portion of the tube to red heat. The cymene bath is replaced by an electric heater, 7 cm. long, made of a block of iron which can be maintained at 174° to 176° C. This uses a current of 0·5 amp. when heating, and remains constant with 0·35 amp. An Osram iron resistance lamp (0·35 amp.) is used in the circuit to maintain the evenness of the current. The advantage of the electric constant-temperature heater is that it can be kept running indefinitely, with no danger of fire or of fumes of cymene escaping into the air. The Bunsen burner is retained for the heating of the substance in the boat, as the process of combustion is easier to watch, and the tube cools more quickly for a subsequent determination. [The heater is obtainable from Firma Kirchenbauer (Apparatebau), Singen (Pforzheim).] J. W. B.

Tests for Nicotine in the Presence of Pyridine and its Derivatives. R. Hofmann. (*Mikrochem.*, 1932, **10**, 53–56.)—1. *With hydroxy-tri-thiocyanate di-amino chromic acid*—The reagent is prepared from Reinecke's salt,

$$\left[\mathrm{Cr}\begin{matrix}(\mathrm{NH_3})_2\\(\mathrm{CNS})_4\end{matrix}\right]\mathrm{NH_4},$$

by recrystallising three times from a dilute acetic acid solution, and then dissolving 1 grm. of the purified salt in 5 c.c. of 8 per cent. hydrogen peroxide solution. The reagent,

$$\left[\mathrm{Cr}\begin{matrix}(\mathrm{NH_3})_2\\(\mathrm{CNS})_3\\\mathrm{OH}\end{matrix}\right]\mathrm{H}\ldots\mathrm{H_2O},$$

(Werner's acid, *Ber.*, **1916**, **49**, 1539) is used in the form of a saturated solution. When a drop of the neutral or slightly acid solution under examination is placed on a microscope slide with a drop of the reagent, large leaf-shaped crystals are formed in the presence of nicotine. The smallest amount recognisable is 0·157γ in a dilution of 1:3800. Pyridine also gives crystals with the reagent, and, although these differ in form from those given by nicotine, yet, with increasing concentrations of pyridine, the two forms become indistinguishable. The highest percentage of pyridine that can be present for the test to be successful is 75 per cent.; of collidine, 90 per cent.; of lutidine, 75 per cent.; and of picoline, 75 per cent. 2. *With silico-tungstic acid.*—The slightly acid (0·1 to 1 per cent. of hydrochloric acid) solution under examination is mixed with a drop of 10 to 12 per cent. sodium silicotungstate. According to the concentration, a cloudiness or white precipitate is formed; if the latter, the solution should be diluted. A few granules of sodium chloride are then added, and in 5 or 10 minutes the nicotine crystallises out in rectangular plates, which are not doubly refracting. The smallest amount recognisable is 0·4γ in a dilution of 1:1,250,000. Pyridine forms larger, more diffractive crystals; lutidine, picoline and collidine do not form characteristic crystals. Photomicrographs of the different crystals are given. J. W. B.

Microchemical Test for Hydrogen Peroxide and for Vanillin. C. Griebel. (*Mikrochem.*, 1931, **9**, 313–315.)—Vanillin hydrochloride forms a blue crystalline compound with hydrogen peroxide. The reaction is used as a test for peroxides, a drop of the solution, or a few granules of the solid, being added to a drop of a 1 per cent. solution of vanillin hydrochloride (0·1 grm. of vanillin dissolved in 10 grms. of 25 per cent. hydrochloric acid and warmed). In 5 or 10 minutes the liquid, if hydrogen peroxide is present, becomes reddish-brown, and, finally, feathery groups of blue-black or violet-black needles crystallise out. Better crystals are formed when the vanillin solution contains alcohol (0·1 grm. of vanillin in 1 c.c. of alcohol and 9 c.c. of 25 per cent. hydrochloric acid). The smallest amount of hydrogen peroxide recognisable in an aqueous solution of 0·02 c.c. volume is 25γ. This test is not so sensitive as the iodine test, but it is specific, and is not interfered with by the presence of other oxidising agents, as is the iodine reaction. *Vanillin.*—As little as 23γ of vanillin can be detected by the test. J. W. B.

Physical Methods, Apparatus, etc.

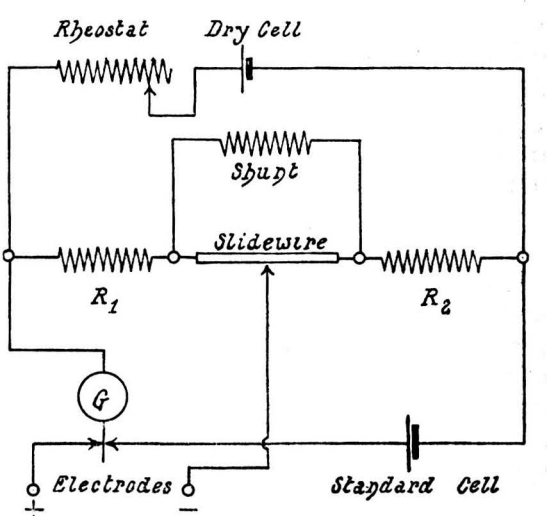
Drawing Reproduction and Lantern-slide Making. H. C. Bennett and R. Lee. (*J. Chem. Education*, 1931, **8**, 2208–2211.)—Kodalith, a new Eastman paper, which has extreme contrast and shows no texture, is recommended for the reproduction of graphs by direct contact. The graphs should be drawn on tracing cloth and a negative prepared on Kodalith. Paper negatives on Kodalith can also be used for the preparation of lantern slides of graphs or typewritten tables of figures. "Slow" lantern plates should be used.

Methods for Studying the Scale Structure of Animal Fibres. J. I. Hardy. (*J. Text. Inst.*, 1932, **23**, 1T.)—The difficulty of observing the scale structure of medullated and pigmented fibres can be overcome by making a celloidin

cast of the hairs or fibres and examining this under the microscope. The method consists in fastening the fibres parallel, without touching each other, over a shallow trough and pouring over them a 20 per cent. solution of celluloid in acetone. After drying for a short time, the celluloid is peeled off, the fibres pulling through the cast and leaving a fissure which does not interfere with the microscopic examination. A series of photomicrographs is given showing the type of result obtained by this method.

Method for Revealing Scale Structure of Wool and Hair. J. Manby. (*J. Text. Inst.*, 1932, **23**, 15T.)—This method is devised to overcome the difficulty that, in examining hairs, the out-of-focus image of the lower scales often interferes with the observation of the upper scales and *vice versa*. The author makes use of the fact that the image of the lower scales can be caused to disappear by mounting the fibres so that the lower half of the fibre is immersed in a medium of approximately the same refractive index as the fibre, but at the same time keeping the upper surface of the fibre clean so that the visibility of these scales is not affected. The method consists in laying the hairs on a glass slide and placing a small drop of the mounting medium at one end and allowing it to drain along the fibre. No cover glass must be used in observing the fibre under the microscope. The mounting medium recommended is composed of 3 grms. of gelatin, 3 grms. of glycerin, and 94 c.c. of water, plus a little phenol or thymol as a preservative. Methods of microscopical technique are discussed and photomicrographs are given.

Automatic *p*H Recorder. C. Morton. (*J. Soc. Chem. Ind.*, 1931, **50**, 436–438T.)—The cell (see Figure), which is made of ebonite, bakelite or porcelain, consists of three compartments with inlets at the top and outlets at the bottom, separated by two porous diaphragms, and containing the sample, saturated potassium chloride solution, and a buffer solution of known *p*H value (*A*), respectively. Two guide-rods, passing lengthwise through all three compartments, keep them in close contact, the completed assembly being held together by means of a screw-clamp, the jaws of which fit into recesses in the end-plates; rubber washers ensure water-tight contacts. Replaceable gold or platinum electrodes, the areas of which are such that the resistance of the cell is approximately equal to that of the galvanometer coils (2,000 ohms), are washed in acetone or ignited over a spirit lamp and mounted in rubber bungs which are then inserted in the end-compartments. For continuous measurements the main supply of sample is conducted



through a siphon-chamber (serving as a by-pass), containing a bag of quinhydrone, from which it overflows into a small cistern (to enable the siphon to act at atmospheric pressure), and thence enters the first compartment. Similar devices attached to reservoirs containing the appropriate solutions (not shown) supply the other two compartments, and ensure automatic flushing. Then pH (at 18° C.) = $(E/0{\cdot}058) \pm A$ (accuracy and "lag" not stated). The surface of the cell should be clean and dry, and care should be taken that the system is not earthed by potassium chloride drippings at the drain outlet. The (Cambridge) recording outfit has the usual potentiometer circuit, but the galvanometer, on deflection, activates a pre-set mechanical relay which moves the slide-wire contact so as to balance the system and thus reduce the deflection. The circuit is thus maintained in balance between limits pre-determined by adjustment of the bridge resistances, the sliding-contact being connected with a recording pen or warning signal. J. G.

Reviews

PRAKTIKUM DER WARENKUNDE. EIN HILFSBUCH FÜR DIE CHEMISCH-PHYSIKALISCHE UND MIKROSKOPISCHE WARENPRÜFUNG. By EDMUND GRÜNSTEIDL, Ph.D. Pp. 196, with 215 illustrations. Vienna: Julius Springer. 1931. Price RM. 11.50.

This is intended as a laboratory guide for a general course for commercial students. For many years the teaching of scientific method to others than those who intend to make it their life's work has been recommended in this country, and this book would appear to be the result of a similar intention in Germany. After a careful perusal the reviewers are not sure that, after all, such efforts can lead to results as useful as was at first hoped.

This book deals with such varied articles as metals, glass, dyes, textiles, oils and foodstuffs. The tests suggested are usually of the simplest description; thus, under Milk, we have only those for specific gravity, the addition of neutralising substances and pasteurisation; and margarine is distinguished from butter by means of a test for sesamé oil. It may be that such elementary work is of value in widening the merchant's outlook, if coupled with carefully arranged lectures, but, after all, it is not necessary to be a second-rate bricklayer in order to appreciate the efforts of the architect, and the question arises whether general lectures in scientific method, coupled with laboratory work of a less specialised kind, would not be of much greater value as a means of acquiring an appreciation of the scientist's outlook.

The printing, paper and general appearance of this book are particularly attractive, and the illustrations are carefully selected and excellently reproduced. The section on microscopy occupies more than half of the total space, but, as an introduction to the subject, suffers from the drawback that it deals too much with actual materials and too little with general principles. Thus, whilst excellent illustrations are given of textiles, starches, sections of plants and powders, no detailed explanations or drawings are given of the different kinds of structure which can be recognised by means of the microscope.

The book will scarcely prove either interesting or useful to any class of British students, but that this is so cannot be regarded as a matter of concern.

G. D. ELSDON.
J. R. STUBBS.

PRACTICAL MICROSCOPY. By L. C. MARTIN, D.Sc., and B. K. JOHNSON. Pp. viii+116, with 88 figures, including 10 plates. London: Blackie & Son. 1931. Price 3s. 6d. net.

This small volume is intended as a guide to the technique of microscopy and photomicrography, and contains much matter that would be obtainable only with difficulty elsewhere. The contents are divided into thirteen chapters, and embrace a wide range of tables, formulae, and methods of utility in the technique of modern microscopy, including magnification, mechanical and optical parts of the microscope, numerical aperture, methods of illumination, photomicrography, the preparation of specimens of various kinds, polarised light, ultra-violet microscopy, and a final highly important chapter on the interpretation of the image observed. It will be obvious from the size of the volume that some of the subjects, notably the preparation of specimens, have been treated briefly, but the methods given are of wide application, and, although concise, are lucid and sound. Numerous references are given throughout the volume to sources of further information on various subjects, and the many illustrations are particularly useful, being well selected and adequately described in the text, whilst the index, which might perhaps be extended with advantage, is almost free from error. The book is thoroughly reliable, eminently useful, and should be in daily demand by the practical microscopist and photomicrographer.

The authors and publishers are to be congratulated upon their joint efforts in producing at such a reasonable price this excellent handbook, the merits of which, when known, will undoubtedly ensure a wide circulation. T. J. WARD.

COLLOID CHEMISTRY—THEORETICAL AND APPLIED. By Selected International Contributors. Collected and Edited by JEROME ALEXANDER. Volume III. First Series of Papers on Technological Applications. Pp. 655. New York: The Chemical Catalog Company, Inc. 1931. Price $10.50.

Volume II of this series was reviewed in THE ANALYST (1929, **54**, 263). Technical chemists have awaited with very considerable interest the appearance of the two volumes devoted to technological applications. The first of these now appears, with 42 papers by distinguished authors, who outline the present position of those colloid subjects on which they are recognised specialists.

Technological applications have been divided into four groups, and the first two occupy the present volume. As the editor states: "The first group consists of subjects of interest to many industries, and comprises eleven papers on *general principles* and six papers dealing with *mechanical* or more specialised matters. The second group (twenty-five papers) may, for want of a better name, be termed *telluric*; for it deals with matters which are of the earth, earthy, beginning with geology and mineralogy, and running to metals, petroleum, asphalt and agriculture."

The first paper is by McBain and Jerome Alexander on "Cohesion and Adhesion," and ably summarises the investigations of the last few years. Clark

follows with a paper on "Some Practical Results of *X*-ray Researches on Colloids." This is well done and provides a useful summary of quite recent work.

The next paper, by Bartell, dealing with the wetting of solids by liquids, is excellent, and it will serve to clarify ideas on the complicated, but most important, subject of wetting. He distinguishes between adhesional wetting, spreading wetting and immersional wetting, and includes a good discussion of contact angles. Wetting phenomena are further treated by Von Terzaghi (The Influence of Elasticity and Permeability on the Swelling of Two-Phase Systems) and by Buchanan, who discusses flotation.

The wide range of subjects is reflected in the papers dealing with catalysis, adsorption by silica gel, colloid factors in water supply, grinding, electrical precipitation of suspensoids, the super-centrifuge, filtration, chemical warfare, colloid phenomena in glass, minerals, geology, porcelain, and sodium silicate solutions, corrosion, colloidal fuel, and coal tar. Such names as E. F. Armstrong, H. S. Taylor, Chwala, Liesegang, Silverman, Benedicks, U. R. Evans, Newton Friend, and Acheson show that the proper authorities have been chosen to review current research.

Washburn discusses ceramic refractories, Searle the colloidal nature of cements and mortars, Alexander, Guertler, Benedicks, Honda and Yap the questions of metals. Three papers are devoted to petroleum, and some over-lapping is the result; but it is interesting to have the views of Dunstan, Morrell and Egloff, and Gurwitsch. The nature of asphalt is treated by Nellensteyn.

The volume closes with a long paper by Gortner under the title: "The Colloid Chemistry of Wheat, Wheat Flour and Wheat-Flour Products." This is a particularly able summary, covering a wide range of subjects.

Mr. Alexander is to be congratulated on getting together so many experts from many countries. One can readily guess at his trials as editor. However, it must be conceded that he has done his part well, and the present volume is a worthy successor to the previous volumes. Criticisms there must inevitably be, but no one scientist could take it upon himself to criticise in detail colloid questions in so many and such diverse fields. The present reviewer has carefully examined those papers coming within the purview of his own interests, and is satisfied. One minor criticism may be permitted. Due, no doubt, to the difficulty of synchronising the receipt of so many papers, there is evidence that some of them are considerably behind work done in 1930–1931. Consideration of the references furnished at the ends of the papers will bear this out.

Taken altogether, this book deserves a place in the library of all chemists who are interested in colloid chemistry and its industrial applications. It is well printed and bound, and typographical errors are few. WILLIAM CLAYTON.

CHEMICAL EMBRYOLOGY. By JOSEPH NEEDHAM, M.A., Ph.D. Vol. I, pp. xxii+613+10 plates. Vol. II, pp. xvi+615–1253+3 plates. Vol. III, pp. xvi+1253–2021+1 plate. Cambridge: University Press. 1931. Price 105s. net.

Embryology has hitherto been regarded as a branch of morphology or anatomy, and no collective work, with the exception of Preyer's small book, entitled *Special Physiology of the Embryo* (1880), has so far been written, which would survey the numerous investigations (Dr. Needham's bibliography, pp. 1728–1970, contains

more than 4500 references) on the development of the embryo from the chemical point of view. Chemical embryology has thus so far lived an intra-uterine existence in scientific journals and specialised monographs. It required a first-class obstetrician to bring chemical embryology into the world. This laborious and difficult task has been most skilfully performed by Dr. Needham, who is already well known as the author of *The Sceptical Biologist*, a work which in centuries to come will probably rival *The Sceptical Chymist* in importance to the history of science. Dr. Needham seems in the habit of producing classics, and there is no doubt whatever that *Chemical Embryology* is, and will remain, a classic for many years.

From the prolegomena (pp. 1–4) to the epilegomena (pp. 1613–1665) the work is impregnated with the idea that scientific knowledge comes gradually to maturity as the result of the accumulated labours of many observers, careful, though in many cases obscure; chemical embryology has certainly been built up by a host of enthusiastic workers who deserve our greatest admiration. Anybody who reads the first two parts (pp. 7–231) of Dr. Needham's treatise, dealing with the historical aspect of the question, will carry away admiration for the many scientists and philosophers and their critics who have helped in building up embryology in general, and chemical embryology in particular. It is the work of pioneers in a field hitherto unknown to many. Again, one may predict that these two sections will, for years to come, remain a history of the subject.

Before dealing with the main section of this work, namely, Part III (pp. 231–1613), one may, perhaps, be permitted to offer some criticism of the manner in which the historical aspect has been dealt with. Dr. Needham commits the common error of the zealous historian, of putting modern ideas into the interpretation of ancient observations. Such dangers must always be guarded against, and one cannot but feel that Dr. Needham has been sometimes misled by ascribing much to the past that really belongs to the present. Incidentally, reference must be made to a curious statement on p. 183. Dr. Needham writes with reference to Boerhaave's *Elementa Chemiae*, that "it will be noted that they are cast in the form of lectures or addresses, as if they had been taken down direct from the lectures of the Professor, a fact which gives them a peculiar charm, when it is remembered how many great men must have listened to them. . . ." Now the *Elementa Chemiae* (Dr. Needham writes *chymiae* on p. 183, but correctly *chemiae* on p. 1750) are not written as *if* they were lectures delivered by Boerhaave, but they are the actual lectures given by him. They were originally published in 1724, without Boerhaave's permission, under the title *Institutiones et Experimenta Chemiae*, but, as they contained too many personal attacks against Van Helmont, Paracelsus and others, Boerhaave republished these lectures under the title *Elementa Chemiae*. Dr. Needham quotes the 1732, Leyden edition, and he must thus have overlooked Boerhaave's *autographed* authority on the back of the title-page of Vol. I. The reviewer trusts he will be forgiven this minor criticism; after all, he has not come only to praise the author of *Chemical Embryology !*

Part III, entitled "General Chemical Embryology," constitutes the bulk of the work. A gigantic undertaking, which, had Dr. Needham not listed in his bibliography (p. 1878) that his *Ing. Diss.*, Cambridge, appeared in 1924, could easily be mistaken for the work of a lifetime. Dr. Needham tells us, however, in his

"Acknowledgments of Indebtedness," that the idea of writing his *Chemical Embryology* originated in 1923 during a conversation with Professor Sir F. G. Hopkins, and thus only nine years have been occupied in the production of this formidable book.

The section, "General Chemical Embryology," is divided into: (i) The unfertilised egg (for some reason or other Dr. Needham has excluded fertilisation from his treatise, a point which will be regretted by many); (ii) and (iii) Embryonic growth (pp. 368–615); (iv) Respiration of the embryo (pp. 615–777); (v) Ontogenesis and its biophysical phenomena (pp. 777–839); (vi–xiii) Embryonic metabolism, including carbohydrates, proteins, nucleins, fats, lipoids, sterols and inorganic constituents (pp. 839–1289); (xiv–xvii) Enzymes, hormones, vitamins, and pigments in ontogenesis (pp. 1289–1383); (xviii) and (xix) Immunology in the life of the embryo (pp. 1383–1456); (xx–xxii) The biochemistry of the placenta (pp. 1456–1565); (xxiii) Blood and tissue chemistry of the embryo (pp. 1565–1595); and (xxiv) Hatching and birth (pp. 1595–1613). This brief summary obviously does not do justice to the work, but it shows the extent of its undertaking. Biologists of all kinds of shades will turn to it for information and find it. The importance of an accurate knowledge of chemical events in the life of the embryo is well realised, and to have all this information at hand will be welcome to the student of genetics, the biologist, the agriculturist, the stock-raiser, the entomologist and the agronomic expert. The Subject Index (pp. 1917–2012) and the "Index Animalium" (pp. 2013–2021) will be found of real assistance. They should be consulted with care. The embryology of the chick has attracted interest since ancient times, and one naturally finds numerous references to *Gallus domesticus*. Compared with it, *Homo sapiens* makes a very poor show in Dr. Needham's "Index Animalium."

Science in general, and biochemistry in particular, are under a very real debt to Dr. Needham for his wide reading, his ability as a writer and scientist, and his great patient industry. The expression of gratitude to Dr. Needham is, however, not complete without a tribute to the Cambridge University Press for the care they have given to the production of this monumental work.

M. NIERENSTEIN.

Publications Received

THE B.D.H. BOOK OF P.P.P. STANDARDS. (Obtainable on request from The British Drug Houses, Ltd., Graham Street, N.1.)

APPLIED CHEMISTRY. Vol. II. FOODS. By C. K. TINKLER and H. MASTERS. London: Crosby, Lockwood & Son. Price 15s. net.

AN INTRODUCTION TO BIOCHEMISTRY. By R. J. WILLIAMS. London: Chapman & Hall. Price 21s. net.

HANDBUCH DER PFLANZENANALYSE. Band II. Spezielle Analyse. 1st Teil. Vienna: J. Springer. Price RM.96.

INDUSTRIAL CHEMICAL CALCULATIONS. By O. A. HOUGEN and K. M. WATSON. London: Chapman & Hall. Price 28s. net.

THE GLYCOSIDES. By E. F. ARMSTRONG and K. F. ARMSTRONG. London: Longmans, Green & Co., Ltd. Price 12s. 6d. net.