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Volume 70 June, 1945
No. 831, Pages 195—230

THE ANALYST

The Journal of The Society of Public Analysts and other Analytical Chemists

Published for the Society by
W. HEFFER & SONS LTD., Cambridge, England

ENTERED AS SECOND CLASS AT NEW YORK, U.S.A., POST OFFICE
PRINTED IN ENGLAND

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held at 5 p.m. on Wednesday, May 2nd, at The Chemical Society's Rooms, Burlington House, London, W.1, with the President, Dr. G. W. Monier-Williams, in the chair. The following papers were presented and discussed: "A Rapid and Simple Method for the Determination of Calcium in presence of Strontium and Barium," by G. W. Osborn; "Lead Printing of Ferrous and Non-Ferrous Metals," by W. B. Wragge; "Reaction of Diazotised *p*-Nitraniline with Phenols: Detection of Tricresyl Phosphate in Edible Oil," by E. Collins, M.A., F.R.I.C.; "A Simple Apparatus for handling Standard Solutions of Bromine in Potassium Bromide," by A. J. Henry, B.Sc., Ph.D.

NEW MEMBERS

John Francis Byrne, A.R.I.C.; Rustom Pirazshaw Daroga, M.Sc. (Bombay), Ph.D. (Lond.), D.I.C., A.R.I.C.; Aubrey Harris Edwards, B.Sc., A.R.I.C.; Harold Johann Thomas Ellingham, B.Sc., Ph.D. (Lond.), A.R.C.S., F.R.I.C., A.M.I.Chem.E.; Ronald Henry Jones, F.R.I.C.; Norman Andrew Marris, M.Sc. (N.Z.), F.N.Z.I.C.; John Arnold Camden McClelland, B.Sc., Ph.D. (Lond.), A.R.I.C.; Arthur Sidney Minton; Stanley Herbert Pitt; John Alfred Ryan.

Methods of Sampling Foodstuffs

THIS was the general subject of the Joint Meeting of the Society with the Food Group of the Society of Chemical Industry on December 6th, 1944. Dr. H. E. Cox, Chairman of the Food Group, was in the chair, and the following papers were read and discussed:

"Some Experiences in the Sampling of Foodstuffs in Bulk," by Mr. J. King.

"The Sampling of Cooked Meals for Nutritional Analysis," by Dr. C. A. Mawson.

"Sampling for Metabolism Studies," by Miss E. M. Widdowson.

Before introducing the first speaker, Dr. Cox referred to the grievous loss sustained in the death of Sir John Fox, and the meeting stood in tribute to his memory.

The Sampling of Foods for Nutritional Analysis

BY C. A. MAWSON

The methods described in this paper were devised for the purpose of an investigation into the nutritive value of British Restaurant meals, instituted by the Ministry of Food in 1942. It was considered sufficient in the first instance to estimate the ascorbic acid contents of the meals, their calorific values and the usual major constituents. The ascorbic acid content was regarded as an approximate index of the water-soluble vitamins present, for it is difficult to construct from ordinary foodstuffs a diet adequate in ascorbic acid but deficient in other labile vitamins. The object of the work was to discover whether the population feeding at these restaurants was receiving a sufficient quantity of good food and whether it was practicable to improve the quality of the food served.

The problem of sampling in communal restaurants is largely sociological. The first essential is to enlist the interest and co-operation of the staff, without which the work will fail. The first reaction of a cooking staff whose products are to be tested is likely to be hostile, because they feel that the investigation may well result in adverse criticism by an amateur who knows nothing of cooking or of their practical difficulties. The initial step should therefore be to meet the superintendent of the restaurant and the meat and vegetable cooks. They should be told that the object of the investigation is to help them to produce the best possible food from the materials available, and it should be pointed out how consideration of the results may help them to overcome difficulties and get the best out of their plant. They

should be told that copies of the reports will be supplied to them and opportunities for discussion furnished. The technique of sampling should be explained carefully and the samplers personally introduced. If these precautions are taken opposition will be rare so long as the investigator shows sympathy and willingness to help with practical problems.

The attitude of the public must also be considered. Difficulties are likely to arise if attempts are made to sample meals actually in the hands of customers. Sampling at the counter before issue causes no resentment, but no attempt should be made to remove food from people's plates, and all plates sampled at the counter must be returned to the kitchen.

Sampling may often have to be done by people without technical training. It is essential that they should know what they are about; if they are to take samples for ascorbic acid estimation they must be told the kind of foodstuffs in which this is likely to occur and must know its general properties. They must appreciate that tact in handling people is one of their most important qualifications. Those familiar with the pitfalls of sampling are apt to assume that knowledge on this subject is widespread, but it is very necessary that untrained samplers should be told beforehand that they must be ruthless in their impartiality. Perhaps the greatest difficulty has been to persuade samplers not to reject a plate of food they have taken because, for example, the amount of potato seems different from that on other plates they see on the serving table. It is not uncommon to find a sampler sorting over several plates before finding one considered a "good average" or even prodding about in the cabbage to make sure there is no excess of stalk or white heart present.

VARIABILITY OF SAMPLES—As soon as we examine the problem of variability it becomes evident that investigations of communal feeding under present conditions of service cannot give accurate results, however painstaking the sampling. One might have thought that a person serving potato or cabbage day after day with the same sort of spoon would deliver approximately the same amount each time. Table I shows how far this is from being true.

TABLE I

PORTIONS OF POTATO AS SERVED (g), WITH MEANS AND STANDARD DEVIATIONS

Restaurant 1	Restaurant 2	Restaurant 3	Restaurant 4
190	130	230	220
210	130	170	190
190	200	120	240
160	180	270	240
210	160	200	200
300	200	200	Mean 220 ± 23
270	120	Mean 200 ± 51	
Mean 220 ± 54	150		
	190		
	280		
	180		
	200		
	150		
	Mean 175 ± 43		

Weight of each portion = mean of 3 weighings on same day.
Mean of all weighings for the 4 restaurants = 196 g ± 44.

Figures for potato servings at four British Restaurants in Reading are given, each figure being the mean of three individual samples taken on one day. In the longest list of thirteen servings of potato the variation is from 120 to 280 g, with a mean of 175. This mean has a standard deviation of ±43 (coefficient of variability, 25%), and therefore has little value as an index of the amount of potato an individual customer is likely to find on his plate. The exact means for the four restaurants are 219, 175, 198 and 218 g, with an overall mean of 196 g; one might think that this last figure would be fairly representative of what might be called the potato-feeding status of the Reading group of British Restaurants. However, for the 31 results as a whole the standard deviation is ±44 (coefficient of variability, 22%).

Variability in the size of servings evidently sets severe limits to the conclusions to be drawn from investigations of communal feeding. If we could detect systematic trends in the size of servings it might help to clarify the picture. Perhaps early servings might be large, and those at the end of the serving period small; on the other hand, an excess of food might be left to the end so that late customers got big helpings. In my experience, however, systematic variation does not occur, and this observation agrees with the findings of the Oxford Nutrition Survey.[1]

SAMPLING FOR VITAMINS—The problem of sampling for estimation of vitamins is a difficult one, especially for ascorbic acid, where one is necessarily estimating a continuously diminishing quantity. The first essential for interpretation of results is that a study should be made of the technique of serving. This point is illustrated by comparing results obtained by the Oxford Nutrition Survey (Table II) with those obtained at Reading. The Oxford workers observed

TABLE II

VARIATION OF ASCORBIC ACID DURING SERVING PERIOD

(Oxford Nutrition Survey)

	mg of ascorbic acid/100 g	
Time	Potato	Cabbage
11.45	9·3	—
12.15	8·7	—
12.45	6·8	0·8
1.35	23·2	4·7
12.00	4·3	—
12.30	10·6	10·7
12.57	13·6	15·0
1.31	19·9	19·3
12.00	3·3	10·2
12.30	6·5	15·0
12.55	9·1	16·4
1.35	11·6	—

that late servings of potato contained very much more ascorbic acid than early servings, whereas we found apparently random variation within much narrower limits. This was due to the fact that at Oxford cooking of potatoes began quite early in the morning and the cooked vegetables were stored and served to the first customers. Later customers received later batches of potato until those who came at the end had potatoes fresh from the pot. At Reading, potatoes—and indeed other vegetables—were cooked in relatively small batches and everyone had an equal chance of receiving vegetables which were either freshly cooked or had been kept on plates in the hot cupboard. It is evident that, to interpret results obtained from food treated so differently, the procedure adopted by the restaurant must be taken into account; appreciation of the nature and effects of cooking and serving routine is an important aspect of sampling.

An additional variable factor is introduced by technical difficulties in the actual sampling. Bray and Thorpe[2] have stated that, if cabbage is sampled as served, the results from random samples may be 70% from the mean. A portion of cabbage is not a uniform piece of tissue, but the process of cooking leads to a considerable exchange of vitamin C between various parts of the leaf; the worse the cooking the more likely is the distribution of the surviving ascorbic acid to be uniform. Table III shows the distribution of vitamin C in raw and cooked cabbage and illustrates variability between different parts of the leaf.

TABLE III

EFFECT OF COOKING ON ASCORBIC ACID

(mg/100 g in Cabbage)

(Dr. E. Huntsman Mawson, National Institute for Research in Dairying, Shinfield)

	1			2	
	Raw	Cooked		Raw	Cooked
Leaves ..	98	65	Outer	162	85
	85	65	leaves .	161	101
Stalk	123	78	Inner	144	83
	127	76	leaves	130	78
			Stalk	128	84
				115	—

The parts of the vegetable were separated after being cooked together.

Table IV shows replicate analyses of whole and sieved spinach beet, of sieved spinach beet which has been reheated, and of cabbage similarly treated. It is evident that the sieving process has made the sampling more uniform.

TABLE IV

EFFECT OF SIEVING AND RE-HEATING ON SPINACH BEET AND CABBAGE

	Ascorbic acid, mg/100 g		
	Whole leaf	Sieved	Sieved and re-heated
Spinach beet ..	11·2	10·6	8·3
	11·6	11·0	8·4
	9·5	9·8	9·7
	6·3	9·5	9·0
Cabbage ..	44·2	36·8	35·8
	34·6	34·9	26·4
	46·3	37·2	32·2
	31·4	34·3	36·2

It is necessary, therefore, that vegetables should be cut up and well mixed before sampling; if this is done rapidly with stainless implements, the loss of ascorbic acid during manipulation is very small, as the sieving experiment clearly shows. The type of product resulting from institutional cooking makes the mixing operation fairly simple, though cabbage skilfully cooked in the home may be more difficult to deal with.

The sampling of raw salad might seem to be very much more difficult than that of cooked cabbage or potato. It usually consists of lettuce and beetroot containing little ascorbic acid, with tomato and possibly raw cabbage containing a great deal. Even so, however, fairly uniform results can be obtained by cutting up and mixing. Dr. M. Kerly considers that the best way to sample raw salad is to weigh and sample each ingredient separately. This is certainly the method of choice if it is technically possible, but it calls for accurate balances and considerable patience.

Table V gives a summary of the vitamin C content of several sets of three samples of

TABLE V

VARIABILITY OF SINGLE SAMPLES

Ascorbic acid, mg/100 g							
Potato		Cabbage		Mince or stew		Cottage pie	
7·2, 6·0, 5·6	6·3	1·8, 1·5, 3·5	2·2	0·9, 1·7, 1·0	1·2	1·5, 1·2, 1·2	1·3
5·6, 9·3, 8·2	7·7	3·7, 2·8, 4·9	3·8	1·2, 1·5, 1·5	1·4	1·2, 1·0, 2·5	1·6
9·5, 10·8, 7·1	9·1	7·7, 9·0, 9·3	8·7	1·9, 1·9, 1·3	1·7	2·3, 2·0, 2·0	2·1
9·5, 9·8, 9·0	9·4	10·4, 11·6, 10·3	10·8	1·6, 2·1, 1·7	1·8	2·9, 1·2, 2·6	2·2
9·2, 11·1, 17·2	12·5	10·2, 11·6, 10·6	10·8	5·0, 4·2, 4·6	4·6		
14·6, 12·9, 11·9	13·1	6·8, 16·8, 16·8	13·5	6·0, 5·3, 7·5	6·3		

various foodstuffs. Each figure is a single determination and each set of three figures represents the three samples taken in one restaurant on a given day. The question is, are these results so variable as to render the average of each group of three meaningless, or can these averages be regarded as indicating the vitamin C content of the food served on the day in question? The figures obtained for cabbage are evidently the least satisfactory, but there seems little doubt that even with cabbage it is possible to say whether the product is good or bad, and this is, after all, the main object of the work. Experience does in fact show that when bad results have been obtained from a restaurant and the staff have then adopted suggestions for better cooking, the improvement shown by analysis has been marked and immediate. An interesting side-light on the significance of grouped means is the fact that

the monthly averages for mg of ascorbic acid per *portion* of cabbage and potato calculated by the Ministry of Food from country-wide estimations in British Restaurants show the well-known seasonal variations for these vegetables. This is the more remarkable when one realises that not only did the sizes of portions vary enormously but also that the range of ascorbic acid content of a portion of cabbage between the different reports sent in was often from nil up to 60 or 80 mg.

SAMPLING TECHNIQUE—My object so far has been to present general considerations in broad outline rather than to give precise directions for sampling, but I will now describe the technique adopted at Reading. The sampling is done at the serving counter just before the plates are issued to customers. Samplers are instructed to stand well away from the counter, to decide on a given plate, and to take that plate irrespective of whether on examination it appears to be an average serving or not. Each item likely to contain ascorbic acid is transferred to the balance, weighed to the nearest $\frac{1}{4}$ ounce, and scraped on to a saucer. The sample is mashed or teased apart with a fork and quickly mixed and one teaspoonful is put into a weighed screw-capped vial. The vial contains 20 ml of 5% trichloroacetic acid containing 2% of metaphosphoric acid. Various liquids have been used by different workers: 10% trichloroacetic acid, 3% and 5% metaphosphoric acid, 20% metaphosphoric acid, 3% trichloroacetic + 2% metaphosphoric acid, dilute sulphuric acid and oxalic acid have all been used with success.

After the introduction of the sample the cap, bearing the same number as the vial, is screwed tightly on and the whole is shaken vigorously for a few seconds. The bottle is then placed in a dark cupboard, or perferably a refrigerator, until it is time to return it to the laboratory. If samples are treated in this way and are kept in the dark, the ascorbic acid content remains substantially unchanged for 24 hr.[3] The bottle is re-weighed immediately on arrival at the laboratory. Homogenisation of the sample is usually achieved easily by means of a flat-ended glass rod. If difficulty is encountered, the addition of a little sand will break up most foodstuffs. Raw salads are particularly difficult to break up and I have found it best to tip the whole contents of the vial into a small mortar containing a fairly large amount of sand and to grind the semi-solid mass. The liquid phase can be poured off with remarkably little loss. The homogenised extract is centrifuged and generally gives a clear extract. Potato extract remains fairly opaque but this does not interfere with the titration; if coloured extracts are obtained, special analytical methods must be applied.[4]

The collection of a whole meal for determination of constituents other than ascorbic acid is carried out by selecting plates as before in as random a manner as possible. The specimens are placed in a glass jar with a well-fitting lid; on arrival at the laboratory the contents can be homogenised with extraordinary rapidity and efficiency by means of a Waring Blendor.[5] Unfortunately I did not have this apparatus at my disposal, so I minced the whole meal twice through a large culinary mincer and then pounded the resulting mass in a mortar until it became as homogeneous as possible. A 100-g portion of this material was dried in air on a water-bath and kept in a vacuum desiccator over sulphuric acid for 24 hr. The dried material was then ground into as fine a powder as possible. It has been found that replicate analyses on this powder give good results; it contains very little water.

The powdered material obtained in this way has been used for determination of protein, fat, calcium, iron and copper. Meals of an acid nature may pick up iron from the mincer; Widdowson has found[6] that the iron content of stewed apples increased greatly during the manipulation with metal kitchen utensils.

When we remember the great variability in size of portions, it is evident that a single complete meal cannot be a satisfactory sample, and this is illustrated by Table VI. If a

TABLE VI

VARIABILITY OF RESULTS FOR WHOLE MEAL

	Calories	Protein, g	Fat, g
Restaurant A	Mean (8 samples) 690 ± 118	Mean 26 ± 8	Mean 16 ± 7·5
Restaurant B	Mean (9 samples) 620 ± 127	Mean 23 ± 6	Mean 16 ± 5

Calories are calculated by Rubner's factors from analytical results. Means are given with standard deviations.

series of seven meals is taken—for example, one meal per day for a week, as recommended by Mapson[5] and Widdowson[6]—much more reliable figures will be obtained. If the day's servings are not specialised in any way, a series of five meals may be sufficient, but in Table VII it

TABLE VII

ASCORBIC ACID IN WHOLE DAYS' RATIONS

	Canteen 1	Canteen 2
Monday	48	27
Tuesday	33	57
Wednesday	33	62
Thursday	45	74
Friday	25	38
Saturday	91	31
Sunday	80	73
	Mean 51 ± 25	Mean 52 ± 20

Means are given with standard deviations.

will be noted that in the first canteen by far the largest intake of ascorbic acid occurred at the week-end, when very large meals were eaten. The possibility of special menus being associated with special days must be borne in mind.

Certain differences in procedure for sampling whole meals have been recommended by Dr. Kerly and Dr. Mapson, who have kindly permitted me to describe their methods. Kerly dries the bulked sample, without grinding, at 60–70° C., minces the dried mixture and powders it in a coffee mill. The powder is suitable for estimation of riboflavin as well as major constituents. The original sample consists of an average meal made up by weighing portions of each constituent taken from the plates of three different people. Mapson would prefer to calculate the average meal by estimating the total weight of each item issued, subtracting the amount uneaten and dividing by the number of customers. This method demands great skill and a high standard of co-operation from all concerned, especially as customers of communal restaurants are very wasteful and the food left on their plates is often in a well-mixed condition. Mapson's alternative is to bulk three or four meals at a time. He advises the use of the wet mix for vitamin B_1 and carbohydrate estimation, to avoid inaccuracies due to heat treatment.

An ingenious method has been used by Macrae[7] for bulk sampling on a large scale. Five complete meals were collected each day for a week and were stored in enamel pails at −20° C. The frozen mass was ground in a mill, dried in hot air and re-ground to give a very uniform and stable product.

The methods described by Macy[8] really apply to metabolic experiments, but one or two points may be mentioned briefly here. When being sampled for minerals the food is dried with added alcohol in a dish at 60° C. and then ground in a ball mill. For carbohydrate and fat determinations the material is chopped in a food chopper and dried at 40° C. in a vacuum oven, after which it is ground in a mortar. For nitrogen determination the sample is dropped into 1/5 to 1/10 vol. of conc. sulphuric acid in a beaker, which is then heated on a steam-bath overnight. This treatment dissolves the sample, which is made up to volume and used directly for Kjeldahl analysis.

I cannot leave this subject without mentioning the method described by Wiehl[9]; though not strictly a method of food sampling, it is so simple and has been so successful that it should be widely known. A group of people representative of the population under test are questioned individually about their food intake during the previous 24 hr. They are helped to give accurate answers by being able to examine an array of plates containing all the likely foodstuffs in various-sized portions, so that a man can pick out, for example, the slice of bread of the correct size and thickness, spread with the correct amount of butter, that he actually had for his tea. Each plate bears a number and the results are entered on a form, calculations being made from food tables. This method can give most informative results, and the consistency of the values obtained even in small groups of people is remarkable.

Although the methods I have been describing have mainly been used for the analysis of communal meals, they are in general applicable to the ordinary restaurant. Where a free choice of menu exists, however, the "whole meal" analysis loses much of its significance, while on the other hand variability of size of servings is likely to be minimal. However, I consider that in the ordinary restaurant such a survey would give information on the quality rather than the adequacy of the food provided.

I am greatly indebted to Mr. C. J. Regan, Chief Chemist to the L.C.C., for many analyses of major constituents of meals, and to Miss Olliver and Drs. Kerly, Kon, Macrae, Mapson and Pyke for advice and criticism.

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THE PATHOLOGICAL LABORATORY
ROYAL BERKSHIRE HOSPITAL, READING

Some Experiences in the Sampling of Food

By J. KING

(Abstract)

The paper was confined to a consideration of canned foods. Two most important factors in sampling were that the sampler should direct control over the operation and that he should be thoroughly familiar with the processes used in the manufacture of the product.

After describing the causes of the most common faults in canned foods the author gave some examples of unusual faults. The first related to the presence of lead in canned beef, some cans of which had been found to be heavily contaminated owing to the occasional use of spirits of salt in the re-tinning of the irons used for re-soldering up the cans. The second related to mould growth due to perforation of the tin through the accidental use of a sheet of defective tin plate; the third concerned the presence of a phenolic flavour in canned jam caused by contamination with printing ink.

Sampling for Metabolism Studies

By E. M. WIDDOWSON

(Abstract)

The author gave an amusing description of the many pitfalls which await the investigator of human metabolism and of the personal discomforts that must be endured in the search for knowledge of the behaviour of the human body in dealing with the food supplied to it. Proper scientific control of the experimental subjects appeared to present numerous problems.

Metabolism studies generally set out to measure the difference between the amount of some substance entering the body and the amount of it that was excreted. Considerable accuracy was required, and an exact duplicate of all that was eaten and drunk must be analysed. The principle to be adopted in cooking and serving was either to serve and weigh each ingredient of a meal separately or to make a sufficiently intimate mixture of the various components, so that any one helping was likely to be representative of the whole dish. At the end of each meal the duplicate samples were weighed and collected in an aluminium bowl. All cups of tea and coffee were measured and a duplicate sample was measured out immediately and poured into a bottle. The pooled food duplicates were weighed and pulped in a large mortar, and suitable aliquots of food and drinks were taken for analysis.

DISCUSSION

Miss M. OLLIVER confirmed Dr. Mawson's experience that, contrary to the findings of some workers, it was possible to obtain representative samples of cooked cabbage for ascorbic acid estimations. It was possible that some workers failed to appreciate that if different batches of the same vegetable served on the same day had been cooked or kept hot under different conditions, they must be treated as separate samples.

With regard to the preparation of samples for the estimation of ascorbic acid, she suggested that the use of trichloroacetic acid should be avoided, that flasks of about 100 ml capacity should be used instead

of the small vials recommended by Dr. Mawson, that vigorous shaking should be avoided, and that final grinding of the sample in acid should invariably be carried out and in presence of sand. The sampling of salad for ascorbic acid assay was certainly difficult, but satisfactory results had been obtained by disintegrating a reasonably large quantity in presence of 20% metaphosphoric acid in a Waring Blendor. When using the Waring Blendor for mixing meals it is most advisable that the glass container should be filled only to about $\frac{1}{3}$ or $\frac{1}{2}$ capacity.

Mr. W. B. ADAM said that in making bacteriological tests on canned foods evidence from the examination of seams should always be included, but it should be realised that the recommended "standard" double seam was not perfect. It might allow very slight leaks on cooling or storage in a small proportion of cans. Experience at Campden had shown that hydrogen swells might develop rather rapidly in canned beetroot but seldom in canned carrots unless in lacquered cans. Seasonal trends in vitamin C content were very noticeable in canned potatoes; it ranged from about 12 mg per 100 g in October to November to about 3 to 4 mg in May.

Mr. R. L. EDWARDS asked if the lead in Mr. King's corned beef was mainly round the edges of the can, or was it evenly distributed. Large amounts of lead had been found along the edges of cans of beef stock, giving erratic results on sampling. All trouble in using the material was avoided by leaving a layer of stock round the sides of the tin when the melted stock was poured out, instead of scraping it all out; any lead from the solder was left in this layer.

Mr. G. E. FORSTNER said that canned beef had often been found to have a high lead content. This was not limited to any one brand or delivery; it had been found at different times and in samples taken from different parts of the country. The portion with the highest lead content was that next to the stuffing hole; the amount decreased appreciably towards the centre, but 10 p.p.m. of lead had been found in the centre of a 6-lb. block of meat.

Dr. MAGNUS PYKE said that the method of surveying food consumption to which Dr. Mawson referred and with which he had coupled his (the speaker's) name could not claim to be food sampling, nor was there any originality in using it. It had been very widely employed in America, principally by Wiehl (see *Millbank Memorial Fund Quarterly*, 1942, **20**, 61). The method was devised to overcome the difficulty to which Dr. Mawson had referred of obtaining for analysis meals which people proposed to eat. Two other popular methods of food survey had been devised to overcome this fundamental difficulty, which was not met with in institutions or canteens where representative specimens could be taken for analysis, but which was, of course, inherent in the study of people domestically or people in various types of industry. It was obvious that one could not take away for analysis a coalminer's dinner which he might be carrying in his pocket. The first and most popular method was that of budgetary survey. All the food going into a household over a period of a week was recorded, its nutritional value was assessed and the total was divided by the total strength of the household combined on a *per capita* basis, where the man was taken as 1, his wife as 0·8 and the children as diminishing fractions down to 0·5.

A second method, mentioned in passing by Dr. Widdowson, required that the subjects under study should weigh and record every single morsel of food and drink they consumed. Moss (*Proc. Roy. Soc.*, 1923–24, **95**, 181), with singular courage, had applied this method to coalminers, but complained of the difficulty of finding men sufficiently co-operative to carry with them on the surface, underground, at home and in the public house a spring balance for the conscientious weighing of their food and drink. Both these two methods suffered from the disability that the subjects under study were not for the full survey under the control of the investigator.

The method to which he wished to refer, although it itself had disadvantages of its own, did at least bring the subject under the more or less complete control of the investigator during the period of the study. Nevertheless, there was in its successful application a certain element of skill, and it was always the object of scientific workers to reduce the element of skill to a bare minimum, so that they could claim to be completely objective. Wiehl's method involved the interrogation of each subject in a factory or elsewhere about every single item he had eaten during the previous 24 hours. Whenever an item was mentioned, a similar quantity was taken from the same type of food, which must be available at the interview, and this amount was immediately weighed. The method, of course, depended on a reasonably accurate memory on the part of the subject and on a general knowledge on the part of the investigator of the type of diet which the people under study did actually eat. The whole must, of course, also be based on proper analytical studies of the types of food consumed. In certain parts of the country, for example Lancashire, foods strange to the eye of a student from, say, London might be encountered, and if these were also unknown to the standard food tables they must be analysed.

Mr. KING, replying, said that in his experience the highest lead content for canned beef was always found near the stuffing hole.

Dr. MAWSON, in reply to Miss Olliver, said that he had never found any difficulty in the use of trichloroacetic acid, but suggested that oxalic acid might also be very effective. He found metaphosphoric acid difficult to work with, but understood that sodium metaphosphate acidified with hydrochloric acid was very useful. He had not found that vigorous shaking of the sample introduced any serious error and considered that the extra oxidation due to air in the sample vessel was likely to be unimportant.

Mr. S. E. MELLING, President of the Society, said that it was his first visit to a Joint Meeting with the Food Group, and it had proved a most enjoyable and interesting experience. He congratulated all the speakers, particularly Miss Widdowson, on the excellence of their papers, and hoped that on a future occasion it might be possible to discuss the relation of sampling to the provisions of the Food and Drugs Act.

The Determination of Free Chlorine and of Chloramine in Water using *p*-Aminodimethylaniline

By A. T. PALIN

The ortho-tolidine method has long been accepted as standard for the determination of chlorine or chloramine in water. With the widespread adoption of chlorination for disinfection purposes, it is probable that tests for residual chlorine are made more frequently than any other tests in the routine control of water purification. Recent work has shown that residual chlorine itself is not a dependable index of germicidal power. It is pertinent to ascertain the form in which the chlorine is present, whether as free active chlorine or in combination with ammonia and other nitrogenous matter, as chloramines.[1]

It has been shown that it is the free chlorine which is most desirable for the disinfection of water. The standard *o*-tolidine test measures free chlorine, and also the less active chlorine combined with ammonia and amino-compounds, and may show false residuals due to interfering substances. In addition, reproducible colours cannot always be obtained, particularly for values of chlorine less than 1·0 p.p.m.,[2] so that the desirability of more specific indicators has long been recognised.[3]

Several methods have been suggested for the differentiation of free chlorine and chloramine, but comparative studies have failed to give concordant results.[4] The Laux–Nickel neutral *o*-tolidine test[5] appears to be specific for free chlorine, but the colours formed may fade rapidly; moreover, the test is not sensitive to residuals of less than 0·2 p.p.m. An amperometric method has been developed by Marks and Glass,[6] and it is claimed that accurate determinations can be made of the amount of free chlorine and chlorine combined with nitrogenous compounds, when present, in a mixture.

In the method now presented use is made of *p*-aminodimethylaniline. This indicator has been proposed by Kolthoff and others,[7,8] as a test for chlorine. The method was investigated in 1936 by Haase and Gad,[9] who found a solution of the reagent in hydrochloric acid to be sensitive to 0·01 p.p.m. of chlorine, but the test was subject to interference by iron, manganese and nitrites. Using the reagent in phosphoric acid soln. gave better results, but interference was not completely prevented. Later work by Byers and Mellon[10] in 1939 indicated that the characteristic colour was given immediately by chlorine, but that with chloramine maximum intensity developed after 6 to 7 min. Tests were carried out in the *p*H range 2·6 to 4·5. The colorimetric standards prepared from acid methyl red solns. originally proposed by Haase and Gad were modified. It was found that iron increased the colour intensity, and with nitrites errors were caused in the opposite direction. They concluded that the method did not appear to have any advantage over the *o*-tolidine method. Standards prepared from acid methyl red and also from dilute potassium permanganate solution have been proved to be relatively unstable and unsatisfactory.[4]

Attention was again focused on the *p*-aminodimethylaniline method by Moore[11] in connection with the determination of the break-point in the chlorination of water. By buffering the sample to be tested to *p*H 6·0, at which *p*H chloramines decompose comparatively slowly, a red "flash" colour was obtained on addition of the reagent when free chlorine was present. On adding a few drops of 10% phosphoric acid, so as to lower the *p*H to 4 or less, chloramines gave a red colour, although this reaction was somewhat slower than the preceding one. *p*-Aminodiethylaniline may also be used, but the dimethyl derivative was found to be more sensitive. Moore placed his test on a semi-quantitative basis by using standards prepared from basic fuchsin and copper acetate.

The present investigation was undertaken to place the reaction on a precisely quantitative basis, and to develop a practical colorimetric method for estimating and for differentiating between chlorine and chloramines, and one not subject to interference by the presence of any other constituent likely to be present in a water.

The method is based on the following facts discovered during the investigation—(1) iodine and chlorine in equivalent amounts give the same colour with *p*-aminodimethylaniline; (2) when the water is buffered to a suitable *p*H, chlorine, but not chloramine, gives a red colour with *p*-aminodimethylaniline. In presence of potassium iodide, and at the same *p*H, the red colour due to chloramine develops. Colour development in both reactions is instantaneous.

REAGENTS—(1) A 0·2% soln. of pure *p*-aminodimethylaniline hydrochloride in industrial methylated spirit (kept in amber bottle). (2) 0·5 *M* phosphate buffer solution, *p*H 6·8, prepared by dissolving 35·5 g of anhydrous Na_2HPO_4 (AnalaR) and 34·0 g of KH_2PO_4 (AnalaR) per litre. The mixture was allowed to stand for several days and any ppt. filtered off (the ppt. appeared mainly to be due to iron). (3) Solid potassium iodide. (4) Dil. iodine soln. (1 ml ≡ 0·05 mg of Cl_2) prepared as required from *N*/10 iodine solution.

METHOD—(*a*) *Chlorine*—Place 100 ml of the sample in a 100-ml Nessler tube containing 2 ml of buffer soln. and 0·5 ml of *p*-aminodimethylaniline soln. Mix, and match the colour immediately by running the standard iodine soln. from a burette into a second 100-ml Nessler tube ("control" tube) containing the same amts. of buffer soln. and *p*-aminodimethylaniline soln. in distilled water. After each addn. of iodine mix with a "plunger type" stirrer. If more than 0·5 p.p.m. of chlorine is present, take less of the sample and place it in a Nessler tube containing the reagents and sufficient distilled water to give a final vol. of 100 ml.

(*b*) *Chloramine*—Put a crystal of potassium iodide in the sample tube, mix, and again match the colours by further addition of iodine to the control tube. The additional iodine soln. required represents chloramine-chlorine. Here, again, if more than 0·5 p.p.m. is indicated, colour matching is made easier by taking less of the sample.

Total available chlorine—The total amount of iodine soln. used gives the total available chlorine.

It is important to make the estimations as quickly as possible.

COMPARISON WITH EXISTING METHODS—*Chlorine*—Solutions of chlorine were prepared in ammonia-free distilled water and the chlorine in p.p.m. was determined.

Method	A	B	C	D
Iodimetric	0·49	0·29	0·12	—
o-Tolidine	0·5	0·3	0·14	0·05
p-Aminodimethylaniline ..	0·48	0·27	0·15	0·07

Chloramine—Solutions of chloramine were prepared in distilled water containing excess of ammonium salt, and the chloramine in p.p.m. was determined.

Method	A	B	C	D
Iodimetric	0·70	0·30	0·12	—
o-Tolidine	0·7	0·3	0·15	0·06
p-Aminodimethylaniline ..	0·68	0·33	0·15	0·08

The iodimetric method was carried out according to the procedure recommended by a Committee Report on Control of Chlorination, Oct. 1943[12] (*i.e.*, starch-iodide method carried out in acetic acid soln.). This method is not sufficiently sensitive for the accurate determination of residuals below 0·1 p.p.m.

Estimations by *o*-tolidine were made according to the improved method of Chamberlin and Glass[13] and recommended by the same Committee. The standard *o*-tolidine test, when used for estimating chlorine or chloramine in distilled water, gave high results; these appeared to be due to the increased acidity of the final colour mixture. These authors have shown that in the *o*-tolidine test increasing the acidity increases the intensity of colour.

The standard *o*-tolidine test gave results agreeing well with the iodimetric and *p*-aminodimethylaniline methods for estimations on natural waters subjected to chlorine and chloramine treatments.

INTERFERING SUBSTANCES—Moore[11] has pointed out that at *p*H values less than 6·0 ferric iron and manganic manganese interfere, and that chloramine, as well as chlorine, will give a colour. Interference was largely eliminated by buffering the samples to *p*H 6·0 (or slightly above), although in his test for chloramines, which depends upon acidifying to *p*H 4·0 or less, interference from these sources would not be eliminated.

In the present study it was found that there was a slow production of colour with chloramine at *p*H 6·0 (*i.e.*, with potassium iodide absent) and also that the reagent was still sensitive to oxidised manganese. These sources of error were avoided more completely by buffering the samples to *p*H 6·8. Buffering to higher *p*H values reduces interference to negligible proportions, although the test becomes less sensitive.

Rate of Formation of Colour with Chloramine (*KI absent*)—The following results were obtained with solns. of chloramine in distilled water. In both tables "Residual p.p.m." represents the chlorine residuals of the solns. found, as described above, after addition of

potassium iodide, whilst below are given the chlorine equivalents of the colours produced without addition of potassium iodide after 1, 2 and 3 min.

	At *p*H 6·0			
Residual p.p.m.	0·50	0·45	0·24	0·11
After standing 1 min. ..	0·03	0·02	0·02	0·01
,, ,, 2 ,, ..	0·06	0·05	0·03	0·02
,, ,, 3 ,, ..	0·08	0·07	0·05	0·02

	At *p*H 6·8		
Residual p.p.m.	0·57	0·26	0·08
After standing 1 min.	0·02	0·01	—
,, ,, 2 ,, ...	0·04	0·02	—
,, ,, 3 ,, ...	0·05	0·03	0·01

Oxidised manganese—The following results were obtained with colloidal solns. of manganese dioxide. The figures in the top row are actual manganese contents, and the figures given below are the chlorine equivalents of the colours produced in the proposed test. Addition of potassium iodide did not increase the interference caused by manganese.

		*p*H 6·0		*p*H 6·8			*p*H 7·4
Manganese, p.p.m.		0·1	0·5	0·1	0·2	0·5	0·5
Equiv. to chlorine	1 min.	0·03	0·19	0·01	0·03	0·06	0·03
(p.p.m.) after	2 ,,	0·05	0·25	0·02	0·05	0·08	0·05
standing	3 ,,	0·06	0·28	0·03	0·06	0·09	0·06

At *p*H 8·5 there was no interference by 2 p.p.m. of oxidised manganese. At high *p*H values, however, there was gradual fading of the colour with chlorine on standing.

The results indicate that no significant errors are caused by amounts of manganese up to 0·2 p.p.m., and interference by greater concentrations can be overcome by suitable choice of *p*H. Where phosphate buffers (*p*H over 7·0) are used with hard waters, precipitation is prevented by adding one or two drops of 10% Calgon soln. In the *o*-tolidine test oxidised manganese in excess of 0·01 p.p.m. interferes.

Ferric iron—With 5 p.p.m. there was no colour after 5 min. standing. Over 0·3 p.p.m. interferes with the *o*-tolidine test.

Nitrites—With 100 p.p.m. (as N) there was no colour after 5 min. Nor was it found that the presence of nitrites decreased the colour intensity with chloramine. Significant amounts of nitrite will not exist in water containing free chlorine.[14] Amounts over 0·1 p.p.m. interfere with the *o*-tolidine test, unless it is carried out in total darkness.

Dissolved oxygen—Moore found that with increasing *p*H value, dissolved oxygen became a more active oxidising agent and gave a colour equivalent to that given by 0·05 p.p.m. of chlorine in 3 min.

This possible source of error was investigated by taking samples of water saturated with oxygen at 16° C. and buffered to *p*H values of 6·0, 6·8, and 8·0. In each test the colour produced even after 5 min. contact with the *p*-aminodimethylaniline reagent was equivalent to 0·03 p.p.m. of chlorine, comparisons being made with controls buffered to the same *p*H values and to which the reagent was added just before matching. For a contact of 1 min. errors were negligible.

Colour and Turbidity—The interference caused by natural colour or turbidity can be overcome by using as the control a sample of water taken before chlorination. If chlorination itself causes a reduction of the natural colour, as may happen in break-point chlorination, the sample of water taken before chlorination may be suitably diluted with distilled water for matching.

Phenols—With 10 p.p.m. of phenol there was no interference for concentrations of chlorine or chloramine up to 0·8 p.p.m. The red colour, first formed, gradually acquired a bluish tinge on standing, due to the slow formation of indophenol.

PRECISION OF THE METHOD—In the range 0 to 0·5 p.p.m. of chlorine the higher residuals can be reproduced to within 0·02 p.p.m. and the lower residuals to within 0·01 p.p.m.

CHOICE OF REAGENT—It is important to have enough *p*-aminodimethylaniline present, otherwise bleaching may take place, giving an apparent chlorine content lower than the true

value. The continued addition of chlorine to a soln. of *p*-aminodimethylaniline will lead to decolorisation of the colour first formed. The colour produced has been attributed to a meriquinone known as Wurster's red, formed by the oxidising action of the chlorine. The decrease in colour caused by excess of chlorine is probably due to the formation of some quinone.[10]

p-Aminodimethylaniline hydrochloride is readily soluble in alcohol, and water, but only sparingly soluble in acetone. Moore used a saturated soln. of the hydrochloride in acetone and added 0·5 ml to 50 ml of sample. It was found that with this reagent a residual of 0·5 p.p.m. of chlorine required approx. 0·75 ml of reagent to 50 ml of the sample for full colour production. The indicator may be used in aqueous soln. with satisfactory results. An alcoholic soln. was used in the present investigation, since, according to Scott,[15] alcoholic solns. permit less decomposition. The soln. used was stable over a period of at least two months.

Effect of *p*H on the Determination—For a given amount of chlorine or chloramine the colour intensity is at its maximum at a *p*H of about 4·5, but both chlorine and chloramine give colours at this *p*H, and there may be considerable interference from manganese, iron, etc. It has been shown that interference by oxidising agents is decreased with rise in *p*H, but at the same time the sensitivity of the test is decreased. The most suitable *p*H was 6·8 (except where the oxidised manganese content of water was unusually high), but it is important that both the sample and control should be buffered to the same *p*H. If this is done, then no matter at what *p*H the test is carried out, the figures obtained will represent the total available chlorine. (Total available chlorine is defined as the amount released on acidification.)

The following results show the values obtained when estimations on the same sample of chloraminated water were made with the sample buffered to different *p*H values but compared with a control buffered always to *p*H 4·5.

*p*H	4·5	6·8	7·4	7·7	8·2	8·6	8·8	9·0	9·2
Residual, p.p.m.	0·63	0·54	0·45	0·41	0·38	0·23	0·15	0·08	0·06

The total available chlorine was 0·63 p.p.m. At *p*H 6·8 for both control and sample the amount was 0·61 p.p.m.

If the colour obtained with a sample at *p*H 4·5, as determined with a control a *p*H 4·5, is taken as representing total available chlorine, as a standard value, then a determination with the sample at another *p*H (*e.g.*, that of the sample itself) and the control at *p*H 4·5 will represent chlorine available at that other *p*H. The method given may thus be modified by substituting in the control a 0·5 *M* potassium dihydrogen phosphate soln. (*p*H 4·5) for the *p*H 6·8 buffer soln. and using no buffer in the sample. The error caused by the depression of *p*H due to the addition of the *p*-aminodimethylaniline hydrochloride is generally insignificant except in waters of low alkalinity. In such circumstances less indicator may be used so long as there is sufficient to ensure full colour production.

Field Test—The methods so far given are essentially laboratory methods. For the routine control of water chlorination, where plant attendants may be required to make regular tests for residual chlorine a simpler procedure is desirable. Various types of colorimeters and permanent colour standards are available for the *o*-tolidine test, and the performance of the residual test becomes a relatively simple matter. There is no permanent colour standard available for the *p*-aminodimethylaniline method, but it was observed that the B.D.H. Lovibond Nessleriser Disc for Nitrites gave a fairly good match, and this was calibrated by means of the standard iodine soln. The following method gave results sufficiently accurate for routine work.

Total available chlorine or chloramine—Put 50 ml of the sample in a Nessler tube containing 1 ml of buffer soln. *p*H 6·8 and 0·25 ml of indicator soln. Match in the Nessleriser, using a blank of untreated water and Nitrites Disc. Divide the number appearing on the disc by 4 (ignoring the gamma) to get the amount of chlorine in p.p.m. Then add potassium iodide to obtain the chloramine.

For residuals over 0·2 p.p.m. Cl_2 it will be found necessary to take less of the sample.

Chlorine or chloramine available at the pH of the water—The procedure is similar to that described above, but with omission of the buffer solution. The number on the disc is divided by $4\frac{1}{2}$ to obtain chlorine in p.p.m.

Discussion—The proposed method has many advantages over the standard *o*-tolidine method. Free chlorine and chloramine can be estimated separately, and the only possible source of error that need be considered is that due to oxidised manganese in amounts greater

than 0·2 p.p.m. This interference is prevented by suitable adjustment of the *p*H at which tests are carried out.

The suggested modification for the estimation of chlorine or chloramine available at the *p*H of the water itself, should be of value in the control of water chlorination. It is known that sterilisation becomes less efficient at higher *p*H values, and it is reasonable to assume that there is a close relation between the germicidal efficiency at a particular *p*H and the amount of chlorine or chloramine available at the same *p*H.

It is also to be noted that in the method given for estimating total available chlorine, it will generally be found that the buffer soln. can be omitted if the *p*H of the water is in the range 6·8 to 8·0, and if the untreated water is used as the control.

The treatment known as Break-point Chlorination, in which chlorine is applied in sufficient amounts to give, after a suitable contact period, residuals of free chlorine, is easily controlled by the *p*-aminodimethylaniline method. The course of the chlorine reaction with ammonia and amino-compounds can be followed. The method would seem to be of particular value in estimating residuals in chlorinated sewage and sewage effluents, where existing methods may be subject to considerable interference.

SUMMARY—A study of the reaction between *p*-aminodimethylaniline and chlorine has shown that it may be developed into a practical colorimetric method for the estimation of chlorine and chloramines in water. If chlorine and chloramines are both present they may be estimated separately.

The possible sources of error have been fully investigated and the method, modified where necessary, can be regarded as free from interference.

In conclusion I wish to thank Mr. N. J. Pugh, C.B.E., M.Inst.C.E., Water Engineer and Manager, for his interest in this work, and, with him, the Chairman and Members of the Waterworks Committee, for their permission to publish the results.

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CITY OF COVENTRY WATER DEPARTMENT *December*, 1944

A Rapid and Simple Method for the Determination of Calcium in Presence of Strontium and Barium

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(*Read at the Meeting, May 2*, 1945)

THE accurate determination of calcium, often present in small amounts as an impurity in barium and strontium salts, is frequently required, especially in pyrotechnics, *e.g.*, calcium in strontium peroxide powders. In view of this it is surprising to find that the method most commonly used is that of Röse and Stromeyer,[1] published as far back as 1860; this method is based on the solubility of calcium nitrate and the insolubility of barium and strontium nitrates in a mixture of abs. ether and alcohol. Treadwell[2] reports on this method that the strontium and barium almost invariably contain some calcium, and the separation must be repeated for accurate work. A modification was suggested by Rawson[3] in 1937, in which nitric acid of sp.gr. was used as a solvent for the calcium nitrate. Noll[4] in 1931 stated that this method

is to be preferred to the use of the ether-alcohol mixture, and this is confirmed by Harwood and Groves,[5] although they suggest an acid of sp.gr. 1·4555, as it dissolves more calcium nitrate. Even these authors, however, do not claim that the strontium and barium are completely from calcium. Yet another method was proposed in 1909 by Bray and Swift.[6] This was based on the pptn. of the barium as chromate from an acetic soln. of the three elements, while the strontium was recovered from the filtrate as chromate by addition of ammonia, and the calcium as oxalate in this filtrate. All the ppts. contain considerable amounts of the other elements, and at least two pptns. had to be made at each stage; this method was therefore tedious and open to error. All the methods mentioned above require the salts to be present in a soluble form and are useless if, as frequently occurs, they are present as sulphates.

Many authors state that barium and strontium sulphates are insoluble in acids, except to a minute degree (ignoring here the well-known solubility of barium sulphate in boiling conc. sulphuric acid), but it has been overlooked that both compounds are entirely and easily soluble in boiling perchloric acid (60%), as also is calcium sulphate. On dilution, however, the barium sulphate is re-precipitated immediately, the strontium sulphate comes down only after vigorous stirring, and the calcium sulphate remains entirely soluble and may be filtered off and determined as oxalate in the filtrate. Advantage has been taken of these facts to separate calcium from admixture as the sulphate with barium and strontium sulphates either together or alone. If the elements are present as other compounds, conversion into the sulphates is usually a simple matter. Expts. are in progress to ascertain the optimum conditions for the separation of barium and strontium sulphates from each other.

Experimental

(A) Separation of Barium and Calcium Sulphates—Pure specimens of barium and calcium sulphates were prepared from barium chloride, calcium carbonate and sulphuric acid, all of analytical reagent quality, and were used in the following expts.

Expt. No. 1—To 0·5 g. of barium sulphate in a 150-ml tall-form beaker and 0·5 g of calcium sulphate in a similar beaker were added respectively 30 ml of perchloric acid (60%), and the solns. were boiled and evaporated to 10 to 15 ml. Both solns., which were quite clear, were diluted to 150 ml. The barium soln. gave a heavy white ppt., but the calcium soln. remained absolutely clear. The barium sulphate was filtered off and determined in the normal way, but only 96% was recovered; the missing 4% was found in the filtrate.

Expt. No. 2—Barium sulphate (0·5 g) in a 150-ml tall-form beaker was treated with 30 ml of perchloric acid (60%), and the soln. was boiled down to 10 to 15 ml. The soln. was perfectly clear, but when it was diluted to 130 ml a heavy white ppt. was formed. Five ml of diluted sulphuric acid (1+1) were added, the solution was boiled, allowed to stand overnight and filtered, and the barium was determined as usual. The recovery was 100%. Several more expts. on these lines confirmed the findings that no re-pptn. of the calcium sulphate took place on dilution and that a 100% recovery of the barium sulphate could be made if a little sulphuric acid was added after dilution; recovery was low by about 5 to 10% if no sulphuric acid was added. On treating 0·5 g of calcium sulphate in a similar manner no pptn. was observed, even after addition of the sulphuric acid.

Expts. Nos. 3–7—As a result of the foregoing expts. mixtures of barium and calcium sulphates in varying proportions were treated with 40 ml of perchloric acid (60%), evaporated to 20 ml and diluted to 150 ml; 1 ml of diluted sulphuric acid (1+1) was added, the solns. were boiled, allowed to stand overnight and filtered through a double No. 542 filter, and the ppts. were washed free from acid, dried, ignited and weighed. The results are given in Table I.

Table I

Taken, g		
Barium sulphate	Calcium sulphate	Found, g Barium sulphate
0·01	0·99	0·0104
0·50	0·50	0·507
0·05	0·95	0·051
1·00	1·00	0·997
0·25	0·75	0·256

The calcium was recovered from the filtrate by adding a slight excess of ammonia, boiling

and adding ammonium oxalate soln. The ppt. was filtered off, washed, ignited and weighed as calcium oxide. Results agreeing with the theoretical were always obtained.

It will thus be seen that by this method not only small amounts of calcium in presence of barium may be determined, but also small amounts of barium in presence of large amounts of calcium.

(B) SEPARATION OF STRONTIUM AND CALCIUM SULPHATES—The greatest difficulty was encountered in preparing a specimen of strontium sulphate free from barium or calcium, since no salts of analytical reagent quality were obtainable and the salts from all the British sources tried were contaminated. At length, however, a sample of reasonably pure strontium bromide was obtained and from this strontium sulphate was prepared.

Expt. No. 8—Three separate portions of 0·5 g of strontium sulphate in 150-ml beakers were attacked by boiling with 40 ml of perchloric acid (60%). The first was removed as soon as the salt was in solution; the second was evaporated to 20 ml, and the third to 10 ml. Then all were diluted to 150 ml. No. 1 remained clear, No. 2 gave a slight deposit only, and No. 3 gave a fairly heavy deposit; this was filtered off and found to contain only 36% of the strontium sulphate. It thus became clear that it would probably be necessary to add sulphuric acid.

Expt. No. 9—Strontium sulphate (0·5 g) was "fumed down" with 20 ml of perchloric acid (60%) until salts appeared. The soln. was then cooled and diluted with boiling water to 200 ml, 10 ml of diluted sulphuric acid (1+1) were added, and the soln. was boiled for 5 min. and allowed to stand overnight. It was washed with 5% sulphuric acid, dried, ignited and weighed. The weight of strontium sulphate recovered was 0·496 g. This result was confirmed very closely by repeated trials; the slightly low result may be due to traces of calcium present or to the slight solubility of strontium sulphate in the wash water.

Expts. Nos. 10–12—Samples (1 g) of strontium and calcium sulphates in varying proportions were "fumed" with 40 ml of perchloric acid (60%) until salts appeared. The solns. were cooled, and diluted with boiling water to 200 ml, 10 ml of diluted sulphuric acid (1+1) added, and the soln. was boiled, stirred for 5 min. and allowed to stand for 2 hr. The ppts. were then filtered off on a double Whatman No. 42 paper, washed with 5% sulphuric acid, dried, ignited and weighed as strontium sulphate. The results are given in Table II.

TABLE II

Taken, g		Found as strontium sulphate, g	Recovery %
Strontium sulphate	Calcium sulphate		
0·50	0·50	0·492	98·4
0·10	0·90	0·098	98·0
0·90	0·10	0·891	99·0

It will be observed that in each test the strontium found was slightly on the low side, and the amount of calcium recovered from the filtrate was correspondingly high. It has not yet been possible to obtain a specimen of strontium salts free from calcium entirely, so that it is not certain if this is a genuine loss of strontium or merely calcium which is present as an impurity.*

Expts. Nos. 13–15—Three samples of strontium peroxide, which had been tested elsewhere for calcium content by the ethyl-alcohol method and also spectrographically, were submitted for checking, as serious differences between the two methods had been found. Two-g portions of the samples were evaporated to dryness with 20 ml of diluted sulphuric acid (1+1) and baked to drive off fumes. The resulting powder was treated with 30 ml of perchloric acid and boiled for 30 min. but did not become clear, as expected; the soln. was then diluted to 100 ml and 10 ml of diluted sulphuric acid (1+1) were added, and the soln. was boiled and allowed to stand for 3 hr. The ppt. was filtered off on a double No. 40 Whatman paper, and the calcium was determined as oxalate in the filtrate by the method given under BARIUM. Results are given in Table III.

Expt. No. 16—Calcium, barium and strontium sulphates (0·5 g of each) were dissolved in 50 ml of 60% perchloric acid, the soln. was "fumed" to dryness, and the residue was diluted to 200 ml with boiling water. Ten ml of diluted sulphuric acid (1+1) were added, the soln.

* For technical reasons the purity of the products obtained has not yet been checked spectrographically.

was boiled, left overnight, and filtered through a No. 4 porosity sintered glass crucible containing a pulp pad, and the residue was washed free from acid. The calcium was recovered from the filtrate as oxalate and calculated as calcium sulphate; recovery = 0·501 g.

TABLE III

	Calcium spectrographically %	Calcium by ethyl-alcohol method %	Calcium by perchloric acid method %
Sample A ..	—	1·25	1·26
,, B ..	0·54	0·82	0·826
,, C ..	1·41	2·18	2·12
			2·19

SUMMARY—Details have been given of a new method for the separation of calcium salts from either strontium or barium salts, or from mixtures of the two. Experimental work shows that a good order of accuracy is obtainable.

These expts. were carried out in the laboratories of Messrs. Internationals Alloys, Ltd., Aylesbury, and thanks are due to the directors for permission to publish. I also wish to thank my colleague, Mr. C. Jukes, who first pointed out to me the phenomenon of the solubility of barium sulphate in perchloric acid, and Mr. W. H. Bennett, M.Sc., F.R.I.C.

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January, 1945

DISCUSSION

Dr. B. S. EVANS asked the author if he had tried the method as a means of purification of precipitates (obtained, *e.g.*, in the determination of sulphur in steel). Whereas years ago barium sulphate had been regarded as the pure precipitate *par excellence*, work done during, say, the past twenty-five years had shown that it was liable to extensive contamination by an ever-growing number of substances—iron, sodium sulphate, sodium chloride, barium chloride, to name only a few. Of course, nothing could be done about barium contamination, but the author's method seemed to show promise of providing a method of purification from, *e.g.*, iron or alkalis, which might be of considerable use.

Mr. R. C. CHIRNSIDE congratulated the author on having devised such an elegant separation of calcium from strontium and barium. He asked if there had been any opportunity of investigating the effect of lead, if present, on the separation. Mr. Osborn had mentioned that the separation of barium and strontium was not so readily effected because of a tendency to the formation of mixed crystals. If the sulphates were analogous to the carbonates, and this seemed likely, he could understand this difficulty. Barium and strontium carbonates readily formed mixed crystals, but the normal form of calcium carbonate was not isomorphous with the other two and one would therefore expect calcium to be more readily separated.

Mr. D. A. LAMBIE asked if the low strontium results might not be due to the solubility of strontium sulphate, which was greater than that of barium sulphate; quantitative results obtained with the latter in sulphate determinations might be due to a compensation of errors.

Mr. J. HASLAM said that the method was likely to be very useful in determining barium and strontium as sulphates. In some of the published methods of separation the recovery of the strontium was not quantitative. He asked whether in these methods any steps had been taken to ascertain if the calcium oxalate precipitated from the filtrate was pure, *e.g.*, by weighing as the monohydrate and subsequent titration of the oxalate radical.

Mr. OSBORN, replying to Dr. Evans, said that he had had no experience in the purification of barium sulphate but thought that the method might prove useful for removing elements which formed soluble perchlorates. Replying to Mr. Chirnside, he said that he would investigate the effect of lead on the separation.* Up to 80% of strontium could be recovered from mixtures containing an equal quantity of barium. He was aware of the danger of the solubility of strontium sulphate, referred to by Mr. Lambie, and had therefore reduced washing to the minimum and had used dilute sulphuric acid. Quantitative results could not be due to compensation, as in the expts. barium was entirely recovered. He agreed with Mr. Haslam that it was desirable to test the purity of the ppts. spectroscopically or chemically; for technical reasons it had not yet been possible to do this.

*Expts. made while this paper was in the press have shown that lead sulphate behaves like barium sulphate in being soluble in perchloric acid; it is pptd. to the extent of 80 to 85% on dilution, and requires addition of a little sulphuric acid to ensure complete recovery. Thus the method may be used for the separation of calcium and lead sulphates, but lead must be allowed for when separating barium and strontium sulphates.

Erratum: ESTIMATION OF THE FRESHNESS OF CANNED SALMON—January issue, 1945, p. 16, lines 23 to 25. *For* "This, however, does not invalidate Fisher's test for straightness of regression lines, ... populations," *read* "This, however, probably does not seriously invalidate Fisher's test for straightness of regression lines, or, in this case, straightness of line of means, although it should be observed that this test requires that the populations tested should be normal and that the pooled variances should be drawn from populations having the same standard deviation."

Notes

THE ESTIMATION OF DRIED EGG IN FRUIT CURD

IN December, 1944, the Ministry of Food requested licensed fruit curd manufacturers to sign an undertaking to conform to the following minimum percentage standard for "Fruit Curd"—Fat, 3·0; citric acid, 0·33; oil of lemon, 0·125 or oil of orange, 0·188; dried egg, 2·0 or shell egg, 6·0. Water, flour (or other starch equivalent) and sugar may be used as required, the statutory minimum of 65% of soluble solids (S.R. & O., No. 842, 1944) being borne in mind. For "Bakery Fruit Curd" the following alternative minimum percentage standards are applicable—(*a*) fat, 6·0; no egg; (*b*) fat, 5·0; dried egg 1·0 or shell egg, 3·0; (*c*) fat, 4·0; dried egg 2·0 or shell egg, 6·0. An enquiry was received from the Ministry as to whether a sufficiently accurate method for the estimation of egg in fruit curd was known, and this led to an investigation in these laboratories of the problem and the adoption of the following method. Extract 20 g of the curd with 100 ml of 95% alcohol in a boiling water-bath under a reflux condenser for about 6 hr. and leave overnight. Filter and re-extract the residue as above. Evaporate to dryness the combined alcoholic extracts, destroy organic matter with sulphuric and nitric acids, and determine the P_2O_5 by precipitation with molybdic acid soln. according to the official method for fertilisers and feeding-stuffs.[1] The method of filtering off the phosphomolybdate ppt. advocated by Dodd,[2] was found convenient. Wash the ppt. with 1% nitric acid followed by 1% ammonium nitrate soln. until free from acid and then two or three times with water. Dissolve the ppt. in excess of 0·1 *N* sodium hydroxide and titrate with 0·1 *N* sulphuric acid, using phenolphthalein as indicator (1 ml of standard alkali ≡ 0·308 mg of P_2O_5). Alternatively, determine the P_2O_5 according to Ibbotson[3] by dissolving the phosphomolybdate in ammonia and weighing the ppt. formed on addition of an acetic acid soln. of lead acetate. Wash the ppt. with *hot* water before weighing. Determine the starch in the alcohol-extracted sample according to the method used by Illing and Whittle[4] which, with slight modifications, is as follows. Add 75 ml of 0·7% aqueous potassium hydroxide soln. and gelatinise the starch by simmering gently for 30 min. Transfer hot to a 200-ml flask, cool, make up to volume and filter. Neutralise, in a centrifuge tube, 20 ml of the filtrate to phenolphthalein with 5% acetic acid. Add 8 ml of 0·1 *N* iodine and 4 ml of either 10% potassium acetate soln. or alcohol, set aside, overnight if necessary, until the ppt. has settled and then centrifuge. Decant the supernatant liquid and, after rubbing up the residue with a glass rod, treat it with 12 ml of a mixture of 10 ml of 95% alcohol and 2 ml of 0·1 *N* sodium thiosulphate, adding the mixture in several small quantities and breaking up the particles with the rod. When decomposition is complete add 25 ml of 80% alcohol and filter through a weighed Gooch crucible, wash with 95% alcohol, dry, weigh as starch and calculate to flour.

The P_2O_5 contents of wheat and rye flours were found by the above method to be 0·077 and 0·064% respectively, whilst dried egg contained from 1·15 to 1·25%. A figure of 1·20 for the latter was used in the calculations of the dried egg in fruit curd, after deducting the P_2O_5 present due to the flour.

The following table shows the results obtained on 3 samples of lemon curd. Sample A was prepared in the laboratory from wheat flour, whilst samples B and C were supplied by manufacturers, who stated that rye flour was added in the preparation of C.

Sample	Total P_2O_5, %	Dried egg %		Flour %	
		added	found	added	found
A	(i) 0·032	2·0	2·2	7·0	7·3
	(ii) 0·032	2·0	2·2	7·0	7·3
B	0·035	2·0	2·5	6·0	5·6
C	(i) 0·028	2·0	1·9	6·9	7·3
	(ii) 0·029	2·0	2·0	6·9	7·3

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April, 1945

THE STORAGE OF SELF-RAISING FLOUR AND THE PROVISIONS OF STATUTORY RULES AND ORDERS, 1944, No. 44

UNDER S.R. & O., 1944, No. 44, a minimum of 0·45% is prescribed for the Available Carbon Dioxide content of self-raising flours which are offered for sale. In some supplementary notes on this point by the Inter-departmental Committee on Food Standards[1] it was concluded that a self-raising flour which shows a

moisture content at packing of 14% "would show practically no deterioration during six months' storage under usual shop conditions." It was further stated that "deterioration of self-raising flour is rapid if the flour used in its manufacture contains appreciably more than 14% of moisture."

It has been shown[2] that the moisture content of a flour will remain constant at 14% in an atmosphere which shows a relative humidity of about 70%. For long periods of the year the average relative humidity in this country is above 70%, and the moisture content of flour stored in paper packages or in bags will tend to rise above 14%. We have tested this by storing two separate mixings of self-raising flour in a substantial brick-built mill warehouse at Birkenhead for 12 weeks during December, January and February 1944–1945. In the first test a freshly-made bulk sample of self-raising flour was thoroughly remixed in the laboratory and packed into twelve 3-lb. paper bags of the normal commercial type. At packing, the moisture content of this flour was 13·2% and its Total Carbon Dioxide figure (S.R. & O., No. 44) was 0·563%. At the end of 12 weeks the flour showed: moisture, 17·2% and Total Carbon Dioxide 0·455%. The Available Carbon Dioxide content was 0·421%, and the flour, if offered for sale, would have contravened the Order. It had in fact lost about 19% of its original aerating value during 3 months' storage, although its initial moisture content was below that suggested as a suitable packing moisture by the Inter-departmental Committee on Food Standards.

The second set was treated similarly but, at packing, had a moisture content of 16·0% and a Total Carbon Dioxide figure of 0·543%. After 12 weeks the corresponding figures were 17·25 and 0·454%. The Available Carbon Dioxide, at 0·423%, was below the permitted limit. Although these two sets of flours differed by 2·8% in moisture content at packing, this had reached substantially the same level before they had been in store for 3 months, and the flours had deteriorated in aerating power to practically the same value.

The bags of flour were stored on a shelf suspended from a girder and were about 8 ft. above floor level. Most of them were not disturbed until the last stages of the test. Intermediate tests during the first 6 weeks were made on not more than 3 bags from each set, and the final (12 weeks) figures were obtained from hitherto unopened bags. The flour in each of these was well mixed before samples of it were taken for moisture and carbon dioxide determinations. The relative humidity inside the warehouse during the period of storage ranged from 72% at 46° F., to 91% at 36° F. The maximum and minimum temperatures recorded were 50° F. and 33° F. respectively. The humidity and temperature readings were taken daily at 11 a.m.

TEST 1. Bags labelled 1 to 12

Date	Duration of storage	Bag No.	Moisture %	Total CO_2, %
31/11/44	Start	—	13·2	0·565
7/12/44	1 week	1	14·3	0·559
14/12/44	2 weeks	2	14·7	0·540
21/12/44	3 ,,	2	15·05	0·515
28/12/44	4 ,,	2	15·6	0·495
11/ 1/45	6 ,,	2	16·45	0·473
25/ 1/45	8 ,,	3	16·5	0·465
15/ 2/45	12 ,,	3	17·2	0·427
		4	17·3	0·453
		5	16·8	0·453
		6	17·35	0·450
		7	17·35	0·460
		8	16·5	0·456
		9	16·8	0·456
		10	17·2	0·445
		11	17·2	0·464
		12	17·2	0·460

TEST 2. Bags labelled 1 to 8

Date	Duration of storage	Bag No.	Moisture %	Total CO_2, %
31/11/44	Start	—	16·0	0·543
7/12/44	1 week	1	15·65	0·527
14/12/44	2 weeks	1	15·4	0·509
21/12/44	3 ,,	1	15·65	0·487
28/12/44	4 ,,	1	16·2	0·473
11/ 1/45	6 ,,	1	16·95	0·440
25/ 1/45	8 ,,	2	17·0	0·445
15/ 2/45	12 ,,	1	17·35	0·431
		3	17·55	0·445
		4	17·2	0·457
		5	17·2	0·454
		6	17·25	0·457
		7	17·2	0·454
		8	17·25	0·454

During the progress of the above tests two paper bags of self-raising flour, of the usual commercial 3-lb. type, were placed for storage on the shelf of a grocer's shop at Willaston, Cheshire. The original moisture content was 13·95 per cent. and Total Carbon Dioxide content, 0·530%. The bags were opened after 6½ weeks, and then gave the following figures.

	Moisture, %	Total Carbon Dioxide, %
Bag 1	15·0	0·467
,, 2	15·45	0·458

Both were below the prescribed limit of 0·45% for Available Carbon Dioxide.

The flours used in these tests were standard commercial mixings containing, as aerating ingredients, sodium bicarbonate and acid calcium phosphate in balanced quantities. There was no prepared chalk (Creta B.P.) in any of these flours. If the normal supplement (7 ozs. of chalk per sack of 280 lb. of flour) had been present in the basis flours, as frequently happens in commercial practice, the Total Carbon Dioxide contents of the self-raising flours at the time of packing would have approached the upper limit of 0·65% allowed by S.R. & O., 1944, No. 44. This illustrates the manufacturer's dilemma. He must add an excess of aerating ingredients to compensate for the possibility of serious loss of carbon dioxide during storage, but this excess must not be such as to give the self-raising flour an original Total Carbon Dioxide content of more than 0·65%.

All moisture determinations were made in an electric oven by heating for 5 hours at 100 ± 1° C. The Total Carbon Dioxide figures were obtained by our routine gasometric method, which involves measuring the increase in pressure that results from the treatment of the flour with an excess of dilute acid in an

evacuated apparatus. The Available Carbon Dioxide values represent the differences between Total and Residual Carbon Dioxide; this last was determined gravimetrically by absorption on soda-lime.

SUMMARY—The suggestion that packing a self-raising flour at a moisture content not exceeding 14% will ensure that it does not undergo such deterioration as to infringe the provisions of S.R. & O., 1944, No. 44, is not supported by the experiments described above. Atmospheric conditions in a good warehouse and in a retail shop are capable of markedly increasing the moisture content. This results in such a decrease in aerating value in a comparatively short time as to render the vendor liable to prosecution.

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February 20th, 1945

THE SO-CALLED "NIERENSTEIN INDICATOR"

IN recent years I have had several enquiries about an indicator of the existence of which (as such) I was not aware. I now find that the substance in question is 2-hydroxy-quinomethane,[1] referred to by Clark[2] as "Nierenstein's indicator."

2-Hydroxy-quinomethane was prepared by Dean and Nierenstein[3] in connection with their work[4] on the constitution of purpurogallin (2,5,6,7-tetrahydroxy-quinomethane) and their synthesis has since been repeated by Green and Nierenstein,[5] who have found it to melt at 257° C., which is higher than the original recorded m.p. 248–250° C.[3] Otherwise they confirmed the former work and in particular the colour changes concerning which Dr. Walpole wrote as follows: "A sample of this indicator was kindly submitted by Dr. Nierenstein for examination and its sensitive range was determined. It is colourless in solution at pH = 2·7, showing a gradual increase of purple colour to pH 3·7. It is similar therefore to some of the azo indicators described by Sörensen, but it is of a sharper colour change than any indicator previously described which changes in this region."

In view of the close relationship between 2-hydroxy-quinomethane and purpurogallin I would suggest that the latter, which is much more easily prepared than 2-hydroxy-quinomethane, should also be investigated, for it, too, may prove a useful indicator. The best method of preparing it is that worked out in 1928 by my assistant, Mr. F. H. Dixon. To 100 g of pyrogallol, dissolved in 400 ml of water, add 40 ml of glacial acetic acid, and cool the solution to below 10° C. Stir vigorously and slowly add 40 g of sodium nitrite in 100 ml of water at such a rate as to keep the temperature at or below 10° C. Continue stirring for 1 hr. after adding the nitrite soln. and then filter off the purpurogallin. On crystallising from glacial acetic acid a yield of 33 g of pure purpurogallin is obtained. Nierenstein and Spiers[6] came to the conclusion that the m.p. of purpurogallin is not a reliable criterion of purity and that it is preferable to determine the m.p. of tetraacetylpurpurogallin, which crystallises from abs. alcohol in yellow needles, melting at 179–180° C.

Lastly, I might draw attention to the colouring matters of galls, which have been shown to be glycosides of purpurogallin.[7] They, too, may prove useful indicators.

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March, 1945

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Ministry of Food

STATUTORY RULES AND ORDERS*

1945—No. 305. The Ice-Cream (Transport) Order, 1945. Dated March 22, 1945. Price 1d.
This Order prohibits the export of ice-cream to Great Britain from Northern Ireland. "Ice-cream" includes water ices and any article, whether frozen or chilled, under whatever description it is sold, which is sufficiently similar to ice-cream as to constitute a substitute for ice-cream.

— **No. 307. Order, dated March 22, 1945, amending the Feeding Stuffs (Regulation of Manufacture) Order, 1944.** Price 2d.
This amending Order, which *came* into force on April 9, 1945, alters the composition of national compounds and livestock mixtures. The principal changes are a reduction in the barley content and a slight increase in the maize content. The maximum fibre content of national compounds is also increased.

* Obtainable from H.M. Stationery Office. Italics indicate changed wording.

1945—No. 353. Order, dated March 27, 1945, amending the Fish (Maximum Prices) (No. 2) Order, 1944. Price 3d.

The *following new definition of "Fillet"* in Article 1 *is provided.* "Fillet" means fish from which all guts, sound, bones, head, tail, fins and flap, and substantially all discoloured flesh, have been removed, and includes, in the case of haddock and whiting only, a block fillet which does not exceed ¾ lb. in weight: provided

(*a*) that the following need not in any case be removed: (i) small pieces of bone necessarily severed in filleting; (ii) any flesh on the upper or dorsal side of a straight line drawn from the vent to the neck on the dorsal side of the pectoral fin joint; and

(*b*) that in the cases of brill, soles, turbot, halibut and other flat fish the flap need not be reckoned.

— **No. 389. The Food Standards (Liquid Coffee Essences) Order, 1945. Dated April 12, 1945.** Price 1d.

(1) *Liquid Coffee Essence or Extract*—The standard shall be as follows:

(*a*) The product shall contain not less than 0·5% w/v of caffeine derived from coffee:
Provided that in any proceedings a product shall be deemed to comply with the requirements of the foregoing sub-paragraph if the defendant proves that not less than 4 lb. of roasted coffee was used in the preparation of each gallon of the product.

(*b*) The product shall not contain extractives from any roasted vegetable matter other than coffee.

(2) *Liquid Coffee and Chicory Essence or Extract*—The standard shall be as follows:

(*a*) The product shall contain not less than 0·25% w/v of caffeine derived from coffee:
Provided that in any proceedings a product shall be deemed to comply with the foregoing sub-paragraph if the defendant proves that not less than 2 lb. of roasted coffee was used in the preparation of each gallon of the product.

(*b*) The product shall not contain extractives from any roasted vegetable matter other than coffee or chicory.

— **No. 464. Order, dated April 27, 1945, amending the Labelling of Food (No. 2) Order, 1944, and Granting a General Licence thereunder.** Price 1d.

This amending Order permits the sale of ice-cream and canned vegetables, without a declaration of the ingredients appearing on the label. Canned vegetables must, however, comply as regards composition with the requirements of the Canned Fruit and Vegetable Order, 1945. A similar exemption from declaration of ingredients is also provided for a limited period for custard powder, free running salt and iodised salt.

Department of Scientific and Industrial Research

INVESTIGATION OF ATMOSPHERIC POLLUTION: SUMMARY REPORT ON OBSERVATIONS DURING THE YEAR ENDED 31ST MARCH, 1944

THE Annual Reports of the Investigation of Atmospheric Pollution are not being published during the war. As in the three previous years, however, a Summary Report has been prepared for the information of the Co-operating Bodies.

The numbers of instruments maintained by the Co-operating Bodies during the last five years were:

	1939–40	1940–41	1941–42	1942–43	1943–44
Deposit gauges	129	106	100	99	102
Automatic filters	11	10	7	8	8
Volumetric sulphur apparatus	12	7	6	7	9
Smoke filters	—	—	—	1	2
Lead peroxide apparatus ..	61	42	40	41	45
Daylight apparatus	22	7	6	7	7

RESULTS OF OBSERVATIONS

DEPOSITED MATTER—The winter of 1942–43 saw the beginning of an increased national effort towards fuel economy, and it was shown in last year's Summary Report that during that winter there was a significant reduction, for the country as a whole, in both deposited matter (including sulphates) and sulphur dioxide. The reduction of sulphates in deposited matter was maintained for the whole of the year ended 31st March, 1944, and, after allowance had been made for the probable effect of differences in rainfall, the rate of emission of sulphates in 1943–4 was estimated to have been about 7% less than in 1941–42. Other forms of deposited matter were not significantly lower in 1943–44 than in 1941–42. The following is a list of stations where there was a marked change in deposited matter in the year ended 31st March, 1944, in comparison with the year ended 31st March, 1942. An increase of 100% or more is recorded as +, and a decrease of 50% or more is recorded as −. S represents the six summer months April to September; W represents the six winter months October to March; SW represents the whole year.

Birmingham—West Heath	Insoluble matter	SW−
Dewsbury—Gas Works	,, ,,	S+
Grimsby	,, ,,	W+
Halifax—5 stations	Tar	W−
2 parks	Sulphates	W−
Keighley—2 stations	Ash	W−
Kingston-upon-Hull—3 stations	Tar	SW+
Central and Suburban	Carbonaceous other than tar	SW−
London—Archbishop's Park and Battersea Park	Sulphates	S−
King Charles Street	All constituents	SW−
Southport—Hesketh Park	Tar	SW−
Stoke-on-Trent—Longton	Insoluble matter	S−

SULPHUR DIOXIDE—If the amount of gaseous sulphur dioxide (from lead peroxide observations) in Great Britain for the winter 1941–42 be arbitrarily taken as 100, the amount in the winters of 1942–43 and 1943–44 was 89 and 97, respectively. In summer, 1943, it was 47, as compared with 49 in summer, 1941. Thus the downward trend in sulphur dioxide did not continue, although there was a small but significant reduction in 1943–44, compared with 1941–42.

The following is a list of stations where there was a significant change in the year ended 31st March, 1944, in comparison with the year ended 31st March, 1942.

	Summer	Winter	Year
	S_5	W_5	
Lead peroxide method :			
Halifax—West View Park	−20%	−25%	−23%
Kingston-upon-Hull	−29%		
Leeds—Headingley	+28%		
Leicester—Westcotes	−40%		
London—Clissold Park	+34%	+24%	+23%
Manchester—Rusholme	−13%		
Rotherham—Technical College	−22%		
Sheffield—Dore	−34%		
Handsworth	+29%		
Stoke-on-Trent—Leek Road	−33%		
Volumetric method : Glasgow—Central		−25%	

RAPID SURVEYS—In response to a number of enquiries from town-planning officers, the Atmospheric Pollution Research Committee has published a leaflet entitled "How to make Rapid Surveys." For

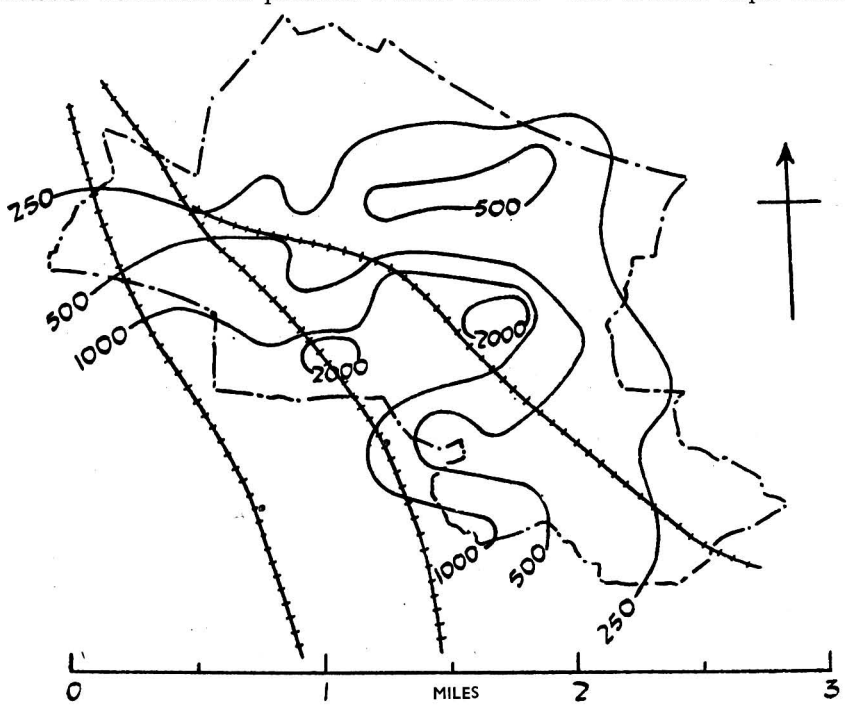


Fig. 1

surveys of dry deposited matter, a large number of 48-hour samples with Petri dishes is recommended; for surveys of smoke, a smaller number of filter-stain samples with a hand pump; for surveys of sulphur dioxide, the lead peroxide method is suggested. The methods are intended to provide the quickest possible information about the relative distribution of pollution. Absolute values of pollution are not aimed at. All these methods have been used with success; Fig. 1 is an example of results obtained by the Petri dish method.

NOTES ON INSTRUMENTS—*Deposit Gauge*—Some observers have reported difficulty in maintaining a watertight connection between the collecting funnel and the bottle, so that rain from outside the funnel has sometimes entered the bottle and has thereby vitiated the results. In a particularly bad example, over 20% of extra water entered in this way. The Atmospheric Pollution Research Committee is testing an alternative method of making the connection. In this test the connector is in the form of an inverted funnel of brass which rests, without cork or bung, in the top of the collecting bottle with its centre tube inside the neck. The rubber tubing which joins this to the glass funnel is wrapped with tape, to retard the action of air and light on the rubber (see Fig. 2).

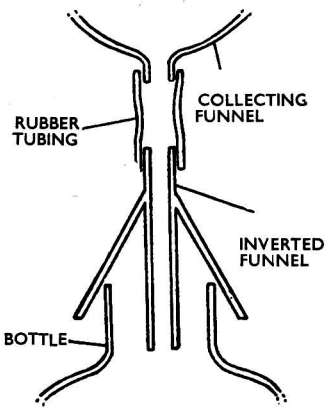


Fig. 2. Connector for Deposit Gauges

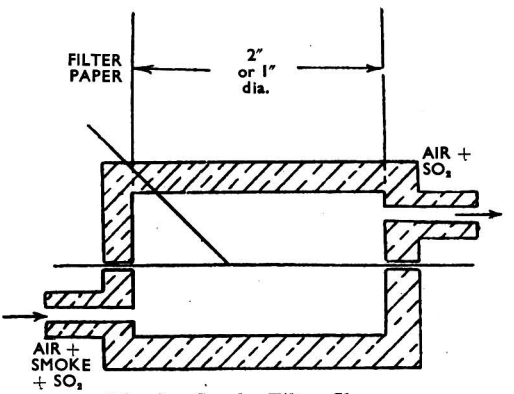


Fig. 3. Smoke Filter Clamp

Smoke Filter—There seems to be an increasing need for instrumental observations of smoke. The concentration of smoke can be observed hour by hour with the automatic filter; the smoke filter, on the other hand, is a much simpler device for observing the daily mean concentration of smoke (see Fig. 3). It can be used in conjunction with the volumetric sulphur apparatus, or independently. By its means, the smoke from 50 to 100 cubic feet of air is transferred to a filter paper as a uniform stain. The weight of material in the stain can be estimated by comparison with the Owen's scale of shades.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Carbohydrate Analysis as applied to Honey. C. D. Hurd, D. T. Englis, W. A. Bonner and M. A. Rogers (*J. Amer. Chem. Soc.*, 1944, **66**, 2015–2017)—In the direct method of Hurd, Liggett and Gordon (*J. Amer. Chem. Soc.*, 1941, **63**, 2656; ANALYST, 1942, **67**, 26) the mono-, di-, and tri-saccharides in a mixture are converted into propionic esters, which are distilled *in vacuo*. Six commercial samples of honey were analysed (A) by this method and (B) by the oxidation methods of Becker and Englis (*Ind. Eng. Chem.; Anal. Ed.*, 1941, **13**, 15; ANALYST, 1941, **66**, 298) and of Jackson and Mathews (*J.A.O.A.C.*, 1932, **15**, 198; *cf.* "*Methods of Analysis*," A.O.A.C., 5th Ed., 1940). The following results were obtained.

Honey	Method	Mono-saccharides, %	Di-saccharides, %	Tri-saccharides, etc., %
Sweet clover	A	72·3	6·1	2·4
	B	76·6	1·1	..
99% Heartsease	A	68·3	10·4	3·9
	B	76·0	1·0	..
Orange blossom	A	70·3	10·8	2·7
	B	76·3	4·4	..
Buckwheat	A	67·4	6·6	1·8
	B	73·8	1·2	..
Tupelo	A	68·8	11·4	4·1
	B	75·2	2·9	..
Cedar honeydew	A	56·5	15·6	9·1
	B	63·2	2·7	..

These results show that the results given by the distillation method are lower for the monosaccharides and higher for the disaccharides than with the oxidation methods when it is assumed that sucrose is the only disaccharide present. Crystallisation of a propionate disaccharide fraction yielded a small quantity of crystals considered to be maltose octapropionate. This confirmed the work of Van Voorst (*Chem. Weekblad*, 1941, **38**, 522), who found maltose in each of a series of samples of honey. The present investigation also indicated the presence of some higher saccharides. It is suggested that the difference in the results for fructose given by two selective oxidation methods is associated with the reducing disaccharides in honey. The general conclusion drawn from the work is that the distillation method is valuable as a check and for indicating deficiencies in other methods.

Determination of Gelatin in Ice Cream. D. Mitchell, E. H. Shaw, Jr., and G. G. Frary (*J. Assoc. Off. Agr. Chem.*, 1945, **28**, 97–105)—Expts. on 22 commercial samples of gelatin showed that silicotungstic acid is a more effective precipitant than metatungstic acid, metamolybdic acid, phosphotungstic acid, phosphomolybdic acid, arsenotungstic acid, or borotungstic acid. On the average it pptd. 99·1% of the gelatin. The best results were obtained at *p*H 1·8 to 4·2, with 3·0 as the optimum. The average % of nitrogen in the 22 samples of dry gelatin was 17·78%, corresponding with a factor of 5·63 for the conversion of nitrogen into gelatin. The method worked out for determining gelatin in ice cream was based on *iso*electric

pptn. of casein at *p*H 4·6, removal of heat-coagulable proteins at *p*H 6·3, pptn. of residual milk proteins with silver nitrate at *p*H 6·3, pptn. of gelatin with silicotungstic acid at *p*H 3·0, and determination of nitrogen in the pptd. gelatin silicotungstate.

Preparation of the reagent—Dissolve 1 g of sodium silicate ($9H_2O$) and 13·93 g of sodium tungstate ($2H_2O$) in 300 ml of water, and add 3 *N* sulphuric acid until a drop of the soln. changes the colour of thymol blue indicator to a definite pink (approx. *p*H 1·8). Boil for 10 min. and leave at room temp. for at least 2 days, filter and adjust the *p*H to 3·0 (not higher) with the glass electrode.

Method—Weigh 150 g of the ice cream into a 600-ml beaker and dilute to *ca.* 400 ml. Heat to 70°–80° C. and transfer to a 500-ml graduated flask. Add 3 ml of 25% acetic acid and shake vigorously. When the pptd. casein tends to settle readily cool to 20° C., dilute to 500 ml, warm to 35° C. and filter through a linen filter. Cool to 20° C. and evaporate a 400-ml aliquot of the filtrate to *ca.* 225 ml. Cool, adjust the *p*H with the glass electrode to exactly 6·3, and boil for 2 min. Transfer the liquid while still hot to a 300 ml graduated flask, add sufficient water to fill the flask to the mark and allow to cool to 20° C. Then readjust the vol. to the mark and filter with suction on a Buchner funnel. Transfer a 250-ml aliquot to a 400-ml beaker and add 1·75 g of solid silver nitrate. Heat and stir with a thermometer until the temp. reaches 70° C. During the pptn. of the residual milk proteins the *p*H will drop spontaneously to *ca.* 5·2. Filter and transfer 200 ml to a beaker. Adjust the *p*H to 2·7–3·0 with 3 *N* H_2SO_4, using bromophenol blue as internal indicator, and add 30 ml of the silicotungstic acid soln. adjusted to the same *p*H. Stir the mixture and leave for at least 4 hr. before filtering. Filter through an ashless filter-paper and determine the nitrogen in the ppt. by the Gunning method (*"Methods of Analyses," A.O.A.C.*, 1940, 26).

The aliquots taken in this procedure correspond with an 80-g sample of ice cream for the gelatin pptn. Since the gelatin is present in solution in milk serum, correct the final aliquot for the vols. of milk protein and fat, based on separate determination on the original sample. In making the volume correction (V.C.), in ml per 100 g of the ice cream, it is assumed that (*a*) only 95% of the total nitrogen in milk is protein nitrogen; (*b*) 0·5% of gelatin in ice cream contributes 0·0804 g of nitrogen per 100 g of ice cream; (*c*) the factor for conversion of milk nitrogen to milk protein is 6·38; (*d*) the sp.gr. of casein is 1·35 and that of milk fat is 0·92 (Garrison, *J.A.O.A.C.*, 1939, **22**, 489). Then

$$\text{V.C.} = \frac{6{\cdot}38 \times (0{\cdot}95 \times \%\,\text{nitrogen} - 0{\cdot}0804)}{1{\cdot}35} + \frac{\%\ \text{fat}}{0{\cdot}92}$$

and the aliquot sample, corrected for vol.,

$$= 80 \times \frac{500}{500 - 1{\cdot}5 \times \text{V.C.}}\ \text{g.}$$

The final calculation for the % of dry gelatin in ice cream $= \dfrac{\text{g of nitrogen in ppt.} \times 5{\cdot}63 \times 100}{\text{aliquot corrected for volume}}$.

In test expts. in which known amounts of gelatin were added to the ice cream mix the following % results were obtained.

Added	0·40	0·50	0·60	0·30
Found	0·41, 0·40	0·50, 0·51	0·61, 0·62	0·29, 0·30

The method is not applicable in presence of sodium alginate because at least half the gelatin is lost by adsorption on the silver alginate ppt. A routine test for sodium alginate in ice cream is therefore recommended. It is based on a qualitative test for pentosan (furfural reaction) in the silver alginate ppt. obtained by adding silver nitrate to the casein-free ice cream serum at *p*H 4·6.

Test for alginate—Weigh 25 g of the ice cream into a 250-ml Erlenmeyer flask and add 75 ml of water. Heat to 70°–80° C., add 0·5 ml of 25% acetic acid, stopper and shake vigorously. Filter, add 0·4 g of silver nitrate to the filtrate, and heat to 70° C. to ppt. silver alginate. Transfer to a centrifuge tube and centrifuge for 5 min. or until clear. Decant and wash the pptd. silver alginate twice with 100 ml of water at 70° C. by centrifuging and decanting, so as to remove all sugars. Transfer the ppt. with 75 ml of 12% hydrochloric acid to a 300-ml flask and heat under reflux for 15 min. to convert the alginic acid into furfural. Cool, transfer the mixture to a 500-ml distillation flask and steam-distil until 10 or 20 ml of distillate have been obtained. During the distillation heat the flask over a low flame and regulate the steam supply to give a vapour at 103°–105° C. Test for furfural in the distillate as follows. (*a*) Apply a few drops of the distillate to aniline hydrochloride paper. A pink colour is obtained in presence of furfural. (*b*) Add 5 ml of Bial's reagent (0·5 g of orcinol dissolved in 250 ml of conc. hydrochloric acid and treated with 13 to 15 drops of 10% ferric chloride soln.) to 10 ml of the distillate and heat for 10 min. in a boiling water-bath. In presence of furfural a greenish-yellow colour, which gradually becomes a green ppt., is obtained. A blank test with 5 ml of Bial's reagent and 10 ml of water should be made simultaneously.

Isolation of Mannitol from the Seeds of the Water Melon. W. M. Higgins and M. P. W. Dunker (*J. Amer. Chem. Soc.*, 1945, **67**, 153–154)—Mannitol was isolated from the alcoholic extract of the de-fatted seeds of the water melon (*Citrullus vulgaris*). The red-brown, viscous extract (4·3%) from 9080 g of the crushed sieved seeds, was successively extracted with boiling ether, alcohol at room temp., and warm alcohol (45° C.), and the final residue was dissolved in boiling alcohol. About 6 g (0·065%) crystallised out, and when recrystallised from 95% alcohol the compound melted at 164·6–166·5° C. (corr.) and had $[\alpha]_D^{26} - 86°$ (*c.*, 1·6) in water. It was identified as *d*-mannitol. Separate extractions of the shells and kernels of the seeds showed that only the former contained mannitol.

Biochemical

Human Milk Fat Component Glycerides. T. P. Hilditch and M. L. Meara (*Biochem. J.*, 1944, **38**, 437–442)—The acids of human milk fat were estimated by standard methods already described in the literature, with the following results (mol. %): decanoic 2·1, lauric 7·7, myristic 9·0, palmitic 23·6, stearic 6·5, arachidic 0·9, decenoic trace, dodecenoic 0·3, tetradecenoic 0·8, hexadecenoic 5·4, oleic 38·2, octadecadienoic 7·1, and unsaturated C_{20-22} 3·4. The probable component glycerides of human milk fat are as follows (mol. %): fully saturated: di-C_{10-14}-monopalmitin 1·7, mono-C_{10-14}-dipalmitin 1·7, mono-C_{10-14}-palmitostearin 5·7; mono-unsaturated-disaturated: mono-unsaturated-C_{10-14}-palmitin 20·3, mono-unsaturated-C_{10-14}-stearin 1·9, mono-unsaturated-dipalmitin 3·5, mono-unsaturated-palmito-stearin 13·9; di-unsaturated-,

mono-saturated: mono-C_{10-14}-di-unsaturated 23·6, palmito-di-unsaturated 19·1; tri-unsaturated 8·6. A comparison of these results with those obtained earlier (Hilditch and Paul, *J. Soc. Chem. Ind.*, 1940, **59**, 138) for the acids of cow milk fat indicates that human milk fat contains less C_{10-14}-saturated and stearic acids, but slightly more palmitic, oleic and other unsaturated acids. The component glycerides in both types of fat are very similar, but human milk fat contains less di-C_{10-14}-saturated glycerides.

F. A. R.

Component Acids of Milk Fats of the Goat, Ewe and Mare. T. P. Hilditch and H. Jasperson (*Biochem. J.*, 1944, **38**, 443–447)—The following results were obtained, using the standard methods of analysis.

	Percentages by weight			Molar percentages		
Acid	Goat	Ewe	Mare	Goat	Ewe	Mare
Butyric ..	3·0	2·8	0·4	7·5	7·5	1·1
Hexanoic ..	2·5	2·6	0·9	4·7	5·3	1·9
Octanoic ..	2·8	2·2	2·6	4·3	3·5	4·4
Decanoic ..	10·0	4·8	5·5	12·8	6·4	7·9
Lauric ..	6·0	3·9	5·6	6·6	4·5	6·8
Myristic ..	12·3	9·7	7·0	11·8	9·9	7·4
Palmitic ..	27·9	23·9	16·1	24·1	21·6	15·4
Stearic ..	6·0	12·6	2·9	4·7	10·3	2·4
As Arachidic	0·6	1·1	0·3	0·4	0·8	0·2
Decenoic ..	0·3	0·1	0·9	0·3	0·2	1·3
Dodecenoic	0·3	0·1	1·0	0·3	0·2	1·2
Tetradecenoic	0·8	0·6	1·8	0·8	0·6	1·9
Hexadecenoic	2·6	2·2	7·5	2·2	2·0	7·2
Oleic ..	21·1	26·3	18·7	16·5	21·6	16·3
Octadecadienoic	3·6	5·2	7·6	2·8	4·3	6·6
Linolenic ..	—	—	16·1	—	—	14·0
Unsaturated C_{20-22}	0·2	1·9	5·1	0·2	1·3	4·0

F. A. R.

Microvolumetric Determination of Iron, with Particular Reference to Blood. W. N. M. Ramsay (*Biochem. J.*, 1944, **38**, 467–469)—In this method organic matter is destroyed by heating with sulphuric, perchloric and nitric acids in a small Pyrex flask, and the digest is diluted and titrated with standard titanous sulphate soln., using a Conway burette, modified so as to permit storage of the reagent under hydrogen. Potassium thiocyanate is used as indicator and carbon dioxide for stirring.

Put 0·2 ml of blood into a digestion flask, add 0·2 ml of conc. nitric acid, 0·2 ml of 60% perchloric acid and 0·5 ml of conc. sulphuric acid. Add a small glass bead and heat gently over a micro-burner. Just before the last of the perchloric acid boils off, the colour of the mixture changes to a deep greenish-yellow for 3 to 5 sec. Continue heating for 30 sec. after the disappearance of this colour, dilute the cooled digest with 5 ml of water (unless copper is present), and titrate with 0·02 to 0·05 *N* titanous sulphate or chloride standardised against pure iron, using 1 ml of 50% potassium thiocyanate soln. as indicator. Adjust the burette so that the tip is just below the surface of the soln., and bubble carbon dioxide into the soln. through a tube 3 to 5 mm longer than the burette.

When copper is present, dilute the contents of the flask, after digestion, with 2 ml of water and transfer them to a narrow test-tube with the aid of two 1-ml portions of water. Add 0·5 ml of conc. sulphuric acid, and saturate the soln. with sodium chloride. Shake with two 2-ml and two 1-ml portions of ether containing 5% (by vol.) of formic acid. Transfer the combined ethereal extracts back to the original flask and evaporate off the ether. Repeat the digestion after addition of 0·5 ml of conc. sulphuric acid and 1 to 2 drops each of nitric and perchloric acids. Finally, dilute the cooled digest with 5 ml of water, and titrate as described above.

In the normal method the standard deviation of a single observation from the mean of 14 with the same sample of blood containing about 50 mg per 100 ml was 0·25 mg per 100 ml, with a coefficient of variation of 0·48%. The procedure modified for copper involved a somewhat greater error. The recovery of iron added to urine ranged from 96 to 105% of the theoretical with 10μg, but was almost theoretical with quantities of 100 μg.

F. A. R.

Determination of Total Base of Serum by Ion Exchange Reactions of Synthetic Resins. B. D. Polis and J. G. Reinhold (*J. Biol. Chem.*, 1944, **156**, 231–236)—Activate a batch of Amberlite resin IR-100, by shaking with 2 ml of 4% hydrochloric acid per g and then wash with water until the filtrate is neutral. Test the activity as follows. Add 0·2 ml of 0·15 *M* sodium chloride to 2 g of resin, wash it through the resin with two 5-ml portions of water, and titrate the filtrate with 0·2 *M* sodium hydroxide. If the titre is lower than the expected value by more than 3 mg-equiv. per litre, re-activate the resin in the tube by washing with 10 ml of 4% hydrochloric acid and then with water until neutral. Put 2 g of dry activated resin into an adsorption tube, the outlet of which is loosely plugged with Pyrex glass wool, add 0·2 ml of serum, and wash with two 5-ml portions of water. To the filtrate add 1 drop of capryl alcohol and 1 drop of 0·04% phenol red indicator, pass a rapid stream of carbon-dioxide-free air through the soln. for 2 min., and titrate with 0·2 *M* sodium hydroxide until the colour matches that of a control soln. consisting of 0·2 ml of serum, 10 ml of water, 1 drop of capryl alcohol and 1 drop of indicator, aerated in the same way. With each set of determinations test a standard consisting of 0·2 ml of 0·15 *M* sodium chloride treated as above. Estimate the amount of base combined as bicarbonate by one of the methods described by Van Slyke and Cullen (*J. Biol. Chem.*, 1917, **30**, 289). The total amount of base per litre of serum in mg-equiv. is given by the expression:

$$\left(\frac{\text{Normality of base} \times \text{ml of NaOH} \times 1000}{\text{Volume of serum}}\right) + \text{CO}_2\text{-combining capacity of serum (mg-equiv. per litre)}$$

—standard salt correction.

Results showed standard deviation ± 1·22 mg-equiv. per litre of serum. The method was compared with the electrodialysis procedure of Keys (*J. Biol. Chem.*, 1936, **114**, 449), and agreement was satisfactory when the carbon-dioxide-combining capacity was higher than 10 mg-equiv. per litre, but there was a marked discrepancy between the two results when the capacity was below this value.

F. A. R.

Detection and Estimation of Uric Acid by 2 : 6-Dichloroquinone-chloroimide. W. R. Fearon (*Biochem. J.*, 1944, **38**, 399–402)—When a very dilute soln. of uric acid is made slightly alkaline and treated with 2 to 3 drops of 0·4% 2 : 6-dichloroquinone-chloroimide soln., the mixture develops a bright yellow colour which changes to red on addition of a few drops of 1% silver nitrate

soln. Although many biological compounds give colours, only cysteine and reduced glutathione form yellow pigments, and these are not changed to red by silver ions.

Dilute the sample of urine, containing 10 to 80 mg of uric acid per 100 ml, 50-fold, and to 5 ml of the diluted urine add 1 ml of *p*H 10 buffer soln. (add 43·9 ml of 0·2 *N* sodium hydroxide to 50 ml of 2 *M* boric acid in 0·2 *M* potassium chloride soln., and make up to 200 ml with 20% sodium chloride soln.) and 0·05 ml of a 0·4% 2 : 6-dichloroquinone-chloroimide soln. in abs. ethanol. Within 1 min. dilute to 10 ml and, within 5 to 10 min., compare the colour with that of 5 ml of standard soln. containing 1 mg of uric acid per 100 ml, treated in the same way. Use either an ordinary colorimeter with the standard set at 20 mm, or a Spekker absorptiometer with the violet glass filter No. 7. Recoveries of uric acid added to urine were about theoretical. Of the interfering substances likely to be present in urine, amino acids, thiols and phenols normally occur in too low a dilution to interfere, and creatinine reacts too slowly to have an effect if the estimation is completed within 10 min. Proteins, reducing sugars, acetone and acetic acid do not interfere. F. A. R.

Estimation of *iso*-Citric and *cis*-Aconitic Acids in Biological Material. H. A. Krebs and L. V. Eggleston (*Biochem. J.*, 1944, **38**, 426–437)—In this method, which permits the determination of a few mg of *iso*citric and *cis*-aconitic acid in presence of similar quantities of citric acid, the sample is first incubated with aconitase to convert the two acids into citric acid, and from the result the amount of citric acid originally present is subtracted. In another portion of the soln., the *cis*-aconitic acid is then converted into the *trans*-form, which is not affected by aconitase, and the incubation is repeated; the difference between this result and the amount of citric acid originally present gives the amount of *cis*-aconitic acid.

Estimation of citric acid—Mix the sample with one-fifth its vol. of 20% metaphosphoric acid and filter after 5 min. Transfer a portion of the filtrate, containing 5 to 10 mg of citric acid, to a 500-ml conical flask, dilute to 80 ml, add 6 ml of 20 *N* sulphuric acid and boil down to about 35 ml. This removes certain interfering substances. Cool, add excess of bromine water (about 3 ml) and leave for 10 min. If a ppt. forms, add more bromine water and leave for a further 20 min. Filter and wash the residue with a few ml of 3 *N* sulphuric acid. To the soln. add 2 ml of 11·9% potassium bromide soln. and, drop by drop, sufficient 4·7% potassium permanganate soln. to produce a permanent dark purple colour. Leave for 15 min. and then add in 1–2 ml quantities a 20% soln. of $FeSO_4.7H_2O$ in 1% sulphuric acid until the colour becomes orange; then add 5 ml excess. Extract the pentabromo-acetone from the soln. with five 5-ml quantities of light petroleum, draw off the aqueous layer and rinse the funnel with two 5-ml portions of light petroleum and add these to the main extract. Discard the aqueous layer after shaking with a further 25 ml of light petroleum. Wash the combined petroleum extracts with four 15-ml portions of water. Shake the extract with 5 ml of 4% sodium sulphide soln. in *N* sodium hydroxide and collect the aqueous phase in a 200-ml long-necked boiling-flask. Shake successively with two 5-ml portions of water, 5 ml of the alkaline sulphide soln., two 5-ml portions of water and, if necessary, with a third portion of the sulphide soln., and then with water. Add to the combined sulphide solns. and washings 1 ml of glacial acid for every 5 ml of sulphide soln. used and a few grains of quartz. Boil to remove hydrogen sulphide and to reduce the vol. to about 7 ml. Cool, add 1·5 ml of 4·7% potassium permanganate soln. and, after 30 sec., sufficient hydrogen peroxide to decolorise the soln. Add 2 ml of conc. nitric acid, 1 ml of 1% iron alum soln. and 5 ml of 0·01042 *N* silver nitrate, and back-titrate with 0·01042 *N* potassium thiocyanate (1 ml ≡ 0·4 mg of anhydrous citric acid).

Estimation of iso-*citric and* cis-*aconitic acids*—Neutralise another portion of the sample, dilute to 30 ml and add 5 ml of 0·1 *M* phosphate buffer (*p*H 7·4) and 5 ml of an aconitase preparation consisting of 1 g of frozen pigeon breast muscle or mammalian heart muscle suspended in 5 ml of water. Incubate at 40° C. for 60 min. with occasional shaking. Prepare a second sample in which 5 ml of water replaces the aconitase and treat it in the same way. At the end of the incubation add 20% metaphosphoric acid to make the vol. up to 50 ml, filter and estimate citric acid as described above. If a = initial amount of citric acid, b = amount of citric acid after incubation with aconitase, and x = sum of the *iso*-citric and *cis*-aconitic acids, then $x = 1{\cdot}117\,b - a$. The larger the amount of citric acid relative to the amounts of the other acids the greater will be the error, but where the amounts of all three acids are of the same order the error may be as low as ±3%.

Separate estimation of iso-*citric and* cis-*aconitic acids*—Transfer a third portion of the sample to a porcelain evaporating dish, acidify with 1 ml of conc. hydrochloric acid and evaporate on a steam bath. Heat for a further 30 mins. after removal of the water, cool and suspend the residue in 10–15 ml of water. Transfer to a 50-ml graduated cylinder, make alkaline to phenol red with 2 *N* sodium hydroxide and add 1 ml excess to hydrolyse the *iso*-citric lactone. Incubate at 40° C. for ½ hr., adjust the *p*H to 7·2–7·4 with *M* phosphoric acid, dilute to 35 ml and incubate with aconitase as described above. If b = amount of citric acid present after treatment with aconitase, b^1 = amount of citric acid present after removal of *cis*-aconitic acid in the sample, then the amount of *cis*-aconitic acid = $1{\cdot}102\,(b - b^1)$. Only when no other substance is present is *cis*-aconitic acid quantitatively removed by this method. In presence of *iso*-citric acid the conversion of *cis*- into *trans*- aconitic acid is incomplete, and the results are therefore too low. F. A. R.

Use of *Lactobacillus arabinosus* 17-5 for Microassay of Pantothenic Acid. E. H. Hoag, H. P. Sarett and V. H. Cheldelin (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 60–62)—Certain difficulties are encountered in the use of *Lactobacillus casei* as the test organism in microbiological assay methods even with modified media and extraction procedures. The organism responds erratically in presence of small amounts of starch, fat and fatty acids, so that it is necessary to digest samples enzymatically and to extract them with ether before the assay. Previous studies have shown that *L. arabinosus* 17–5 responds more rapidly to graded amounts of pantothenic acid than does *L. casei*, and this organism is not known to be stimulated appreciably by starch or fatty acids. The basal medium (medium A) used in the earlier part of the work was that of Pennington, Snell and Williams (*J. Biol. Chem.*, 1940, **135**, 213; ANALYST,

1940, **65**, 661) with the glucose and sodium acetate increased to 40 and 36 g per litre respectively, and the organism was maintained on yeast agar stab cultures. To prepare cultures for the inoculum, transfer cells from a suitable stab culture into a tube containing 5 ml of medium A diluted with 5 ml of water containing 1 μg of pantothenic acid, 5 mg of yeast extract (Difco) and 5 mg of liver extract (Lederle). Incubate the inoculum at 37° C. for 16 to 24 hr. and dilute 1 ml to 15 ml with 0·9% saline. For assays, add one drop of the dilute suspension to each test culture. Addition of yeast and liver extracts appears to promote subsequent rapid growth and high acid production in the test cultures. The grown culture may be stored in a refrigerator for 24 hr. before inoculation into test cultures. Inocula of *L. casei* incubated for *ca.* 24 hr. are comparable in growth to 16-hr. cultures of *L. arabinosus*. Prepare the sample extracts by digesting finely ground or blended samples for 24 hr. at 37° C. with taka diastase and papain (Cheldelin *et al.*, *Univ. Texas Publ.*, 1942, No. 4237, 23). With medium A steep quantitative curves are obtained both for turbidity (measured as optical density) and acid production with *L. arabinosus* in response to the addition of 0·01 to 0·2 μg of pantothenic acid per 10 ml of culture. After 14-hr. growth *L. casei* cultures are only slightly turbid, whereas those of *L. arabinosus* have practically attained max. growth. Comparison of the acid production of the two organisms gives similar results. Improvement of the basal medium for measurement of pantothenic acid in pure soln. could not be effected either by change of concn. of the ingredients or by adding glutamic acid, asparagine, tryptophan or B vitamins other than riboflavin. Riboflavin, although not essential for growth of the organism, produced slight stimulation in the early stages of growth and was therefore retained. The medium finally selected for use with a wide variety of materials has the following composition—alkali-treated peptone, 10 g; glucose, 40 g; sodium acetate 24 g; acid-hydrolysed casein (technical), 4 g; autolysed yeast (Norit-treated), 2 g; rice bran concentrate (Vitab, Norit-treated), 15 g; cystine hydrochloride, 200 mg; riboflavin, 200 μg; salt solns. A and B (Pennington *et al.*, *loc. cit.*), 10 ml each; water, to 1 litre. Reports of the stimulatory effects of starch and fatty products upon the growth of the two organisms have been generally confirmed, although it was found that *L. arabinosus* is not appreciably stimulated by 25 or 150 μg per tube of oleic acid, Wesson, corn, wheat-germ, cod-liver, linseed or olive oils. or by 5 or 15 mg of starch. One sample of technical oleic acid produced *ca.* 75% stimulation. In contrast *L. casei* was stimulated by the abovementioned substances to an extent ranging from 25% with starch to over 200% with corn and wheat-germ oils. With foods of high fat content it is desirable to extract the sample with ether before assay. The pantothenic acid contents of yeast extracts were much higher when determined by the chick method than when determined by *L. arabinosus*, although the two methods agreed closely with rice bran. The lower values obtained for yeast confirm previous results with *L. casei*, and attempts to increase these values with Mylase P or a fungal proteolytic enzyme were unsuccessful. A. O. J.

Estimation of Vitamin C in presence of Iron Salts. O. Gawron and R. Berg (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 757)—In work on isolated vitamin C-iron salt systems it was found that interference by ferrous and ferric iron can be avoided if titration of the vitamin with dichlorophenolindophenol is made in a suitable medium. Thus vitamin C can be determined in presence of ferrous iron if acetic acid is the titration medium, and in presence of ferric iron if metaphosphoric acid is present. Since ferrous iron reduces dichlorophenolindophenol in presence of metaphosphoric acid, the vitamin aud the ferrous iron can be determined in the same aliquot. Titration with dichlorophenolindophenol soln. of 1-ml portions of a soln. of ferrous sulphate containing 0·64 mg of ferrous iron per ml with 25 ml of 8% acetic acid, 3% or 6% metaphosphoric acid and 8% acetic acid containing varying amounts of metaphosphoric acid as titration media showed that in presence of 1% metaphosphoric acid or more the titre was equiv. to the amount of iron present. Similar expts. with 1 mg of vitamin C and 0·75 mg of ferric iron in 25 ml of 8% acetic acid with and without metaphosphoric acid showed that the reduced dye is re-oxidised by the ferric iron unless metaphosphoric acid is present. It is therefore apparent that vitamin C and ferrous iron can be determined in one aliquot by titrating first in acetic acid, then adding metaphosphoric acid, and continuing the titration. As an example of the application of the method, a capsule containing 35 mg of vitamin C and 15 mg of ferrous iron in the form of ferrous sulphate was dissolved in 25 ml of water, and a 1-ml aliquot was mixed with 20 ml of 8% acetic acid and titrated with dichlorophenolindophenol soln. The titre was equiv. to 34·7 mg of vitamin C in the original sample. After addition of 10 ml of 6% metaphosphoric acid the additional vol. of dye soln. required to complete the titration was equiv. to 15·1 mg of ferrous iron in the original sample. The dichlorophenolindophenol soln. was made by dissolving 50 mg of the ether extracted dye in 200 mg of water containing 42 mg of sodium bicarbonate. The applicability of the method to biological systems, pharmaceutical products and food products, where interfering substances in addition to iron may be present, remains to be investigated. A. O. J.

Agricultural

Determination of Trace Elements in Soils and Plants. I. Boron and Manganese. V. V. K. Sastry and B. Viswanath (*J. Indian Chem. Soc.*, 1944, **21**, 370–375)—*Preparation of the sample.* (*a*) *Soil*—Fuse 2 g of finely powdered soil with 10 g of anhydrous sodium carbonate. Extract with 25 ml of water (double-distilled water is used throughout), warm and add 10-ml portions of 4 *N* sulphuric acid until the melt is disintegrated and the residue colourless. Filter and dilute to 100 ml. (*b*) *Plant material, e.g., tobacco*—Ash 2 g, triturate the residue with 5 ml of 3·6 *N* sulphuric acid, filter and dilute to 100 ml. *Boron determination*—The colorimetric method used is based on that of Smith (ANALYST, 1935, **60**, 735) and that of Olson and De Turk (*Soil Sci.*, 1940, **50**, 257), the measurements being made on a Pulfrich photometer with a light blue filter (L3–37). To 25 ml of the soil soln. add 150 ml of 95% ethyl alcohol, shake and filter. Add 100 ml of water to the filtrate, make just alkaline with potassium carbonate and distil to a small volume. Transfer the residue to a platinum dish, evaporate to dryness and ignite. Triturate the residue with 5 ml of 0·36 *N* sulphuric acid and dilute the soln. to 50 ml. To a 5-ml portion add 45 ml of sulphuric acid (98·5%) and cool. Add 2·5 ml of quinalizarin soln. (0·01%), cork lightly and leave at room temp. for 30 min.

Prepare a blank from 5 ml of water and compare in the photometer with the sample soln., using 30 cm vessels. Correct for the reagent blank and determine the boron by reference to a calibration curve. When working with the plant ash soln., take 25 ml, evaporate nearly to dryness, dissolve in 20 to 25 ml of 0·36 *N* sulphuric acid, dilute to 50 ml, take a 5-ml portion and continue as above. *Manganese determination*—Heat 25 ml of soil or plant ash extract on a water-bath with 5 ml of conc. sulphuric acid to eliminate chlorides. Add 5 ml of conc. nitric acid and 1 ml of phosphoric acid and heat until all nitrous fumes are removed. Add 20 ml of water and 0·2 g of sodium periodate, boil and keep undisturbed until the pink colour is fully developed. Cool, dilute to 50 ml and compare with a blank in the photometer, again using the light-blue filter. Refer the instrument reading to a curve prepared by measuring solutions of known manganese content. L. A. D.

Gas Analysis

Determining Oxygen in Hydrocarbon Gases. K. Uhrig, F. M. Roberts and H. Levin (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 31–34)—The method is a development of that of MacHattie and Maconachie (*Ind. Eng. Chem., Anal. Ed.*, 1937, **9**, 364), in which the oxygen is made to combine with reduced copper kept moist with ammoniacal ammonium chloride soln., but the original colorimetric copper determination is replaced by a simple iodimetric procedure. The apparatus, for details of which the original paper must be consulted, consists essentially of a lift pump in which oxygen-free nitrogen is produced from commercial nitrogen, a reaction cell where the oxygen in the sample combines with copper, and measuring apparatus consisting of a Shepherd burette, calibrated receivers and a three-leg mercury manometer. In the lift pump nitrogen from a cylinder causes a continuous circulation of ammoniacal ammonium chloride soln. (equal parts of conc. ammonia and sat. ammonium chloride soln.) through a sponge-like pad of copper ribbon whereby the nitrogen is freed from oxygen. The reaction cell is charged with copper ribbon which has been freed from grease by heat and from copper oxide by ammoniacal ammonium chloride soln. Before the apparatus is used the air is completely replaced by oxygen-free nitrogen and the receiver for the sample is evacuated, charged with purified nitrogen and evacuated again. For samples containing as little as 10 p.p.m. of oxygen a 5-gallon bottle forms a suitable receiver and 5000 ml a suitable sample size. If the sample contains more than 1% and less than 5% of oxygen it is measured in the burette. If it contains more than 5% it must be diluted with nitrogen and sufficient time allowed for complete mixing. For such samples corrections must be made for deviations from the gas laws. All samples are charged into the receivers at 1000 mm pressure, and samples containing less than 1% of oxygen are taken directly out of the receivers and measured by pressure difference. The copper in the reaction cell is kept moist with ammoniacal ammonium chloride soln., and the sample is passed through the cell at *ca.* 150 ml per min. When sufficient has passed through, any sample remaining in the tubes is swept into the reaction tube by a stream of nitrogen, the reaction liquid is run into a beaker, and the tube is rinsed with ammoniacal ammonium chloride soln. until all the cuprous oxide has been dissolved and transferred into the beaker. The combined reaction liquid and washings is boiled to remove most of the ammonia, and, after cooling, it is acidified with acetic acid, boiled, cooled and treated with potassium iodide crystals. The liberated iodine is titrated with 0·025 *N* sodium thiosulphate, with starch as indicator, from a micro-burette graduated to 0·01 ml. Each ml of 0·025 *N* sodium thiosulphate ≡ 0·14 ml of oxygen at 0° C. and 760 mm. Expts. with known mixtures of oxygen and hydrocarbons showed that the method is accurate. Olefines and diolefines do not interfere, but samples containing unsaturated gases must be analysed promptly because oxygen appears to be consumed by unsaturated compounds, although not by acetylene. Hydrogen sulphide, mercaptans and sulphur dioxide interfere, but they are easily removed by successive scrubbing of the sample with silver nitrate soln. and potassium hydroxide pellets. Scrubbing with potassium hydroxide alone fails to prevent absorption of oxygen by mercaptans. A. O. J.

Organic

Colorimetric Estimation of Amino Nitrogen. J. A. Russell (*J. Biol. Chem.*, 1944, **156**, 467–468)—In the colorimetric estimation of amino nitrogen by the naphthaquinone sulphonate method of Frame, Russell and Wilhelmi (*J. Biol. Chem.*, 1943, **149**, 255; ANALYST, 1943, **68**, 378), the bleaching procedure has been found unsatisfactory, the concns. of acid and thiosulphate previously recommended being sufficient to destroy the coloured condensation product as well as the unchanged reagent. To overcome this difficulty, the bleaching reagent has been modified. Prepare the samples, neutralise and develop the colour as described in the original publication, but use 2% instead of 1% borax soln. as the buffer. After colour development, cool, dilute the sample to about 13 ml, and add 1 ml of 0·3 *N* hydrochloric acid containing 0·04 *M* formaldehyde and 1 ml of 0·05 *M* sodium thiosulphate. Dilute to 15 ml and measure the colour 10 to 30 min. after addition of the reagents F. A. R.

Quantitative Analysis of Alkyl Lithium Compounds. H. Gilman and A. H. Haubein (*J. Amer. Chem. Soc.*, 1944, **66**, 1515–1516)—The method is a modification of that of Ziegler *et al.* (*Ann.*, 1929, **473**, 31), in which RLi compounds in admixture with other lithium compounds are determined by differential titration of two aliquots after hydrolysis; one titration is for total lithium hydroxide and the other for lithium hydroxide present after destroying the RLi compound. In the present method the RLi compound is removed by reaction with benzyl chloride, leading to the formation of three coupling products:

$$n\text{–}C_4H_9Li + C_6H_5CH_2Cl \rightarrow n\text{–}C_8H_{18} + n\text{–}C_4H_9CH_2C_6H_5 + C_6H_5CH_2CH_2C_6H_5.$$

This quantitative procedure cannot be used with aryl lithium compounds, or with methyl lithium or phenylethynyl lithium, the last two probably being insufficiently reactive. The procedure is as follows. Filter the soln. of the alkyl lithium compound (Gilman, Langham and Moore, *J. Amer. Chem. Soc.*, 1940, **62**, 2327) under nitrogen, preferably through a sintered glass funnel, to remove insol. material. Withdraw a 5- or 10-ml aliquot, by means of a rubber suction bulb connected with a pipette, and hydrolyse in 10 ml of water. Titrate with standard acid (phenolphthalein as indicator) to obtain the total alkali. Add another 5- or 10-ml aliquot to

10 ml of dry ether containing 1 ml of benzyl chloride (purified by drying over phosphorus pentoxide and then distilling at reduced pressure). As the alkyl lithium soln. drops into the benzyl chloride a yellow colour flashes through the liquid; if the RLi soln. is concentrated a white ppt. of lithium chloride forms and the yellow colour disappears. The ethereal soln. may become warm enough to boil, but is not cooled. Allow the mixture to stand for 1 min. after the addition, then hydrolyse and titrate with standard acid. Take care not to overstep the end-point in this titration, since the aqueous layer is decolorised before the ethereal layer; this may be overcome by vigorous shaking near the end-point. Coupling of benzyl chloride and the RLi compound is slow in solvents other than ether. For the analysis of RLi compounds prepared in light petroleum dissolve the benzyl chloride in a relatively large vol. of ether. Lithium *n*-butoxide did not cleave benzyl chloride under the experimental conditions. E. M. P.

Analysis of Ternary Mixtures of Three Isomeric Heptanes. V. A. Miller (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 5–12)—The method depends upon the determination of the solution temp. of the hydrocarbon mixture in diethyl phthalate and in nitrobenzene with an accuracy of ±0·01° C. The data are then used with a series of calibration curves prepared with mixtures of known composition to determine graphically the % of each of the three constituents in the unknown mixture. The three isomers investigated were 2,4- and 2,2-dimethylpentane and 2,2,3-trimethylbutane. Preliminary study showed that the difference between the aniline points of 2,2- and 2,4-dimethylpentane is only 0·35° C., and other solvents were examined to find one giving a larger difference. Considerations of purity, stability, working range and sharpness of end-point excluded all but 12 of 97 solvents examined; of these only acetyldiethylamine and diethyl phthalate gave a conveniently large difference, and diethyl phthalate was finally chosen for its much sharper end-point. The soln. temp. was measured in a small closed glass tube mounted in another glass tube serving as an air-bath, which, in turn, was supported in a large well-stirred bath of a 50% soln. of ethylene glycol in water in a 4300-ml unsilvered, wide-mouthed, vacuum-jacketed vessel. The bath was maintained at constant level and contained an electrically driven pump-type stirrer, a mercury thermometer reading to 0·1° C., a knife-type immersion heater and a freely suspended coil of copper tubing through which carbon dioxide could expand to lower the temp. of the bath. For the air bath a glass tube of 3·5 cm in diam. and 23 cm long, was freely supported in the bath. This tube was constricted near the top to form a seat to support the sample tube. This reaction tube was made by sealing a glass tube, diam. 1·6 cm and 14 cm long, to a 24/25 ground-glass joint, and it reached to within 4·8 cm of the bottom of the air jacket. Temperature measurements were made with a standardised Leeds and Northrup platinum resistance thermometer (No. 8163-A) with a G2 Mueller bridge (No. 8069) and a type R galvanometer (No. 2500-*a*) in a modified Julius suspension. With this arrangement readings could be made to 0·001° C. (0·0001 ohm), but for this work readings were made to the nearest 0·01° C. The top of the ground-glass joint was blown into a bulb, and into this was sealed a glass tube carrying the glass-sheathed resistance thermometer and another tube carrying the stirrer through the rings of which the thermometer tube passed. An all-glass apparatus was devised by means of which the dry solvent could be stored in an atmosphere of nitrogen and dispensed directly into the reaction tube. Repeated purifications by distillation were thus avoided. For precise details of the apparatus and its manipulation the original paper must be consulted, but the procedure is essentially as follows. Cleanse the tube holding the thermometer and stirrer first with acetone and then by repeated evacuation and flushing with dry nitrogen. Heat the reaction tube while a stream of nitrogen is passing through it and allow it to cool while maintaining the stream of nitrogen and finally passing it through the pipette used for measuring the hydrocarbon or mixture. Under conditions which preclude the introduction of air, measure the hydrocarbon into the reaction tube, add the measured amount of solvent from the solvent dispenser, insert the thermometer and stirrer and place the tube in the air-bath, which has previously been evacuated and filled with nitrogen. Adjust the temp. of the liquid bath to *ca.* 1° C. above the expected solution temp., and operate the stirrer so that it does not break the surface of the liquid. Measure the solution temp. while the temp. of the liquid is rising and while it is falling by alternate operation of the immersion heater and the cooling coil in the bath. Construct calibration curves for the pure hydrocarbons and for binary mixtures of known composition. These diethyl phthalate point calibration curves are all that is required for binary mixtures, but for ternary mixtures a second set is required. Plot the composition and diethyl phthalate point data for the three sets of binary mixtures on the edges of a large triangular graph and draw straight lines through the *iso*-solution temp. points on two sides of the triangle to form a series of *iso*-solution temp. lines. With all the solvents tested, the solution temp. of 2,2,3-trimethylbutane is lower than that of the other two hydrocarbons. For most solvents the solution temp. of 2,4-dimethylpentane is higher than that of the 2,2-compound, but with a few it is lower. These solvents will give *iso*-solution temp. lines with a slope opposite to that of the diethyl phthalate lines, thereby giving sharp intersections and more accurate readings. Nitrobenzene was chosen as the second solvent. The procedure for finding the composition of a ternary mixture is as follows. The composition of the binary mixture of 2,2,3-trimethylbutane and 2,4-dimethylpentane giving the same solution temp. in nitrobenzene as that found for the ternary mixture is ascertained from the calibration curve, and this point is located on the appropriate side of the triangular diagram. The composition of the binary mixture of 2,2,3-trimethylbutane and 2,2-dimethylpentane giving the same solution temp. in nitrobenzene as the ternary mixture is similarly ascertained from the calibration curve and located on the corresponding side of the triangle. The line joining these points will represent all ternary mixtures of the three hydrocarbons with the same nitrobenzene point. Data derived from the calibration curves for the diethyl phthalate points are plotted on the triangle in the same way, giving a line representing all ternary mixtures having the same diethyl phthalate point as that found by expt. The intersection of the two lines indicates the composition of the ternary mixture. Since it is difficult to obtain perfectly dry samples by distillation of small fractions of unknown mixtures of the hydrocarbons, it was thought advisable to standardise the

procedure by using hydrocarbons saturated with water at 30° C. The solvent, however, must be kept dry. A. O. J.

Measuring the "Existent Corrosivity" of Used Engine Oils. R. G. Larsen, F. A. Armfield and L. D. Grenot (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 19–24)—The most practical test material for ascertaining the corrosivity of an oil at a given time would appear to be metal deposited and mounted in such a manner that its removal by corrosion could be followed visually. The reduction in length of an acutely tapered wedge of corrodible metal would measure the corrosion penetration into the surface. Test-strips were made by depositing alternate wedges of lead and copper and of lead alone on glass microscope slides, and tests with corrosive oils showed that with wedges containing both lead and copper the lead is selectively attacked, as it is in corroded copper-lead bearings. Glass-supported metal wedges have the advantage that the end of the wedge can be clearly observed by transmitted light, but they are fragile and not easily made. More convenient test-strips were made by plating lead on copper in steps of varying thickness (0·08, 0·18, 0·36, 0·61, 0·94, 1·52 and 2·54 μ). To prepare such strips, deposit lead on a copper strip from a lead anode in a plating bath containing basic lead carbonate (120 g), 50% hydrofluoric acid (192 g), boric acid (84 g), glue (0·15 g) and enough water to make 800 ml. Make the first and thinnest deposit with 8·9 cm of the strip immersed and, after rinsing and drying the strip, apply the subsequent steps by lowering the strip into the bath in 1·27 cm increments. The method can be adapted to the plating of sheets of metal to be cut later to the desired width, and if the strips are to receive much handling they may be coated with hard paraffin, which is readily removed by the hot oil. The advantages of the test are that it can be applied without removing the oil from the crank case of the engine and that it is made under the environmental conditions of the engine without an intermediate cooling period. Since the strip-test measures existent corrosivity of the used oil for bare metal, there is little correlation between the observed corrosion and the loss suffered by the bearings that have remained in the oil from the outset, because the bearing is protected by surface films formed before the oil becomes corrosive and thus is not subject to corrosive action to the same extent as the test-strip. Correlation of corrosion of test-strips with corrosion of bearings not protected by films has not yet been made owing to the difficulty of ascertaining when such films are absent. Sap. value and increase of viscosity indicate oxidation of the oil and not its corrosivity. Acid value, although an unsatisfactory index of corrosivity where protective films are formed, could measure existent corrosivity only if all oils formed acids of the same type and corrosivity. As a rule correlation between the strip-test and the acid value is poor, and there is evidence that acids are corrosive only when peroxides are present. For a given oil in a given engine there is sometimes a relation between corrosivity and acid value, but the relation is not universal. Sulphur compounds may interfere with the test by forming dark deposits on the strips, and when a strip is left in the crank case of a stationary engine condensed water may cause local corrosion above the oil level. The test-strip may be immersed in oil withdrawn from the engine, *e.g.*, to follow the development of corrosivity in an oil that is being used in an engine operating under changing conditions, but, to reproduce the conditions in the crank case, the strip must be rotated in the oil and allowance may have to be made for corrosive vapours that may be present in the crank case but not in the oil withdrawn. Samples should be tested soon after their removal from the engine because, owing to esterification and polymerisation, many oils lose their corrosivity upon long standing. Relation between corrosion and time of immersion is linear, but the relation between corrosion and temperature is not so simple. In spite of this, graphs may be constructed to convert a corrosion value determined at any time and temperature to the value corresponding with another time and temperature (within the range covered) with acceptable accuracy. Pending further investigation it may be stated that the removal by the oil of the first two steps of deposited lead in 1 hr. at 138° C. indicates incipient corrosion, and the removal of four or more steps indicates definite corrosion of unprotected bearings. The strips may be made with other pairs of metals, the more corrodible metal being deposited upon the less corrodible metal. A. O. J.

Quantitative Isolation of Hemicelluloses from Coniferous Woods. L. E. Wise (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 63–64)—Recently Jayme (*Cellulosechem.*, 1942, **20**, 43) has developed a method for isolation of holocellulose depending upon partial delignification of wood by an aq. acetic acid soln. of sodium chlorite, the chlorine dioxide removing much of the lignin without affecting the cellulose or hemicelluloses. The fibrous residue is an impure holocellulose from which the lignin and lignin degradation products may be removed almost quantitatively by subsequent operations, and this holocellulose serves as a suitable starting point for the quantitative isolation of hemicellulose fractions. Heat 10-g samples of air-dried unextracted wood (prepared by passing through a Wiley mill) to 60° C. with a soln. containing 500 ml of water, 50 ml of acetic acid and 50 g of sodium chlorite. Allow delignification to proceed for 24 hr. at *ca.* 30° C., stirring the mixture occasionally. Collect the nearly white residue in a suction filter, wash it with ice water and extract the hemicelluloses in an atmosphere of nitrogen, first with dil. potassium hydroxide soln. and finally with a strong soln. To effect this, transfer the holocellulose into a wide-mouthed Erlenmeyer flask carrying an outlet tube with a stopcock and closed by a rubber stopper carrying a dropping funnel and gas inlet tube reaching nearly to the bottom of the flask. Pass dry nitrogen through the flask for 20 to 30 min., then gradually add 500 ml of 4 to 5% potassium hydroxide soln. (previously heated to 85° C.) without interrupting the nitrogen stream, which should be maintained for 15 min. longer. The treatment with potassium hydroxide soln. may be made in as many fractions as seems desirable. Close all stopcocks and shake the flask at intervals during 24 hr. Make the final extraction similarly but with *ca.* 170 ml of cold 24% potassium hydroxide soln. Precipitate the hemicellulose from each fraction by adding acetic acid and alcohol. When necessary, free the hemicellulose from residual lignin by adding bromine drop by drop to the soln. (or suspension) in cold acetic acid until, after shaking, a slight excess of bromine persists. After 20 min. destroy the bromine by adding excess of 95% alcohol, which serves also to ppt. the hemicellulose. Separate the hemicellulose by centrifuging, wash it thoroughly

with alcohol and ether, and collect it in a 1G3 Jena sintered glass crucible. The final fibrous residue is α-cellulose. The sum of the various fractions (corrected for ash content) gives the hemicellulose content of the unextracted wood. It is recognised that the boundary between resistant hemicelluloses (cellulosans) and true cellulose is an arbitrary one, and that changes in the extraction technique may result in the isolation of more or less hemicellulose, but the sum of α-cellulose and hemicelluloses, *i.e.*, holocellulose, remains reproducible with the two coniferous woods studied. The following figures (%) were obtained with redwood and (Douglas fir)—Lignin, 31·30 (25·80); alcohol extract, 21·83 (4·20); water extract of the residue from alcohol extraction, 1·80 (3·20); α-cellulose, corrected for ash and lignin, 35·75 (50·09); hemicelluloses, corrected for ash, 9·63 (15·10); acetyl, 0·28 (0·76). The method affords for the first time a direct proximate gravimetric determination of the hemicelluloses. Acetyl values are included because these groups are removed during the isolation of hemicellulose.

A. O. J.

Inorganic

Determination of Bismuth with Phenyldithiobiazolone Thiol. A. K. Majumdar (*J. Indian Chem. Soc.*, 1944, **21**, 347–351)—Like dimercaptothiobiazole (Majumdar, *Id.*, 240–244; ANALYST, 1945, **70**, 189), phenyldithiobiazolone thiol gives coloured ppts. with metals of the hydrogen sulphide group, and the compound has been studied as a reagent for the colorimetric and gravimetric determination of bismuth. The potassium salt of the reagent, prepared according to Busch (*Ber.*, 1894, **27**, 2511) is used in 0·5% aqueous soln. in the colorimetric method. Other reagents and the colorimetric procedure are similar to those of the dimercaptothiobiazole method (see above). When determining 0·26 mg of bismuth, the following amounts, in mg, of other ions are the maximum permissible unless the nitric acid concn. is increased:—Cu^{++}, 0·07; Co^{++}, 2; Ni^{++}, 9; Zn^{++}, 10; Cd^{++}, 0·5; Pb^{++}, 1; Hg^{++}, 0·5; Ag^{+}, 0·3; As^{+++}, 0.05; Sb^{+++}, 0·1; Sn^{++}, 0·2; Cl', 50. The following amounts are not harmful—100 mg of Mn^{++}, Fe^{++}, nitrate, oxalate, tartrate, or sulphate, 1 ml of nitric acid (sp.gr. 1·4), 1 ml of phosphoric acid (sp.gr. 1·27) or 10 ml of sulphurous acid (saturated). The peak of the absorption curve of the colour produced is at 460 *mμ*, and the system obeys Beer's law when the bismuth concn. is between 65 and 650 p.p.m. Notes on the solubility in organic solvents of the compounds formed by the reagent with several metals are given. The reagent gives good results in the gravimetric determination of bismuth. *Method*—Add to a bismuth soln., containing 10 ml of *N* nitric acid in each 100 ml, 1% aqueous soln. of the reagent (potassium salt) until no further pptn. occurs. Heat and stir until the ppt. coagulates, and filter on a weighed Gooch crucible, washing with hot water. Dry at 100° C. and re-weigh. The compound weighed is $(C_8H_5N_2S_3)Bi.\frac{1}{2}H_2O$ and the bismuth factor is 0·2340. Zinc and hydrogen sulphide metals are also quantitatively pptd.

L. A. D.

Determination of Manganese in Caustic Soda. D. Williams and R. V. Andes (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 28–31)—The metal (usually 1 p.p.m.) is oxidised with periodate after acidification with phosphoric acid to pH_2, which prevents interference of chloride and the turbidity due to pptn. of silica. With very low manganese concns. preliminary extraction of the oxinate with chloroform is more reliable. *Direct method*—Mix 20 g of 50% lye with 50 ml of water, add 50 ml of 1 : 1 phosphoric acid and 0·4 g of potassium periodate, and boil for 20 min. Cool, dilute to 100 ml in a graduated cylinder, and measure the transmission in a photoelectric colorimeter (Wratten 58A filter). Read the μg of manganese on a calibration curve prepared by treating aliquots (0, 10, 20, 50, 100 μg Mn) as above after addition of 50 ml of water. *Extraction method*—Dilute 100 g of 50% lye to 600 ml in a 1000-ml beaker. Add 1 ml of 10% sodium sulphite soln., 90 ml of 12 *N* hydrochloric acid and phenolphthalein, neutralise, and add 5 ml in excess. Set aside for 5 min. while preparing the blank (600 ml of water and 5 ml of acid). Stir in 5 ml of a 1% soln. of 8-hydroxyquinoline in *iso*propanol, followed by 15 ml of ammonia (0·90 sp.gr.), cool and transfer to a 1000-ml separator. Add 30 ml of chloroform and shake vigorously for 60 sec. Allow to clear and draw off the lower layer into a 250-ml wide-mouthed conical flask. Repeat the extraction with 15 ml of chloroform and again with 10 ml; make a fourth extraction if the last extract is appreciably coloured. Add 5 ml of 70% perchloric acid and 5 ml of 30% hydrogen peroxide to the combined extracts and evaporate slowly to 1 or 2 ml. Do not evaporate to dryness, nor add more of the organic reagents than specified, to avoid violent explosions. Cool, add 10 ml of filtered 50% sodium dihydrogen phosphate soln., 100 ml of water, 0·4 g of periodate, and a glass bead. Boil down to 90 ml, cool, and dilute to 100 ml in a graduated cylinder. Proceed as above, putting the blank through the same procedure. Prepare the curve by adding 2 ml of perchloric acid to a series of aliquots as before, beginning the treatment with the addition of the sodium phosphate, and plot the data obtained. The smallest division of the graph should correspond to 1 μg of manganese and 0·2% light transmission.

W. R. S.

Analysis of Manganese Bronze. H. Ravner (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 41–43)—A rapid and accurate method for copper, lead, tin, iron, and nickel in one and the same portion; manganese and aluminium are determined in separate ones. To 2 g of sample in a 400-ml Vycor beaker (iron-free), add in order 15 ml of water, 1 ml of hydrofluoric and 10 ml of strong nitric, acid; cover with a platinum lid, set aside till dissolved, and boil off nitric fumes. Rinse the lid and dilute to 200 ml, add one drop of hydrochloric acid, and electrolyse at 1 amp. If no lead peroxide is visible after a few minutes, add 10 ml of a 0·16% lead nitrate soln. and electrolyse for 30 min. Add 8 ml of diluted sulphuric acid (1+1) and continue until the copper is deposited. Plunge the electrodes into 200 ml of cold water, and weigh after drying. Evaporate the electrolyte in a 300-ml platinum dish with 10 ml of diluted sulphuric (1+1) and 5 ml of hydrofluoric acid until white fumes are evolved; rinse down the dish and again evaporate. Dissolve the residue in 50 ml of water, transfer to a 250-ml beaker, dilute to 125 ml, and pass hydrogen sulphide for 30 min. Filter off the stannic sulphide with pulp and wash it with 1% sulphuric acid containing hydrogen sulphide. Digest the filter and ppt. with sulphuric and nitric acids and finally expel the nitric acid by repeated evaporation. Add 30 ml of water, transfer to a tin reduction flask, reduce with hydrochloric acid and 8 g of test

lead, and titrate the tin by the usual method. To the filtrate from the sulphide ppt. add 15 ml of diluted (1+1) sulphuric acid, pass carbon dioxide, and boil off hydrogen sulphide; cool under carbon dioxide and titrate iron with ceric sulphate and o-phenanthroline. Add 10 ml of 20% citric acid soln. to the titrated liquid and determine nickel as usual with glyoxime. Manganese is titrated with sodium arsenite (persulphate-silver nitrate method). For the determination of aluminium, the electrolyte freed from copper is treated with a little sulphurous acid and pptd. with ammonia at the methyl red end-point. W. R. S.

Microchemical

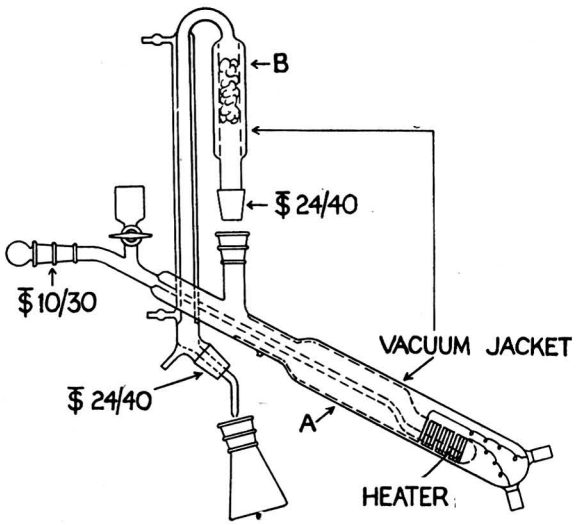
Applications of Spot Reactions (IV). Note on the Detection of Elementary Sulphur and Selenium. F. Feigl and N. Braile (*Chemist Analyst*, 1944, **33**, 28–31)—When a drop of an organic solvent or an alkali sulphide containing dissolved sulphur is placed on a test-paper impregnated with thallous sulphide, a reddish-brown thallium polysulphide, resistant to dilute acids and to hydrogen peroxide, is formed. On treatment with one of these reagents uncombined thallous sulphide dissolves, leaving a brown fleck of polysulphide. Any suitable solvent may be used to extract the sulphur from the sample. Selenium behaves similarly, giving a dark brown or black fleck. *Thallium sulphide paper*—Prepare freshly. Soak filter paper (Schleicher & Schüll No. 589 or Whatman No. 42) for several min. in 0·5% thallium carbonate or acetate soln. Drain and dry with warm air. Warm ammonium sulphide to *ca.* 80° C. in a beaker and place the paper over the mouth. After a few min. (when fully blackened), cut into strips. *Method*—Place a drop of the extract on a test paper and allow it to evaporate (warm air may be used). Place the paper in 0·5 *N* nitric acid and move to and fro until it becomes white (*ca.* 30 sec.). A brown or black fleck indicates sulphur or selenium, the intensity depending on the amount present. The spot is stable if the paper is thoroughly washed and dried. In 1 drop (0·05 ml), 3 μg of sulphur or 1 μg of selenium may be detected. To detect free sulphur in rubber, treat a small sample with carbon disulphide for 2 or 3 min. and test a drop of the soln. as above. Other applications are briefly described. J. T. S.

Applications of Spot Reactions (V). Note on the Identification of Calcium Sulphate. F. Feigl and N. Braile (*Chemist Analyst*, 1944, **33**, 76–77)—The test depends on the conversion of "insoluble" calcium sulphate into lead sulphate, which is identified by the specific sodium rhodizonate reaction. Strontium sulphate, but not barium sulphate, behaves likewise. *Method*—Place some particles of the sample on wet filter-paper at the bottom of a Gooch or sintered glass crucible. Treat the particles with 2 or 3 drops of lead nitrate soln. (2%, diluted with an equal vol. of 0·2 *N* nitric acid). After 1 min. suck off the liquid and wash the paper thoroughly under gentle suction by dropwise addition first of 2 ml of 0·2 *N* nitric acid and then of 3 ml of water or alcohol. Place the paper on a glass plate and treat first with fresh 0·2% aqueous sodium rhodizonate soln. and then with a buffer soln. of *p*H 2·79 (1·5 g of tartaric acid and 1·9 g of sodium bitartrate in 100 ml of water). A red colour indicates calcium sulphate. In absence of aluminium sulphate, calcium sulphate filler can be detected in the ash from a few mg of paper. Other applications are briefly described. J. T. S.

Colorimetric Estimation of Small Amounts of Ammonia by the Phenol—Hypochlorite Reaction. J. A. Russell (*J. Biol. Chem.*, 1944, **156**, 457–461)—Put 1·5 ml of the sample, containing 0·5 to 6 μg of ammoniacal nitrogen, into a calibrated colorimeter tube and add one drop of 0·003 *M* manganous salt soln., 1 ml of alkaline phenol reagent, and 0·5 ml of hypochlorite soln. To prepare the *phenol reagent*, mix 25 g of crystalline phenol with water, add with stirring 54 ml of 5 *N* sodium hydroxide and dilute to 100 ml. To prepare the *hypochlorite soln.*, grind and sift 25 g of bleaching powder and dissolve it as far as possible in 300 ml of hot water. Add, with stirring, 135 ml of 20% potassium carbonate soln. previously boiled to free it from ammonia, mix, heat for a short time at 90° C., cool and dilute to 500 ml. Test a small portion for calcium ion by adding a little potassium carbonate soln. and heating to boiling; if the test is positive, add more carbonate to the mixture. Filter, and store in brown bottles in the refrigerator. The soln. should be clear and should contain 1·3 to 1·4 g of free chlorine per 100 ml. (Dilute 2·00 ml with 10 ml of water, add 2 ml of 5% potassium iodide soln. and 1 ml of glacial acetic acid, and titrate with 0·100 *M* sodium thiosulphate soln.; 7·5 to 8·0 ml of thiosulphate should be required.) Both these solns. should be cold when added to the sample to avoid loss of ammonia. After mixing the solns., place the tubes immediately in a boiling water-bath for about 5 min., cool, dilute to a convenient volume, and measure the absorption in a photoelectric colorimeter, with a filter transmitting maximally at 625 mμ. A detectable colour is given by as little as 0·1 μg of nitrogen. The method can be used in conjunction with the Conway micro-diffusion technique, the contents of the inner cell of the units being rinsed into a colorimeter tube. The contents of the blank Conway units may be used as blanks in the colorimetric method if standards are run simultaneously in Conway units. F. A. R.

Micro-determination of Nitrates by the Devarda Method. R. Kieselbach (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 764–766)—Ammonia formed by the reduction of nitrates is distilled off, absorbed in boric acid soln. and titrated with 0·01 *N* hydrochloric acid with bromocresol green—methyl red as indicator. Though not essential, the special apparatus is convenient. Electrical heating permits accurate control of the distillation, while air escaping from the inmost tube prevents bumping. With a sample of small vol., steam introduced at the No. 10/30 standard joint may be used to effect the distillation. Pipette 10 ml of 2% boric acid soln. into the receiver and add 2 drops of indicator soln. (10 ml of 0·1% bromocresol green plus 2 ml of 0·1% methyl red, in 95% ethanol). Fit a fresh plug of glass wool in spray-trap B. Introduce the sample into the distilling flask A, add 0·5 g of Devarda's alloy, and rinse down to bring the total vol. to 20–25 ml. Connect the spray-trap, condenser and receiver. Add through the funnel 10 ml of 20% sodium hydroxide soln. (ammonia- and nitrate-free. Dissolve 200 g in 1 litre of water, add 1 g of Devarda's alloy, boil for 10 min., cool, and make up to vol.). Close the stopcock immediately. Heat the mixture until vigorous effervescence begins, then leave for at least 5 min. Heat

gently until foaming subsides and then distil 10 ml. Lower the receiver, continue to distil for 30 sec., and rinse the delivery tip. Titrate the distillate with 0·01 *N* hydrochloric acid to a colourless end-point. If the sample contains ammonia, add the alkali, boil and cool thoroughly before adding



Devarda's alloy. Nitrites, if present, must be determined separately and deducted from the total. Carbon dioxide does not interfere. The method is accurate and precise (99·8% for 0·05 mg-equiv. of nitrate), and a single determination can be made in 20 min. J. T. S.

Micro-determination of Nitric Oxide in Gases. R. Kieselbach (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 766–771)—The gas is scrubbed with alkaline permanganate soln., which oxidises and retains the nitric oxide. The soln. is then analysed by the micro-Devarda method (Kieselbach, *Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 764; see preceding Abstract). A modified Shaw scrubber (*Ind. Eng. Chem., Anal. Ed.*, 1934, **6**, 479) avoids excessive back pressure and is efficient down to 2 p.p.m. of nitric oxide. A semi-automatic portable apparatus assembled in a cabinet is described. Gas enters through a glass-wool filter and flows through the scrubber at 300 ± 2 ml per min. A flowmeter-manometer system with electrical contacts controls the rate of flow by operating a relay which causes a small, reversing electric motor to open or close the pinchcock-type feed valve. Traps prevent loss of liquid should the pressure rise suddenly or the control fail. A manometer indicates the pressure on the apparatus and another connected between the flowmeter and the scrubber allows the flowmeter reading to be corrected for back pressure. The flowmeter is calibrated against a back pressure equal to that created by the scrubber so that the correction is normally zero. A hygrometer is also incorporated. A 0·5% potassium permanganate—0·5% sodium hydroxide soln. is used in the scrubber. When the apparatus has been flushed out with the sample, a stream of gas is passed through the scrubber for a known time, during which the back pressure is observed for any abnormality. The flow is then stopped and the contents of the scrubber are washed into the micro-Devarda flask (Kieselbach, *loc. cit.*). Ten ml of 6% oxalic acid soln. are then added, the mixture is evaporated to 20 ml (preferably using a spray trap) and the determination is completed as usual. The accuracy and precision with olefine-free gases is 99%. Excessive reduction of permanganate by unsaturated compounds (olefines) in the sample causes serious errors. The concn. of the soln. may be raised to 5% permanganate–5% sodium hydroxide; if this is insufficient, olefines are removed by preliminary scrubbing with sulphuric acid saturated with silver sulphate. Higher oxides of nitrogen interfere, and are removed by preliminary scrubbing with 0·5% sodium hydroxide soln. J. T. S.

Micro-analysis of Silicates. Analysis of 18th Century Chinese Porcelain. P. E. Wenger and Z. Besso (*Helv. Chim. Acta*, 1944, **27**, 1038–1044)—Micro-analysis had to be adopted to avoid ruining the specimens. The method used is based on that of Hecht-Donau (*Anorganische Mikrogewichts-analyse*, Vienna, 1940, p. 326). The material was finely powdered and three portions were taken. Silica was determined in the first portion, oxides of iron, aluminium, calcium and magnesium in the second, and the alkalis in the third. As applicable in general, the method is as follows.

(1) *Determination of silica*—Weigh 10 mg of the sample into a small platinum crucible and cover with 0·15 g of anhydrous sodium carbonate. Cover the crucible and ignite at 950–1000° C. for at least 1 hr. Cool in the oven, add 3 ml of water, cover with a pierced watch-glass and cautiously add hydrochloric acid dropwise. When gas evolution ceases, heat on the water-bath for 30 min., rinse the watch glass and evaporate to dryness. Successively repeat the evaporation with two 1-ml portions of conc. hydrochloric acid. Treat the residue on the water-bath with 4 to 5 ml of diluted hydrochloric acid (1+3), filter hot through a platinum filter-stick, and wash with three 0·5 ml portions of diluted hydrochloric acid (1+3) and then with small quantities of hot water. Retain the filtrate and washings. Dry the residue first on the water-bath and then on the hot plate, add 3 drops of conc. sulphuric acid and evaporate to dryness. Heat at 950–1000° for 3 hr., cool, place first in the desiccator and then for at least 10 min in the balance case. Weigh at the 25th min. after cooling. Re-heating to obtain constant weight is usually unnecessary. Evaporate with 4 to 5 ml of hydrofluoric acid and 0·5 ml of nitric acid, then with 0·1 ml of sulphuric acid and 1 ml of hydrofluoric acid. Ignite at 950° C. to constant weight. The loss in weight gives that of "insoluble silica." Evaporate the filtrate and washings and take up with conc. hydrochloric acid. Filter and ignite as above to obtain the weight of "soluble silica." A single evaporation with a few drops of hydrochloric acid is sufficient, and the ignition-time may be reduced to 30 min.

(2) *Determination of aluminium, ferric, calcium and magnesium oxides*—Weigh 20 mg of the sample into a platinum crucible and add 0·5 ml of water and 2 ml of hydrofluoric acid. Heat on the water-bath for 30 min., cool, add 0·2 ml of conc. sulphuric acid and continue heating to constant vol. Add 1 ml of hydrofluoric acid and heat until its odour has gone. Drive off the sulphuric acid by gentle heating on an aluminium block. Add 1·5 ml of water and heat on the water-bath, if necessary adding a drop of hydrochloric acid to obtain solution. Filter into a porcelain crucible which has been weighed with a filter-stick. Add diluted ammonia (1+3) dropwise in the cold until pptn.

begins and then add a drop of hydrochloric acid to dissolve the ppt. For every 10 mg of iron or aluminium add, with stirring, 1·5 to 2 ml of 8-hydroxyquinoline soln. (Dissolve 4 g in 8 g of glacial acetic acid by gentle warming. Add 88 g of boiling water and heat on the water-bath until clear.) Cover the crucible, heat on the water-bath for 5 min., add 1 ml of 50% ammonium acetate soln. and 5 drops of 10% ammonia, and leave on the water-bath for a further 10 min. Add a few drops of ammonia, leave for 5 min. and filter. Wash the ppt. thoroughly with 5 or 6 small portions of hot water (retain the filtrate and washings). Heat with a Teclu burner, beginning with a small flame, and hold at max. temp. for 30 min., then cool for 5 min. on one nickel block, for 5 min. on another, for 10 min. in the desiccator and finally for 10 min. in the balance case. Weigh. Treat the crucible similarly to find its tare and thus obtain the weight of aluminium and ferric oxides. Evaporate the filtrate and washings in a Pyrex capsule. Cover with a watch-glass, add 2 ml of *aqua regia* and warm very gently on the water-bath. Rinse the watch-glass and evaporate to dryness. Transfer to a platinum crucible and ignite gently until white. Take up with a few drops of dil. hydrochloric acid, filter and transfer to a weighed Emich beaker. The vol. should not exceed 2 to 3 ml. Heat to boiling, add 0·5 ml of 3% oxalic acid soln., then 10% ammonia dropwise with stirring until just opalescent. Add 1 drop of methyl red and, if necessary, 1 drop of hydrochloric acid to render slightly acid. Warm, dilute to not more than twice the original vol., and add ammonia dropwise until the colour is yellow. Leave for 2 hr., filter, wash with small portions of very dil. ammonium oxalate soln. and then cold water (retain the filtrate and washings). Dry for 30 min. at 110° C. in a Benedetti Pichler drying apparatus and weigh the calcium as $CaC_2O_4.H_2O$ at the 20th min. after cooling. Evaporate the filtrate and washings to dryness in a Pyrex capsule. Cover with a watch-glass and add 2 ml of *aqua regia*. When the reaction ceases, rinse the watch-glass, evaporate to dryness and steadily raise the temp. to 200° C. Add 0·5 ml of *aqua regia* and again evaporate to dryness. Take up with water containing 1 drop of dil. hydrochloric acid and transfer to a beaker weighed with a filter-stick. The vol. should not exceed 1 ml; evaporate if necessary. Add 0·2 ml of 15% ammonium chloride soln. and 0·5 ml of 10% disodium hydrogen phosphate soln. Boil and add ammonia, with stirring, until alkaline. Leave for 30 min., wash with small portions of ammonia and then with methyl alcohol. Dry to constant weight at room temp. in a current of air and weigh the magnesium as $MgNH_4PO_4.6H_2O$.

(3) *Determination of alkalis*—Weigh 20 mg of the sample into a platinum crucible. Add 0·2 ml of conc. sulphuric acid and 4 ml of hydrofluoric acid and heat first on the water-bath and then on a nickel block until white fumes appear. Add 1 ml of hydrofluoric acid and evaporate to dryness. Take up with 2 ml of warm double-distilled water (used throughout in this determination) and filter into a large platinum capsule containing 0·15 g of fresh calcium oxide and 10 ml of water. Evaporate to one-third vol., filter, warm and ppt. the bulk of the calcium with ammonium carbonate. Filter into a platinum capsule, evaporate the filtrate to dryness and drive off ammonium salts by gentle heating. Take up the residue with a few drops of water and 1 drop of hydrochloric acid and ppt. the remaining calcium with ammonium oxalate. Leave for 2 to 3 hr., filter into a platinum crucible, evaporate and heat to drive off ammonium salts. Take up with warm water and filter into a platinum crucible (previously heated to 750° C. and weighed). Add 2 or 3 drops of conc. sulphuric acid and evaporate first on the water-bath, then on an aluminium block. Heat at 700–750° for 1 hr. and weigh the alkaline sulphates. Dissolve the residue in 2 or 3 ml of water. For every mg of sodium add 8 ml of water and 12 ml of uranyl acetate reagent. (Dissolve 3·2 g of uranyl acetate and 10 g of magnesium acetate in 2 ml of dil. acetic acid, 50 ml of alcohol and 30 ml of water. Heat on the water-bath, cool, make up to 100 ml, leave for 48 hr., filter and store in a brown bottle in the dark.) Leave for 16 to 24 hr. in the dark, filter through a Pregl filtering tube, wash first with the reagent, then with abs. alcohol and dry at 110° C. Weigh the sodium as $NaMg(UO_2)_3(C_2H_3O_2)_9.6H_2O$.

J. T. S.

Physical Methods, Apparatus, etc.

Determination of Formaldehyde in Presence of Acrolein and Other Aldehydes by the Polarographic Method. G. C. Whitnack and R. W. Moshier (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 496–498)—In alkaline media, the polarographic wave of formaldehyde occurs between the two waves of acrolein and encroaches on the first of these. This encroachment may be prevented by using a lithium hydroxide—lithium chloride base soln. containing the maxima inhibitor and by keeping the concn. of aldehydes low. *Base soln.*—Add 1 ml of 0·2% alcoholic methyl red and 1·5 ml of 0·02% alcoholic bromocresol green to 1 litre of a soln. which is 0·1 *N* in lithium hydroxide and 0·01 *N* in lithium chloride. *Method*—Add the sample (containing 1 to 6 mg of formaldehyde) to the base soln. in the cell so that the final vol. is 105 ml. Polarograph between applied voltages −1·40 and −1·70. (A manual instrument, the Fischer Electropode, was used. The drop rate was 6·3 sec., the drop wt. 0·0045 g, and the galvanometer sensitivity one-fifth.) Measure the height of the formaldehyde wave, which appears in the range −1·50 to −1·65 volts. The height is proportional to the concn. of formaldehyde. Removal of dissolved oxygen is unnecessary, but for accurate work the temp. should be kept constant. No loss of formaldehyde occurs on leaving up to 30 min. before polarographing, but acetaldehyde and acrolein must be determined immediately after adding the sample to the base soln. With 6 mg of formaldehyde, not more than 0·75 mg of acrolein may be present, but with 1·2 mg of formaldehyde, 2·2 mg of acrolein and 2·74 mg of acetaldehyde do not interfere. The accuracy is ±2%. Propionaldehyde may also be present, but gives low results unless the determination is made immediately after adding the sample to the base soln.

J. T. S.

Paraffin Wax Absorptiveness of Paper. T.A.P.P.I. Tentative Standard T467 m-45. Anon. (*Paper Trade J.*, 1945, **120**, Feb. 15, *T.A.P.P.I. Sect.*, 57)—The sample (3 × 8 in.) is conditioned and weighed, and placed between sheets of waxed blotting paper (water absorptiveness by the T.A.P.P.I. Standard T432 m, 9–20 sec.; thickness, 0·0195–0·0205 in., substance, 80 ± 4 lb., 19 × 24/500) in contact with the felt sides. The blotting paper is previously immersed for 30 min. in paraffin wax (m.p. 54·4°–55·6° C.) at 71° ± 2° C., allowed to drain for 1 hr. at 105° ± 2° C., and cooled. The pad is placed between 2 cold-rolled

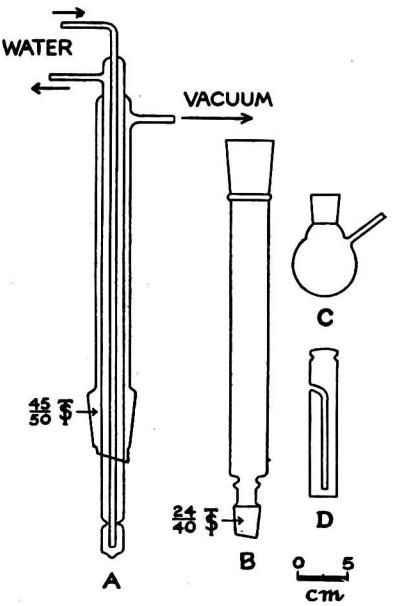
steel plates (thickness, 0·25 in.) which are taken directly from an oven at 105° ± 2° C., and the resulting combination is replaced in this oven for 1 hr. The increase in wt. of the conditioned sample, expressed as a % on the wt. of the unwaxed sheet, is recorded. J. G.

New Method for the Determination of the Suitability of Component Parts for V-Board Production. Buoyancy Test. R. W. K. Ulm (*Paper Trade J.*, 1944, **119**, 31st Aug., *T.A.P.P.I. Sect.*, 87–88)—The method is based on the fact that hard-sized papers become more buoyant, and soft-sized papers less buoyant, when immersed in water. It is concluded that, assuming rosin and/or wax sizing is used to obtain the specified wet strength, then if the sample definitely loses wt. in the test it is sized suitably for V-board production. The magnitude of the change in wt. is of less importance, and no definite conclusions can as yet be drawn from it. J. G.

Estimation of the Inflammability of Treated Paperboard. Anon. (*Paper Trade J.*, 1944, **119**, 27th July, *T.A.P.P.I. Sect.*, 39)—A revision of T.A.P.P.I. Suggested Method T 461 sm-43 applicable to samples not more than 1/16 in. thick, which have been treated to prevent the spread of flame when ignited. In general papers which have a mean char length of over 4·5 in., with a max. value for any one specimen of over 5·5 in., are not considered sufficiently flame-resistant to be effective, but it is not intended that this statement should represent an official standard of flame-resistance. J. G.

Additional Weight Factors of Pulps. J. H. Graff (*Paper Trade J.*, 1944, **119**, 12th October, *T.A.P.P.I. Sect.*, 155–156)—In the evaluation of mixtures of fibres (*e.g.*, in pulp or paper) by counting the average numbers in fields observed under the microscope, it is necessary to multiply the % of each fibre estimated to be present by a "weight factor" in order to obtain the % present by wt. This factor allows for differences in the dimensions of different fibres (*cf.* Graff, *id.*, 1940, **110**, 11th Jan., *T.A.P.P.I. Sect.*, 23). Standard factors for some common fibres have been determined, and are as follows: rag, 1·00; groundwood, 1·30; coniferous western sulphite, 1·23; coniferous unbleached and bleached sulphite and kraft, 0·90; coniferous southern kraft, 1·57; coniferous alpha, 0·70; gumwood chemical pulps, 1·03; hardwood soda, 0·50; hardwood sulphite, 0·60; hardwood kraft, 0·70; hardwood alpha, 0·55; bagasse, 1·58; raw straw, 0·64; unbleached and bleached straw, 0·36; cane (unbleached and bleached), 0·75; bamboo (unbleached and bleached), 0·56; esparto (unbleached and bleached), 0·51; flax (unbleached and bleached), 0·49; flax hurds, 0·35; jute (unbleached and bleached), 0·55; manila and sisal (unbleached and bleached), 0·61; ramie, 0·51; mitsumata, 0·32; kozo, 1·38; virgin cotton linters (unbeaten), 1·50. J. G.

An Extractor for Use with Reduced Pressure. W. F. Barthel (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 53)—When used under vacuum the usual Soxhlet apparatus floods and the siphon does not function properly. The apparatus described permits the low-temperature extraction of sensitive



plant materials with solvents of high normal b.p. by heating reflux under reduced pressure. A wad of cotton wool is placed evenly at the bottom of thimble D. The sample (20 to 30 g) is then introduced, followed by a second wad and a circle of wire screening retained by the projections at the upper end of D. The thimble is inserted into B and rests on the projections near the bottom of B. Condenser A and boiling flask C are then attached. Solvent is introduced into C through the side tube, which is then closed with a sound cork carrying a capillary to give smooth ebullition. An ice-trap in the vacuum line prevents loss of solvent. The apparatus was used on the water-bath with solvents of b.p. 90–125° C. (760 mm). The solvent passes through the thimble at *ca.* 2 ml per min. By dispensing with the capillary and adding boiling-chips the extractor may be used at atmospheric pressure. J. T. S.

Reviews

TEXT-BOOK OF PHARMACEUTICAL CHEMISTRY (BENTLEY AND DRIVER) REVISED BY J. E. DRIVER. 4th Ed. Pp. 644. London: Oxford University Press, 1945. Price 21s.

This work is essentially a text-book for those studying pharmaceutical chemistry in Great Britain and other parts of the British Empire and who have already reached Intermediate Science standard. The publication of the Second to the Sixth Addenda to the British Pharmacopoeia 1932 has necessitated the revision of much of the subject matter of previous editions. Unfortunately the revision was completed before references to the Seventh Addendum, which became official from February 1st, 1945, could be included.

The book is divided into three main parts. Part I gives a general account of methods for the determination of the purity of pharmaceutical substances. The chapters of this

section dealing with hydrogen ion concentration were written by Dr. E. B. R. Prideaux. Part II deals primarily with inorganic compounds in common use in pharmacy and pharmacopoeial tests for possible impurities. Part III treats of organic preparations, tests for purity, quantitative determinations, alkaloidal assays and organic and inorganic analysis, and contains a long section on other substances of pharmaceutical interest such as vitamins and hormones.

Analysts will be interested in the copious references to original papers in THE ANALYST and "*The Quarterly Journal of Pharmacy and Pharmacology*" throughout the book. The text is marred by occasional inaccuracies; *e.g.*, calciferol is not vitamin D_1, as is stated several times, but D_2. A serious omission noted is the absence of any reference to the importance of drying substances before melting-point determinations are made, although nearly two pages are devoted to this subject. The book, however, is well written and produced and should be of considerable use to workers in pharmaceutical fields generally as well as advanced students.

S. K. CREWS

THE DISCOVERY OF THE USES OF COLOURING AGENTS IN BIOLOGICAL MICRO-TECHNIQUE. By J. R. BAKER, D.Sc. Monograph of the Quekett Microscopical Club. Pp. 22. London: Williams & Norgate, Ltd. 1945. Price 1s. 6d.

This monograph is not only a valuable addition to the history of micro-technique in biology but will also interest the general analyst. It deals with the use of colouring matters under six headings and describes the development of tests in each: (1) Non-vital Dyeing (Leeuwenhoek, 1714). (2) Injection of Coloured Substances (Ruysch, 1723). (3) Vital Colouring of Pre-existing Structures (Trembley, for the *Hydra*, 1774). (4) Colouring by Phagocytosis (von Gleichen, 1778). (5) Histochemical Colour Tests (Link, iron tests for tannin, 1807). (6) Impregnation with Opaque Substances (Krause, use of silver nitrate, 1844).

The most interesting point brought out by Dr. Baker is the way in which colour tests, now well known, have been forgotten and then re-discovered and called by the name of the later discoverer. Thus the Adamkiewicz test for proteins (1878) is merely a modification of Raspail's test (1828) with sugar and sulphuric acid; both depend on a reaction of an aldehyde with tryptophan. In the Adamkiewicz reaction glyoxylic acid, present as an impurity in the acetic acid used in place of sugar, supplied the aldehydic group. Incidentally, Dr. Baker states that in his experience Raspail's original test is more sensitive for histochemical work than the modern test with vanillin and hydrochloric acid. Raspail also described (1829) what was later termed the xanthoproteic reaction and the so-called Liebermann's reaction for proteins. Again, Franz Schulze of Rostock demonstrated in 1850 the use of zinc chloride with potassium iodide and iodine to distinguish between cellulose and cuticular substances, but the reagent is now commonly known as "Hertzberg's Stain." Similarly, Runge discovered in 1834 that solutions of salts of "Kyanol" (aniline) stain spruce wood yellow, but the method was not introduced into micro-technique until 1867. Several other striking instances of re-discovery are cited, and the author attributes this neglect of earlier work to histochemical methods being overshadowed by the use of dyes and to the publication of *The Origin of Species*, which had caused interest to be centred on the morphological aspect of biology.

It could not have been an easy task to prevent the large number of facts collected for the monograph from becoming a repellent list, but Dr. Baker has succeeded in weaving them into an eminently readable account. At the end there is a useful detailed bibliography containing 73 items.

EDITOR

FORMATION OF A GROUP DEALING WITH BIOLOGICAL METHODS OF ANALYSIS

IN pursuance of the policy for the formation of Groups for special branches of analytical chemistry, the Council has decided to form a Group to be known as the Biological Methods Group. The objects of the Group will be to promote discussion of all aspects of the design, technique and computation of quantitative assays in which measurements of the response of living organisms, or parts thereof, to the substance assayed provide data from which the result is calculated. The Group should be of special interest to members of the Society concerned with vitamins, hormones, insecticides, chemotherapy, industrial toxicology and the toxicology of new drugs, pharmacology and statistics. The Group will deal with methods of analysis and not with substances as such.

Members of the Society who wish to become members of the Group are asked to notify the Hon. Secretary of the Society, 7–8, Idol Lane, London, E.C.3. Members who have already notified Mr. E. C. Wood need not make further application.

ADVICE TO AUTHORS

THE Council has approved the following notice by the Publication Committee, which is here given in condensed form.

The Society publishes papers concerned with all aspects of analytical chemistry, inorganic and organic, including chemical, physical, biological, bacteriological and micro methods. Papers on these and allied subjects, by members of the Society or non-members, may be submitted for presentation and publication; they may:

(1) record proposals for new methods and the investigations on which the proposals are based;
(2) record the results of original investigations into known methods or improvements therein;
(3) record analytical results obtained by known methods where these results, by virtue of special circumstances, such as their range or the novelty of the materials examined, make a useful addition to analytical information;
(4) record the application of new apparatus and new devices in analytical technique and the interpretation of results.

Communications.—Papers (which should be sent to the Editor) will normally be submitted to at least one referee, on whose advice the Publication Committee will be guided as to the acceptance or rejection of any communication. Papers or Notes accepted by the Publication Committee may not be published elsewhere except by permission of the Committee.

Abstracts.—The MS. should be accompanied by a brief abstract of about 100 to 150 words indicating the scope and results of the investigation.

Proofs.—Proofs should be carefully checked and returned within 48 hours. Two galley proofs* will normally be sent out, one of which should be retained by the Author.

Reprints.—Ten Reprints are supplied gratis to the Author. Additional reprints may be obtained at cost if the Author orders them directly from the printers, W. Heffer & Sons Ltd., 104, Hills Road, Cambridge, at the time of publication. Details are sent to Authors with the proofs.

Notes on the writing of papers for THE ANALYST

Manuscript.—Papers and Notes should preferably be typewritten.

The title should be descriptive and should set out clearly the scope of the paper. Degrees are now omitted after the names of Authors in the headings of papers.

Conciseness of expression should be aimed at; clarity is facilitated by the adoption of a logical order of presentation, with the insertion of suitable paragraph or sectional headings.

Generally, the best order of presentation is as indicated below:

(*a*) General, including historical, introduction.
(*b*) Statement of object of investigation.
(*c*) Description of methods used. Working details of methods are usually most concisely and clearly given in the imperative mood, and should be given in this form, at least while economy of paper is pressing, *e.g.*, "Dissolve 1 g in 10 ml of water and add . . .". Well-known procedures must not be described in detail.
(*d*) Presentation of results.
(*e*) Discussion of results.
(*f*) Conclusions.

followed by a short summary (100 to 250 words) of the whole paper: items (*e*) and (*f*) can often be combined.

Illustrations, diagrams, etc.—The cost of setting up tabular matter is high and columns should therefore be as few as possible. Column headings should be brief or replaced by a number or letter to be used in combination with an explanatory footnote to the table.

Sketches or diagrams should be on white Bristol board, not larger than foolscap size, in Indian ink. Lettering should be in light pencil.

Tables or graphs may be used, but normally not both for the same set of results. Graphs should have the co-ordinate lines clearly drawn in black ink.

References.—References should be numbered serially in the text and collected in that order under "REFERENCES" at the end of the paper. They should be given in the following form:

1. Dunn, J. T., and Bloxam, H. C. L., *J. Soc. Chem. Ind.*, 1933, **52**, 189T.
2. Allen, A. H., "*Commercial Organic Analysis*," Churchill, London, 1882.

Notes on the Presentation of Papers before Meetings of the Society are appended to the "ADVICE," copies of which may be obtained on application to the Secretary, 7/8, Idol Lane, London, E.C.3

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* During the paper shortage two copies of the MS. will not be insisted on, nor will two galley proofs be sent.

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Printed for the Society of Public Analysts and other Analytical Chemists by W. Heffer & Sons Ltd., Hills Road, Cambridge. **Communications to be addressed to the Editor, C. Ainsworth Mitchell, The Close, Weedon, Aylesbury, Bucks.**