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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

A JOINT Meeting of the Society with the Food Group of the Society of Chemical Industry was held at 2.30 p.m. on Wednesday, December 6th, 1944, at The Chemical Society's Rooms, Burlington House, London, W.1. The chair was occupied by Dr. H. E. Cox, Chairman of the Food Group. The subject of the meeting was "Methods of Sampling Foodstuffs for Analysis," and the following papers were presented and discussed:—"Some Experiences in the Sampling of Foodstuffs in Bulk," by Mr. J. King; "Sampling of Cooked Meals for Nutritional Analysis," by Dr. C. A. Mawson; "Sampling for Metabolism Studies," by Dr. E. M. Widdowson.

NEW MEMBERS

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Obituary

SIR JOHN J. FOX

JOHN JACOB FOX, eldest son of Mark and Hannah Fox, was born in London on April 12th, 1874, and died on November 28th, 1944. He received his scientific education at the Royal College of Science, South Kensington, and at Queen Mary College, London, taking the B.Sc. degree by research in 1908 and the D.Sc. degree two years later. He was elected a Fellow of the Institute of Chemistry in 1916. He entered the Government Service in 1896 and was appointed to the permanent staff of the Government Laboratory in 1904. He became Superintending Chemist in 1920, Deputy Government Chemist in 1929, and Government Chemist in 1936.

His early years in the Government Laboratory were spent in the Crown Contracts Department, where he was concerned, at first, with the analysis of a great variety of materials, but soon he was chosen by Sir Edward Thorpe to assist him in the work of the Departmental Committee set up by the Home Office to ascertain how far the danger of plumbism might be diminished, or removed, by substituting for white lead either less soluble compounds of lead or "leadless" glaze. At the outbreak of the first world war Fox, now in charge of the Crown Contracts Department, was called upon to meet a greatly increased volume of work for the Armed Forces. He devised rapid methods of analysis for both ferrous and non-ferrous alloys, enabling the Department to carry on this work without serious increase of staff. At the same time his wide knowledge of chemistry enabled him to advise Departments on many matters relating to the war effort, such as captured materials and seized cargoes. Soon after the end of the war the Safeguarding of Industries Act was passed. The Act threw a considerable volume of additional work upon the Laboratory. Fox was called upon to organise the Section dealing with this work. It was largely owing to his encyclopaedic knowledge of organic chemistry and his sound judgment that the administration of the Act proceeded smoothly. About this time his attention was also directed to official matters of more than usual interest, including the causes of decay of buildings, pollution of rivers by drainage from tarred roads and the cleaning and restoration of wall paintings in buildings under the charge of the Office of Works—notably in the Chapter House at Westminster and in the Orangery at Hampton Court. Later still he was asked to undertake the organisation of Sections of the Laboratory concerned with the duties on silk and artificial silk and on hydrocarbon oils.

As Government Chemist he sat upon the Departmental Committee appointed by the Home Office to enquire what steps were desirable and practicable to be taken against the

danger arising to the public, especially children, from the use of celluloid articles. He was also greatly concerned with the extension of the uses of duty-free alcohol in industry. At the outbreak of the present war he threw himself with great energy into scientific matters connected with its prosecution. This aspect of the work of his last years cannot yet be described in detail, but it can be stated that he served on numerous Departmental Committees and was a member of the Hydrocarbon Oil Duties Committee at the time of his death.

Throughout his official career Fox found time to undertake a great deal of research, some of it arising from ideas suggested by his official work. His interest in research lay, at first, in organic chemistry, but subsequently his mind turned towards spectroscopy and its relation to molecular structure. He was interested in the ultra-violet absorption spectra of alkaloids, of sulphur, of the halogens and of light elements. Later, infra-red spectroscopy occupied his attention. In collaboration with some of his colleagues he published many papers in this branch of chemistry.

Fox had a very alert mind, a photographic memory and abundant energy. Besides the numerous research institutions to which he was appointed in his official capacity he gave his time freely to the work of scientific societies. He was a Past President of the Royal Institute of Chemistry and of the Oil and Colour Chemists Association and a Vice-President of the Society of Chemical Industry. He served on the Council of the Chemical Society and was a manager of the Royal Institution. He always had at heart the welfare of Queen Mary College, its students and its old students. He became a Fellow of Queen Mary College in 1937.

He was always willing to help; he never allowed red-tape to interfere with his official contacts with industry. He gladly saw the representatives of chemical manufacturers and other traders and freely gave his knowledge and experience to help them to overcome those difficulties inseparable from governmental control. Although his whole life was devoted to the service of chemistry, he yet found time to act as treasurer of his church for many years. He was kind and generous to his colleagues and lost no opportunity to encourage those young members of his staff who showed a lively interest in chemistry. His enthusiasm for chemistry remained to the end, when he could still be seen moving from room to room of the Laboratory asking, suggesting, encouraging.

His services to the country were rewarded by the honour of the O.B.E. in 1920, of the C.B. in 1938 and of a Knighthood in 1944; his services to chemistry were acknowledged by the Royal Society in 1943 when he was elected a Fellow. He leaves a widow, a son and a daughter.

A. G. FRANCIS

The Riboflavin Content of Tea

and some Results for the Pantothenic Acid Content

BY E. A. M. BRADFORD AND E. B. HUGHES

IN January of last year Drummond and Moran,[1] in a communication entitled "Unconsidered Trifles in Our Diet," drew attention to the riboflavin content of tea, of the order of 9 μg per g, and pointed out that the daily quantity of this vitamin so supplied "would certainly not be negligible in the case of the habitual tea drinker." We therefore thought it desirable to determine the riboflavin content of a wide range of individual (*i.e.*, unblended) teas and have found, as results reported here show (Table IV), that from black teas (Indian type) the amount of riboflavin yielded in ordinary infusions, as consumed, is fairly regular—about 9 μg per g of dry tea—and from China tea (2 samples) a rather lower amount—about 6–7 μg per g.

The assays were made by the microbiological method of Snell and Strong,[2] using *Lactobacillus helveticus*, with the exception that, in the culture medium, yeast supplement prepared from pressed (bakers') yeast was used in place of the Difco yeast extract, which was not available. As the method of preparing this supplement from yeast may be of use to other analysts, the procedure is described.

EXPERIMENTAL

PREPARATION OF YEAST SUPPLEMENT—Grind 50 g of fresh pressed yeast* to a paste with 25 g of sand and a few drops of distilled water. Transfer this paste with distilled water

* It is essential to use a yeast proved suitable by trial.

(total vol. used, 200 ml) to a 500-ml conical flask, plug the flask, steam for 2 hr., autoclave at 15 lb. for 45 min. and cool. Decant the liquor and treat 50 ml of it with basic lead acetate and so on, as described by Snell and Strong for Difco yeast extract, and, after filtration from the basic lead acetate precipitate, make up to 50 ml with distilled water. Use 20 ml of this de-riboflavinised extract in each 250 ml of medium. We have found it necessary to make the yeast extract fresh (starting from pressed yeast) for every batch of medium.

PROCEDURE FOR THE DETERMINATION OF THE RIBOFLAVIN CONTENT OF TEA INFUSIONS—The assays of riboflavin content were made on infusions of tea prepared as the tea is usually "made" for drinking (1 part of tea with 40 parts of boiling water) so that results represent the amounts of riboflavin ingested in the drinking of tea; the infusion was diluted, as required, for the determinations.* Trials on such infusions without preliminary treatment other than dilution showed that results obtained at different "levels" were sometimes erratic, and that there was apparently some factor causing interference with response of growth of organism to the riboflavin in the infusion; it was with the object of minimising this effect (more necessary for unblended tea) that the method of pre-treatment of the infusion ("creaming"), later described, was devised.

The most satisfactory method of overcoming the uncertainty would be to use, for the standard curves, a "blank" of tea infusion de-riboflavinised by the action of light, to which the standard amounts of riboflavin had been subsequently added. This was tried (on alkalised solution) but, presumably owing to interference by the colouring matter of the tea infusion, the action of light had to be long—as much as 4 days in daylight. Such a procedure is obviously unsuitable, chiefly because of uncertainty of complete de-riboflavinisation. Consequently another method was sought, preferably one which would leave the tea infusion as little altered as possible. The principal constituents likely to be causing irregularity in the results are caffeine and the polyphenols ("tea-tannin") and accordingly tests were made to ascertain their effects.

EFFECT OF CAFFEINE AND OF POLYPHENOLS IN THE ASSAY—*Caffeine*—A solution of caffeine of approximately the same concentration as contained in the 1·25% tea infusion, namely, 0·05%, was tested. The results† (Table I) showed neither stimulation nor inhibition of growth of the organism. The figures recorded in these tables are also of interest in indicating the degree of precision of this microbiological method.

TABLE I

ml of 0·05% caffeine soln.	Riboflavin added μg	ml of *N*/10 acid produced (duplicates)	Riboflavin determined μg
0·5	0·1	5·4; 5·6	0·100
1·0	0·1	5·3; 5·5	0·098
2·0	0·1	5·4; 5·5	0·099
3·0	0·1	5·6; 5·4	0·100
4·0	0·1	5·5; 5·4	0·099
5·0	0·1	5·6; 5·6	0·102
—	—	(blank) 0·3; 0·4	—

Polyphenols—It has been shown by Bradfield and Penney[3] that the polyphenols of tea are composed of two main fractions, one of which can be extracted from tea infusion by ethyl acetate. These ethyl acetate soluble polyphenols appear to form the major portion of the polyphenols that separate as a precipitate with caffeine when tea liquor "creams" (*i.e.*, becomes cloudy or opaque on cooling, without addition of milk).

This ethyl acetate soluble fraction of tea polyphenols was used in place of tea infusion for the purpose of testing its action, and results (Table II) showed that it has a distinct inhibitory effect.

Tea infusion that had been extracted with ethyl acetate was also tested in the same way as unextracted tea infusion. Results showed amounts of riboflavin of the order of the results given in Table IV, but there was still some irregularity. This indicates that the ethyl acetate soluble fraction of the polyphenols is not the sole cause of the interference and that the residual polyphenols also have a similar effect.

As in the "creaming" of tea portions of both of these fractions of the polyphenols separate (with caffeine as "caffeine theotannate"), it was thought that removal of part of the

* All results were calculated from standard curves prepared for each batch.

† The full procedure specified by Snell and Strong, including autoclaving, was applied in every test.

polyphenols in this way might be sufficient to allow of satisfactorily regular results for riboflavin content being obtained. Some teas, chiefly, it seems, those that have a low proportion of their polyphenols soluble in ethyl acetate, do not form much "cream" and may scarcely "cream" at all—yet infusions of such teas submitted to the creaming technique did give more satisfactory results.

TABLE II

ml of 0·2% polyphenols soln.	Riboflavin added μg	ml of $N/10$ acid produced (duplicates)	Riboflavin determined μg
0·5	0·1	4·9; 4·9	0·085
1·0	0·1	4·7; 4·7	0·080
1·5	0·1	4·2; 4·6	0·075
2·0	0·1	3·8; 4·0	0·065
3·0	0·1	3·0; 3·2	0·05
4·0	0·1	1·1; 1·2	0·01
5·0	0·1	0·2; 0·15 (blank)	nil

METHOD OF "CREAMING" TREATMENT OF THE INFUSION FOR ASSAY—To 50 ml of the 2·5% infusion were added 2·5 ml of a 2·0% solution of caffeine,* the mixture was kept cold (0°–5° C.) overnight and then centrifuged while cold. The clear liquor was then decanted and 25 ml of it were diluted to 100 ml., giving the equivalent of a 0·60% infusion. (Actually dilution to 200 ml instead of to 100 ml was found more suitable, and this was done for the determinations recorded in Table IV.) Table V shows the degree of uniformity of results obtained at different "levels." Table IV gives results as determined for a number of teas, to the nearest μg per gram.

TABLE III

ml of 0·60% "creamed" infusion	ml of $N/10$ acid produced (duplicates)	Riboflavin μg per g
0·5	1·7; 1·8	9·6
1·0	3·4; 3·4	9·6
2·0	6·8; 6·9	9·5

TABLE IV

Tea No.	Country of origin	Type†	Riboflavin μg per g	Pantothenic acid μg per g
1	N. India	B.P.S.	10	26
2	,, ,,	B.P.	10	29
3	,, ,,	B.O.P.	9	31
4	,, ,,	P.F.	10	36
5	,, ,,	B.O.P.	7	28
6	,, ,,	O.F.	8	36
*7	,, ,,	—	9	20
*8	,, ,,	—	7	19
*9	India	—	11	21
10	Ceylon	B.O.P.	10	40
11	,,	,,	7	—
12	S. India	,,	8	—
13	,, ,,	,,	7	—
14	Kenya	,,	9	—
15	Nyasaland	B.P.	9	—
*16	,,	—	11	14
17	China	Keemun	7	—
18	,,	Lapsang	6	—
*19	,,	Gunpowder (green)	6	15

† B = Broken F = Fannings O = Orange P = Pekoe S = Souchong

* These samples were four years old.

It is of interest to note that practically the whole of the riboflavin passes into tea infusion prepared as tea is usually "made" for drinking; for example, a sample of finely ground tea extracted by boiling for 1 hour under a reflux gave about the same result (11 μg) as was obtained from the ordinary infusion (10 μg).

* This addition is not essential generally, but is made in case the tea should containin sufficient caffeine for pptn. of the "caffeine theotannate"; also it sometimes hastens precipitation.

Occurrence of Pantothenic Acid in Tea—As there appears to be no record of the occurrence of pantothenic acid in tea, it was thought desirable to proceed also with this determination[4] (using a different medium but employing *Lactobacillus helveticus*), and for this we used "creamed" tea infusions (see above).

As in preparing the medium for riboflavin, so also for these pantothenic acid tests we found it necessary to prepare the yeast extract from pressed yeast, and used the following procedure.

Alkali-treated Yeast Extract—75 g of pressed yeast with 1000 ml of distilled water were steamed for 2 hr., cooled and centrifuged and the supernatant liquor was removed and concentrated (under reduced pressure, water pump) to 180–200 ml. This corresponds approximately to the 20 g of Difco yeast extract specified in the instructions[4] for the preparation of the medium; subsequent procedure was as there described.

Results—In Table IV (last column) are the results found for the pantothenic acid content of twelve of the teas listed in the Table. These quantities are appreciable, and tea may therefore be considered a useful source of riboflavin and of pantothenic acid. For example, on the common basis of 1 teaspoonful of tea (2 to 2·5 g) plus a little "for the pot"—say 1/4th—for a cup of tea (generally about 5–6 oz. of infusion) the amounts of these vitamins so supplied are about 25 μg and 75 μg respectively. These quantities are exclusive of amounts derived from the milk added.

We desire to thank J. Lyons & Co., Ltd., in whose laboratory this work was carried out, for permission to publish.

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The Laboratories
J. Lyons & Co., Ltd., London, W.14 *November*, 1944

Determination of Hydrogen Sulphide in Foods and Waters

By DENIS DICKINSON

The detection and determination of hydrogen sulphide in presence of organic matter presents many difficulties. Most of the proposed methods are modifications of the iodimetric, lead acetate, or methylene blue reactions. Some attention has also been paid to Lestelle's argentometric method[1] which, since the reaction involves a characteristic colour change, could be most useful. The end-point, however, has hitherto been difficult to determine except by electrochemical means.[2] Horner[3] employed the method in neutral solution, nitric acid equiv. to the quantity of hydrogen sulphide added being liberated,[4] but it could not be used in presence of sulphur dioxide. I suggested titration of the sulphide with ammoniacal silver nitrate soln., using *p*-dimethylamino-benzal-rhodanine as external indicator,[5] and the present paper describes the development of this method and its application to the determination of very small quantities of hydrogen sulphide in canned foods and polluted waters.

Experimental—Preliminary expts. indicated that it was essential to separate the hydrogen sulphide from organic matter by distillation in an inert atmosphere. Kapp[6] showed the danger of losing the sulphide by oxidation by dissolved oxygen, and, to avoid this, all oxygen must be removed from the apparatus prior to the distillation, and, if dilution of the distillate is necessary, only recently boiled water must be used.

Separation of Hydrogen Sulphide by Distillation—Fit a 1500-ml round-bottomed flask with a cork, bored to take a still-head and a narrow glass tube extending to the bottom of the flask. Connect a condenser to the delivery of the still-head and a delivery-tube to the condenser. Add about 750 ml of distilled water and a few fragments of porous pot to the flask and pass a slow stream of pure nitrogen through the water. It has been the practice to use commercial nitrogen from a cylinder after purification by passing through two

gas wash-bottles containing alkaline pyrogallol. With the nitrogen flowing, heat the water and boil for about 5 min. Turn out the burner and increase the flow of nitrogen to prevent air being drawn into the apparatus during cooling. When the water has cooled sufficiently, add rapidly the measured quantity of material under test to the flask. Provided that the flow of nitrogen is maintained, the cork may be removed from the flask without fear of any considerable quantity of air diffusing into the apparatus. If the material is not an acid food, add also approx. 1 g of potassium dihydrogen phosphate. Replace the cork as quickly as possible and immerse the delivery tube in *ca.* 10 ml of dil. ammonia (1 vol. of sp.gr. 0·880 : 2 vols. of water) in a conical flask. As the bulk of the hydrogen sulphide is driven off almost as soon as the liquid begins to boil, the flow of nitrogen should be discontinued when heating of the flask is resumed. Allow the distillation to proceed until about 30 ml have distilled, and finally sweep out the apparatus with a rapid stream of nitrogen maintained for about 1 min. The distillate may, if desired, be made up to a standard volume with recently boiled, and cooled, distilled water.

Detection and Determination of the Hydrogen Sulphide in the Distillate—The reagent soln. used is *N*/80 silver nitrate containing a slight excess of ammonia. The indicator is a 0·03% soln. of *p*-dimethylamino-benzal-rhodanine in acetone. Titrate the distillate, or an aliquot, with the ammoniacal silver nitrate soln., adding the reagent cautiously until it is no longer possible to be sure that further pptn. of brown silver sulphide results from each drop of the reagent. Test the soln. for excess of silver by "spotting" the indicator on to a filter-paper and, *before* the spot dries, covering or streaking it with a drop of the soln. being titrated. The end-point is indicated by the appearance of a faint mauve colour in this spot-test. A little practice is needed to judge this end-point precisely, but, since one drop of the silver soln. is equiv. to only about 0·01 mg of hydrogen sulphide (as sulphur), considerable latitude is allowable.

Accuracy of the Method—This has been assessed by determination of the recoveries obtainable from pure solns. in presence and absence of organic matter and sulphur dioxide. These pure dil. solns. were prepared by distilling 1 ml of AnalaR ammonium sulphide in the apparatus and collecting about 200 ml of distillate. It was sometimes necessary to repeat this distillation to obtain a colourless soln., and a fresh soln. had to be prepared each day, as it was impossible to prevent slow oxidation of the sulphide. The concn. of this soln. was determined by titration with silver nitrate soln., as described above. Comparison of the result with an iodimetric determination was satisfactory when the soln. was freshly prepared, but, on standing, the concn. of hydrogen sulphide determined by the silver method decreased, whereas that determined by iodine remained unchanged. This is thought to indicate oxidation of the sulphide to thiosulphate. Table I illustrates the efficiency of recovery from pure solns. by the distillation method described above.

TABLE I

Hydrogen sulphide taken, mg ..	0·26	0·27	0·57	1·08	1·42	2·68
,, ,, found, mg ..	0·24	0·28	0·59	1·03	1·30	2·46
Percentage recovered ..	92	104	104	96·2	91·5	91·9

The potassium dihydrogen phosphate was added in each test to assist the liberation of the hydrogen sulphide. Recoveries in presence of organic matter of various kinds were also satisfactory, as shown in Table II.

Determinations were made with foods containing sulphur dioxide to decide two points, *viz.*, whether a large quantity of sulphur dioxide would give a false positive result due to possible auto-reduction of the sulphur dioxide[7] and whether interaction between sulphur dioxide and hydrogen sulphide would occur during distillation and give a low result for hydrogen sulphide. The figures in Table III show that neither of these reactions occurred to any appreciable extent[8]; even when the sulphur dioxide present was more than 100 times the quantity of hydrogen sulphide, 89% recovery of the latter was obtained.

Collection of the distillate in a closed flask fitted with a Peligot tube containing ammonia soln. did not increase the efficiency of recovery.

APPLICATIONS OF THE METHOD—Two immediate uses for the method have been found. The first, which was actually responsible for its evolution, was an investigation of the blackening of canned peas, when sulphide bacteria were suspected as a possible cause.[9] On another occasion definite sulphur spoilage was found in a can of potatoes. There was no possible doubt in this instance, the contents of the can yielding 77·5 p.p.m. of H_2S. As sulphide spoilage organisms are extremely difficult to culture on artificial media, a chemical method

for the determination of minute quantities of hydrogen sulphide is very useful when there is doubt.

The method has also been applied to the investigation of stream pollution. From this it is estimated that hydrogen sulphide in a polluted stream can be detected by smell when the concn. is as little as 5 p.p.m., even in presence of the other odours associated with anaerobic decomposition.

TABLE II

	Hydrogen sulphide, mg		Hydrogen sulphide recovered, %
	Added	Found	
Canned peas, 100 g	nil	0·12	—
Do.	1·34	1·24*	92·5
Do.	0·54	0·48*	89
Canned potato brine, 100 ml	nil	nil	—
Do.	0·26	0·24	92
Canned beans, 50 g	nil	0·07	—
Do.	1·76	1·61*	91·5
Canned soup (oxtail), 50 g	nil	nil	—
Do.	1·2	1·18	98·3
Canned fresh peas, 50 g	nil	nil	—
Do.	1·01	0·91	90
Canned plums (from hydrogen swell), 100 g	nil	nil	—
Do.	0·97	0·99	102
Strawberry jam	0·85	0·82	96·5

* Net, after allowing for the quantity naturally present.

TABLE III

Fruit product	SO_2 present, mg	Hydrogen sulphide, mg		Hydrogen sulphide recovered, %
		Added	Found	
Blackcurrant jam	1·9	nil	nil	—
Strawberry ,,	0·8	2·13	1·90	89
,, ,,	0·8	2·13	2·00	94
,, ,,	1·6	0·85	0·76	88·5
,, ,,	13·6	nil	trace	—
,, pulp	52·5	nil	trace	—
,, ,,	52·5	0·36	0·32	89

SUMMARY AND CONCLUSIONS—Minute quantities of hydrogen sulphide are separated from organic materials by distillation in an inert atmosphere. The quantity of sulphide is then determined by titration of the distillate with ammoniacal silver nitrate soln., using *p*-dimethylamino-benzal-rhodanine as external indicator. Oxidation of hydrogen sulphide in such dilute solns. takes place rapidly and it is thought that this fact accounts for the recoveries obtained being generally up to 10% low.

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UNIVERSITY OF BRISTOL RESEARCH STATION
CAMPDEN, GLOUCESTERSHIRE

September, 1944

A New Method for the Determination of Strychnine in Cases of Poisoning by Strychnine or Nux Vomica

By S. R. NAIDU and P. VENKATRAO

Although various modifications have been suggested, the Stas-Otto process still remains the best method for the routine extraction of alkaloids from viscera. Unless, however, much care is taken, the extraction is not quantitative and, even if quantitative, the extract is contaminated with protein and fatty matter and not suitable for direct weighing. The removal of this fatty matter or proteins by the well-known method of digestion of the alkaloidal residue with sulphuric acid and re-extraction of the alkaloid, leaves a pure product, but our experience has shown that this treatment leads to partial destruction of strychnine and it is not possible to say how much of the alkaloid has been destroyed in any particular case.[1]

In this laboratory we have used an alternative method of purification, which consists in warming the strychnine residues with a few drops of strong potassium hydroxide soln. and extracting the alkaloid directly with ether. We have found that this treatment not only yields clean alkaloidal residues of strychnine, but also appears to have no destructive effect on the alkaloid. Reference to the literature has shown that not only strychnine, but also brucine, is quite unaffected by aqueous or alcoholic potash.[2]

It is well known that visceral material can be brought into complete solution by treatment with aqueous or alcoholic potash, the fats being saponified and the proteins dissolved; this treatment would be unlikely to affect any strychnine or brucine that might be present. This consideration suggested an alternative method to the Stas–Otto process for the quantitative extraction of strychnine from viscera, and as a result of repeated tests the following method has been evolved.

Method—(1) *Alkaline destruction of the viscus*—Treat an aliquot of the minced, well-mixed material in an Erlenmeyer flask with 5 ml of 40% potassium hydroxide soln. and 5 ml of 90% alcohol for every 10 g of the sample taken. Place a funnel over the mouth of the flask with the stem downwards and heat on a steam-oven for 30 min. to 1 hr., until the whole material is completely digested and a homogeneous soln. is obtained.

(2) *Extraction of the alkaloidal substances with ether*—As soon as digestion of the visceral matter is complete, cool the contents of the flask and transfer them to a separating funnel, washing any residue in the flask first with distilled water and then with ether. Make up the vol. of ether in the separating funnel to *ca.* 50 ml, shake and allow the ether to separate. Draw off the lower aqueous layer into a second separating funnel and again shake with further 50 ml of ether. Repeat this extraction until a few drops of the ethereal layer show no trace of alkaloid after evaporation and testing with Mayer's reagent. About 7 to 8 extractions with ether are sufficient for complete extraction of the alkaloid.

(3) *Re-extraction of the alkaloidal matter from ether with dilute acid*—Distil the ether from the combined ethereal extracts, leaving about 75 ml in the residual portion. Transfer this residual ether, with the alkaloids, to a separating funnel, and rinse any residue in the flask thrice with 5-ml portions of ether into the separating funnel. Extract the alkaloid in the ethereal soln. with 25-ml portions of *N*/10 sulphuric acid until the acid extract shows no trace of alkaloid when tested with Mayer's reagent. About 4 or 5 extractions are sufficient for complete extraction of the alkaloid. Collect the combined aqueous sulphuric acid extracts in a beaker and warm over a boiling water-bath to expel all traces of ether and alcohol.

(4) *Precipitation of the alkaloid from the acid solution with Mayer's reagent*—Cool the aqueous sulphuric acid extract and treat it with Mayer's reagent, drop by drop, until there is no further precipitation, avoiding any large excess. Set aside for 2 hr., filter off the ppt. under suction on a Gooch crucible, re-transfer the filtrate to the beaker and treat it with Mayer's reagent, drop by drop, and collect any ppt. on the same Gooch crucible after 1 hr. Wash the ppt. with water acidified with a few drops of dilute sulphuric acid, and transfer the entire contents of the Gooch crucible to the same beaker in which precipitation was effected.

(5) *Liberation of the alkaloid from the precipitate with Mayer's reagent*—Treat the aqueous suspension in the beaker with a 5% soln. of sodium sulphide, drop by drop. After each addition shake the suspended ppt. and let it stand for a few minutes, and then test a drop of the supernatant liquid with a drop of lead acetate soln. on a watchglass; the appearance of a light brown colour indicates that sufficient sodium sulphide has been added. Rinse the contents of the watchglass back into the beaker, and warm on a steam-oven for *ca.* 30 min.,

occasionally stirring with a glass rod so that the ppt. and suspended matter are thoroughly broken up. Then acidify with dil. sulphuric acid, filter and wash, collecting the washings in a separating funnel, until a drop no longer gives a ppt. with Mayer's reagent. (Grandval and Lajoux's method.[3])

(6) *Extraction of the liberated alkaloid*—Make the contents of the separating funnel alkaline with sodium hydroxide and extract the alkaloid with 20-ml portions of chloroform until extraction is complete, as shown by testing the aqueous liquid with Mayer's reagent. Distil the combined chloroform extracts, adding a few drops of rectified spirit towards the end, and dry and weigh the residue. The weight thus obtained gives the total amount of strychnine in cases of strychnine poisoning. The purity of the residue is shown by its m.p.

Results in Test Expts.—The method was first tested with known quantities of strychnine without viscus, the solns. being treated with varying quantities of alkali and the alkaloid extracted as described above. The results are given in Table I.

Table I

Strychnine taken mg	40% KOH added ml	90% alcohol added ml	Strychnine recovered mg	m.p. of strychnine °C.
15	5	5	15	269
35	8	8	35	269
40	12	12	40	269

The method was then tried on known quantities of strychnine added to specimens of stomach and liver, and the following results (Table II) were obtained.

Table II

	Viscus taken g	Strychnine added mg	Strychnine recovered mg	m.p. of strychnine °C.
Stomach ..	20	18	18·0	269
Liver	20	24	23·4	268
Stomach ..	20	25	24·8	269
Liver	20	28	28·0	268
,, ...	30	38	38·0	268

Nux Vomica Poisoning—In cases of *Nux Vomica* poisoning the method yields the total alkaloids (strychnine and brucine) in a pure condition. From these total alkaloids strychnine may be separated by treatment with the requisite quantities of sulphuric and nitric acids, as prescribed in the British Pharmacopoeia. Weigh the total alkaloids and treat them with sulphuric and nitric acids in the proportion of 15 ml of 3% w/v sulphuric acid and 2 ml of nitric acid of 1·42 sp.gr. for every 250 mg of the mixed alkaloids. Maintain the mixture at 17° C. in a thermostat for 30 min. and then transfer it to a separating funnel containing 20 ml of 20% sodium hydroxide soln., shake for 2 min., then add 20 ml of chloroform and shake again. Separate the chloroform soln. and wash it first with 5 ml of 20% sodium hydroxide soln. and then with 20 ml of water. Continue the extraction of the alkaloids with successive quantities of 10 ml of chloroform, until extraction is complete, washing each chloroform soln. with the sodium hydroxide soln. and the water used for washing the first chloroform soln.

Distil the chloroform from the combined chloroform solns., adding 5 ml of rectified spirit towards the end. Weigh the strychnine residue and multiply the weight by the factor 1·02, as prescribed by the British Pharmacopoeia, to make up for the loss of strychnine in the B.P. method of treatment.

Mixtures of known quantities of strychnine and brucine with viscera were treated by the alkaline digestion and extraction method described above, and the total alkaloids thus obtained were treated by the British Pharmacopoeia method for isolation of strychnine. The results are given in Table III.

Table III

Strychnine added mg	Brucine added mg	Total alkaloids recovered mg	3% w/v H_2SO_4 ml	HNO_3 (1·42 sp. gr.) ml	Strychnine recovered ×1·02 mg	Melting point °C.
35	35	69·5	5	0·7	34·7	269
25	25	51·0	3	0·4	24·9	269
15	15	31·0	2	0·3	14·5	268

The method was then tried with viscera to which varying quantities of Tincture Nux Vomica, previously assayed by the British Pharmacopoeia method aud containing 122 mg of strychnine per 100 ml of the tincture, were added. The viscera, with the Tincture Nux Vomica added, were subjected to the above alkaline digestion method, the total alkaloids being extracted as described above. The alkaloids thus obtained were treated by the B.P. method for the destruction of brucine, and the residual strychnine was weighed. The purity of the residue was shown by the m.p. The results are given in Table IV.

TABLE IV

Tincture of Nux Vomica ml	Total alkaloids mg	3% w/v HSO_4 ml	HNO_3 (1·42 sp.gr.) ml	Strychnine found × 1·02 mg	Strychnine by B.P. method in original tincture mg	m.p. °C.
25	59	4	0·5	29·8	30·5	269
30	72	4·5	0·6	36·7	36·6	—
35	82	5·3	0·7	42·4	42·7	268
36	82	5·3	0·7	43·6	43·9	269
44	109	6·5	0·9	53·4	53·7	268

CONCLUSIONS—The Stas-Otto process remains the best method for the detection of alkaloids in viscera. When the alkaloids have been proved to be strychnine in cases of strychnine poisoning, or strychnine and brucine in cases of Nux Vomica poisoning, after extraction by the Stas-Otto process of a portion of the viscus, the method of alkaline digestion of proteins and fats and direct extraction of the alkaloid or alkaloids from an aliquot portion of the viscus is suitable for the quantitative determination of these alkaloids and of strychnine in particular.

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LABORATORY OF CHEMICAL EXAMINER TO GOVERNMENT
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Determination of Thickness and Composition of Tin-Lead Alloy Coatings on Steel (Terne Plate)

BY J. W. PRICE

PUBLISHED methods for the determination of coating composition of terne plate involve complete solution of the specimen in dil. acid,[1] or solution of the coating (together with a small amount of the steel) in hot conc. sulphuric acid[2] and subsequent determination of tin and lead. The first of these methods takes a long time, while the second could be simplified by knowing the coating weight, whereby it would be possible to find the composition by determining only one of the constituents.

During the course of an investigation on hot-dipped tin-lead alloy coatings on steel, it was found that all such coatings can be selectively dissolved in cold hydrochloric acid containing antimony trichloride, as described by Clarke for pure tin coatings,[3] thus affording a rapid means of determining coating thickness by loss of weight. The rate of solution of the coatings was found to be considerably slower than with tinplate, the reaction continuing for some time after gas evolution had ceased; the time taken was of the order of 1 min. for each 1 lb. of coating per basis box,* or rather longer with alloys very high in lead.

Preliminary examination of coating thickness on sheets of commercial terne plate showed large variations (in some instances 100%) in thickness over individual sheets, so that it was apparent that it would be necessary to determine both coating weight and tin or lead on the same sample. It was, therefore, decided to investigate the possibility of determining the tin dissolved in the stripping soln. after determining coating weight.

* The basis box is a unit of area equal to that of 31,360 sq.in. of sheet (*e.g.*, 112 sheets each 20 in. × 14 in.) and the area of coated surface in a basis box is 62,720 sq.in. A coating weight of 1 lb. per basis box is equiv. to 11·2 g per sq. metre on each face.

ACTION OF THE STRIPPING SOLUTION ON TIN AND LEAD—Solution of tin in cold hydrochloric acid containing antimony trichloride takes place partly by replacement by antimony, and partly by accelerated attack by acid, with evolution of hydrogen. It was found that part of the tin remained in the antimony ppt. after solution appeared to be complete, but that on boiling the soln. this residual tin also dissolved, leaving the pptd. antimony entirely free from tin, as found by solution of the ppt., reduction with nickel and titration with iodate. For example, 1 g of tin produced 0·48 g of precipitate on dissolving in the cold, and this was reduced to 0·40 g on boiling. (For replacement reaction, 1 g of tin would liberate 0·67 g of antimony.)

Titration, with standard iodate, of a cold diluted soln. containing 0·1 g of tin gave tin recoveries of only 70–80%, even when the tin was dissolved in absence of air; after boiling, cooling and diluting, again in absence of air, rather more tin was found, but still 10–15% was not titrated. Addition of an extra 0·5 g of powdered antimony gave approx. correct results, but with further amounts of antimony the results were too high. It is clear that the presence of powdered antimony during the reduction of tin solns. can cause serious errors. Finely divided antimony and solns. of antimony trichloride have been found to react with iodine under certain conditions, causing high results. Also it is known that high or low results may be obtained according to the particle size and amount of the antimony present.[4,5,6] The effect of particle size does not come into the question in the solution of tin in hydrochloric acid containing antimony trichloride, as the pptd. antimony is in spongy form.

Solution of lead in hydrochloric acid containing antimony trichloride was found to proceed entirely by replacement without gas evolution, the pptd. antimony tending to adhere to the surface of the lead. Using thin lead foil (~0·001 in.), solution was complete in a few min. on warming and loosening the antimony ppt. by means of a glass rod. Solution of 1 g of lead liberated the theoretical amount of antimony, 0·39 g.

TITRATION OF SOLUTIONS CONTAINING TIN AND LEAD—Varying proportions of tin and lead were dissolved in the stripping soln. and titrated under the following standard conditions: *Solution required*: 800 ml of HCl (sp.gr. 1·16), 200 ml of water and 20 g of Sb_2O_3. The metals were dissolved in 100 ml of the soln., boiled for 10 min. in a conical flask connected to a supply of carbon dioxide[7] and cooled, 100 ml of water, 5 ml of 1% starch soln. and 1 ml of 10% potassium iodide soln. were added, and the tin was titrated with *N*/20 potassium iodate. Under these conditions stable end-points were obtained, the addition of potassium iodide being essential. The amounts of tin found by titration are given in Table I. It was found that the presence of 0·4 g or more of pptd. antimony obscured the end-point and led to erratic results. (*Cf.* Expts. 6, 7 and 8.)

TABLE I

EFFECT OF AMOUNT OF PRECIPITATED ANTIMONY ON TIN TITRATION

Expt. No.	Wt. of tin g	Wt. of lead g	Wt. of antimony pptd. g	Iodate used in titration ml	ml of iodate/ mg of tin (theoretical value 0·324)
1	0·0627	—	0·025	16·9	0·270
2	0·0527	0·0555	0·043	15·15	0·287
3	0·0542	0·1340	0·075	16·4	0·302
4	0·0584	0·2960	0·14	18·25	0·313
5	0·0559	0·5410	0·24	17·2	0·318
6	0·0521	1·0470	0·44	17·3	0·332
7	0·0482	0·9620	0·40	15·0	0·312
8	0·0490	0·9760	0·41	15·6	0·319
9	0·0262	0·5240	0·22	8·2	0·313
10	0·0387	0·7700	0·32	11·8	0·305

Low results were obtained throughout until more than 1 g of lead (liberating 0·39 g of antimony) was present, a high figure being then obtained; but the error decreased with increasing amounts of lead up to 1 g., tin being kept constant. The last two results (Expts. 9 and 10) show that the titration is affected by the tin/lead ratio (5% of tin) as well as by the total weight of metal present. However, as shown in Table II, where the total weight was kept constant at about 0·7–0·8 g, consistent results were obtained to within 1% of the tin content over the range of 10–25% of tin. It was, therefore, possible to standardise the iodate empirically against known weights of tin and lead and to determine tin in mixtures in this

range of composition to within about 0·1–0·2% of the total weight. These compositions cover the normal grades of commercial terne plate (12 and 25% of tin) and the degree of accuracy is quite sufficient for this type of material.

TABLE II

ACCURACY OF RESULTS UNDER STANDARD CONDITIONS

Wt. of tin g	Wt. of lead g	Tin %	Iodate used in titration ml	ml of iodate/ mg of tin	Tin from aver. value of iodate %	Error %
0·0718	0·7050	9·24	22·7	0·316	9·19	−0·05
0·0725	0·7200	9·15	22·95	0·317	9·11	−0·04
0·0712	0·7200	9·00	22·8	0·320	9·06	+0·06
0·1293	0·6490	16·62	41·45	0·320	16·74	+0·12
0·1930	0·5810	24·94	61·2	0·318	24·87	−0·07
				Av. 0·318		

METHOD FOR TERNE PLATE—Select a sample of known area to give a coating weight of not more than 0·8 g, degrease with a solvent, or, preferably, cathodically in 1% sodium carbonate soln., dry and weigh. Attach to a nickel wire and immerse in 100 ml of the stripping soln. in a shallow dish. When the coating is dissolved, quickly remove the loose deposit with a rubber-tipped glass rod, keeping the specimen immersed in the soln. When the steel is clean, remove it by means of the wire, wash with a little cold water, dry and weigh. Transfer the soln., with the deposited antimony and the washings, to a conical flask, connect to a supply of carbon dioxide[7] and boil for 10 min. Cool, dilute with 100 ml of water, add 5 ml of 1% starch soln. and 1 ml of 10% potassium iodide soln. and titrate with *N*/20 potassium iodate. Standardise the iodate against tin plus lead in approx. the same proportions, dissolved in the stripping soln. and treated as above. Coating weight and composition can be determined by this method in 30–40 min.

DISTRIBUTION OF COATING THICKNESS AND COMPOSITION IN TERNE PLATE—Two sheets of commercial terne plate, one carrying a 3 lb. coating (Pb 90 : Sn 10) and the other a 2 lb. coating (Pb 80 : Sn 20), were investigated in detail. After removal of all edges, the sheets were cut into 3-in. squares which were all analysed, some by stripping and titration of the tin as described above (Method A), and others by solution of the coating in hot conc. sulphuric acid and determination of lead as sulphate and tin by reduction with nickel (Method B).[2] It was not possible to use the results of lead determination as a check on the tin figures, as an appreciable amount of iron was dissolved during solution of the coating and this was not determined. Coating weight was taken as the sum of the tin and lead determinations, and the tin % was found from this. A few specimens were completely dissolved in dil. acid (Method C),[1] but this method, while being considerably longer, offered no advantage over Method B. Results are shown in Figs. 1 and 2, which are pictures of the surface of the sheet, analysis figures representing the coating on both sides. In each square is given the method of determination (A, B or C) followed by the coating weight in oz. per basis box and by the tin content %. A distinct variation in tin content, not related to the variation in coating thickness and beyond the experimental error, was found by all the methods of analysis.

The extent of the tin-iron alloy layer on terne plate was investigated by anodic treatment in sodium hydroxide soln. under controlled conditions in which the alloy is unattacked while the free metal of the coating is dissolved.[8] On removing the coating, the characteristic appearance of the tin-iron alloy layer, as seen on tinplate, was found to be entirely absent on terne plate with coating weights of 4 lb. or less per basis box, practically no weight loss being observed on treating the anodically stripped specimen with hydrochloric acid containing antimony trichloride, in which the tin-iron alloy is soluble. A sample of heavy-gauge sheet, with a 13 lb. coating of 25% tin alloy, showed the normal alloy layer associated with tinplate on anodic treatment, but the weight of the layer was only about 2 oz. per basis box. It appeared, therefore, that any correction for the weight of iron removed by solution of the alloy layer in the stripping soln. is negligible with terne plate. The variation in tin content over a sheet (Figs. 1 and 2) may be due to segregation of the alloy during solidification, but the distribution of coating composition over sheets examined appears to be quite unsystematic.

OTHER APPLICATIONS OF THE METHOD—The method described for the analysis of terne plate has been found to be applicable to the analysis of electro-deposited tin-lead alloys on steel, and to the determination of tin in tin-lead solders by rolling samples to foil, in which form they are readily soluble in hydrochloric acid containing antimony trichloride. Alloys containing more than 25% of tin could be analysed by dissolving with the sample enough lead foil to reduce the tin/lead ratio to between 1 : 3 and 1 : 10.

FIG. 1. TOPOGRAPHICAL DISTRIBUTION OF COATING WEIGHT (OZ. PER BASIS BOX) AND TIN CONTENT (%) ON 3-LB. TERNE PLATE (10% TIN). (AVERAGE OF BOTH SIDES.)

A 49 9·5	B 51 9·8	B 52 9·8	A 51 9·9	B 53 9·7	B 62 9·5	A 63 9·4
C 47 9·8	B 42 9·9	B 41 10·0	A 48 9·9	B 44 10·0	A 54 10·0	B 55 9·6
B 39 10·0	B 41 9·8	A 32 9·6	B 44 9·8	A 41 10·1	B 52 —	C 57 9·6
A 43 9·5	B 40 9·3	B 46 9·4	A 54 10·1	B 54 9·6	B 53 9·6	A 56 9·5

FIG. 2. TOPOGRAPHICAL DISTRIBUTION OF COATING WEIGHT (OZ. PER BASIS BOX) AND TIN CONTENT (%) ON 2-LB. TERNE PLATE (20% TIN). (AVERAGE OF BOTH SIDES.)

A 25 17·6	B 25 18·1	B 25 18·1	A 29 18·4	A 29 18·4	B 31 18·1	A 33 18·1
B 29 18·1	A 29 18·7	A 29 18·7	B 32 17·9	B 33 18·5	A 32 18·8	A 34 18·8
A 29 18·6	A 29 18·9	B 31 18·1	B 31 18·2	A 32 18·2	B 31 18·7	A 34 18·0
B 30 18·5	A 31 18·5	A 33 18·3	B 32 18·4	A 32 18·6	B 32 18·0	A 33 18·5
A 31 18·9	B 32 18·6	A 32 18·3	A 34 18·4	B 34 18·6	B 34 18·8	A 35 18·7

SUMMARY—1. The thickness of tin-lead alloy coatings on steel can be determined by weight loss on treatment with cold hydrochloric acid containing antimony trichloride. The amount of tin-iron alloy layer present in terne plate is generally so small that no correction is necessary for the iron dissolved by the stripping soln.

2. The composition of the coatings can be found by determination of tin in the soln., after stripping, by titration with iodate under standardised conditions, using an empirical factor.

3. Commercial terne plate coatings vary in thickness by as much as 100% over single sheets; coating compositions also vary, independently of thickness.

This work forms part of a series of researches on tin alloy coatings being carried out in the laboratories of the Tin Research Institute. I am indebted to the International Tin Research and Development Council for permission to publish. Approval for publication has been granted by the Director-General of Scientific Research and Development, Ministry of Supply.

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TIN RESEARCH INSTITUTE
FRASER ROAD, GREENFORD, MIDDLESEX

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Estimation of the Freshness of Canned Salmon by means of the Carbon Dioxide Value of the Drained Muscle Tissue

BY F. CHARNLEY

IN a study of spoilage in meat Lyubin and Lebedeva[6] followed the progress of the decomposition by measuring the amount of carbon dioxide evolved by a given weight of minced tissue. Their expts. were mainly on uncooked meat, but from tests on samples of spoiled meat that were subsequently boiled they found that spoiled samples, even after cooking, gave off a greater amount of carbon dioxide than did cooked, unspoiled samples. In view of this result and of the unsatisfactory results given by other tests for spoilage, it was decided to investigate the suitability of this method for determining spoilage in canned salmon.

METHODS—The general plan of the investigation on canned salmon was similar to that followed in determining the relation between the acid value of the oil of canned herring and examiner's rating for freshness,[2] *i.e.*, carbon dioxide values of samples of canned salmon were compared with the ratings of experienced examiners. The examiner's ratings were determined prior to the carbon dioxide values and were recorded as follows: A—1=Good, B—2=better than average, C—3=average, D—4=poorer than average, E—5=poor, S—6=stale and T—7=Tainted. In one set of expts. the ratings were the averages of ratings given by two examiners, while in the second set the ratings represent those of one examiner only.

Carbon dioxide values were determined by the diffusion method, using Conway micro-diffusion units.[4] Approx. 5 g of the drained muscle tissue, taken from the interior of the sample, was rapidly broken or minced and introduced into the outside annular space of a weighed Conway unit. The cover of the Conway dish was quickly replaced and the dish and contents were weighed. One to 1·2 ml of $N/10$ barium hydroxide was then rapidly introduced into the interior chamber of the Conway unit and the soln. was quickly spread evenly over the bottom of the chamber. The cover was replaced and the dish and contents were introduced into an incubator maintained at 50° C. After 30 min. the sample was removed from the incubator and allowed to cool to room temp., and the residual baryta soln. was titrated from a burette, graduated in 1/20 ml, with $N/10$ hydrochloric acid, phenolphthalein being used as indicator.

In these expts. a mixture of 75% of paraffin and 25% of petrolatum was employed as sealing compound. Subsequent tests in water at 50° C., however, showed that with this mixture there was some leakage of gas. Further tests on the same set of samples, using as sealing compound this mixture and a mixture made up to 50% of paraffin and 50% of beeswax (with which there was no gas leakage when the Conway unit was immersed in water at 50° C.), revealed no significant difference in the mean results for carbon dioxide with the different sealing compounds. The values obtained with the first sealing mixture may therefore be regarded as safely comparable with those obtained with the second sealing mixture, *i.e.*, the 50% paraffin and 50% beeswax at present employed by this laboratory.

The *carbon dioxide value* is expressed as the number of mg of carbon dioxide per g of muscle tissue obtained by the foregoing procedure.

RELATION BETWEEN CARBON DIOXIDE VALUE AND EXAMINER'S RATING—Table I gives a summary of the crude data obtained in these expts. Since the samples represented by these

data were made available through routine inspections, part of the data, *viz.*, groups 1 to 3 inclusive, represent samples of keta salmon (*Oncorhynchus keta*) and the remainder, groups 4 to 5, refer to samples of pink salmon (*Oncorhynchus gorbuscha*). Also, it should be noted that group 1 consists of samples drawn from 5 different codes and that the ratings in groups 1 to 3 refer to the averages of the ratings of two examiners. Similarly, group 4 consists of 3 different codes and the ratings in groups 4 and 5 are the scores of one examiner only.

TABLE I

SUMMARY OF DATA RELATING CARBON DIOXIDE VALUE OF CANNED SALMON AND EXAMINER'S RATING FOR FRESHNESS

(X = examiner's rating. Y = carbon dioxide value)

Group	Sample size	ΣX	ΣY	ΣX^2	ΣY^2	ΣXY
1	14	56·5	1·395	232·25	0·14495	5·7015
2	6	28·5	0·902	136·75	0·13935	4·3190
3	18	102·0	3·215	600·00	0·68280	19·3920
4	9	35·0	0·632	145·00	0·04757	2·5280
5	12	39·0	0·599	129·00	0·03069	1·9230
Total	59	261·0	6·743	1243·00	1·04536	33·8635

From columns 2, 3 and 4 of Table I a simple calculation gives the means of the individual groups and of the total distribution relating carbon dioxide value and examiner-rating. On plotting corresponding means, as shown in Fig. 1, it was thus found that the mean carbon dioxide value is apparently connected through a linear relation with mean examiner's rating. There were, however, a number of points to clear up in connection with these data before it was felt safe to set up a tentative grading plan for grading freshness of canned salmon by means of carbon dioxide values.

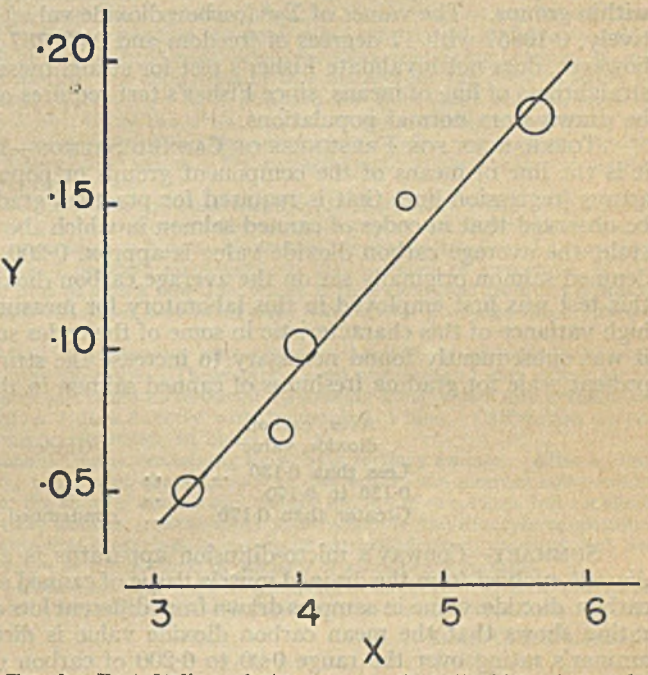

FIG. 1. Best fit line relating mean carbon dioxide value and mean examiner's rating of samples of canned salmon. X = Examiner's rating; Y = carbon dioxide value. Areas of circles are proportional to sample sizes.

First, when the usual method of least squares was applied it confirmed the hypothesis that the relation between the means is linear.

Secondly, it was shown that the codes combined in groups 1 and 4 gave homogeneous groups. Thirdly, since examiner's ratings, like *p*H values of the aqueous liquid of canned salmon, are very considerably influenced by the state of spawning migration of the salmon, it might be contended that the relation between carbon dioxide value and examiner's rating is also mainly the result of this effect. That the carbon dioxide value, however, is reasonably free from seasonal effects may be inferred from the results listed in Table II, which shows an excerpt from the laboratory report of examination of six samples of keta salmon packed in November, 1942. The averages for firmness (3·2 corresponding to an average penetration of 21·8 mm)[3], free aqueous liquid, free oil, intensities of the red and yellow colours of the muscle tissue, and the water-markings all indicate that this salmon was in a very advanced stage of its spawning migration at the time it was caught and packed. The average carbon dioxide value, however, is only 0·058, showing that there was no post-mortem spoilage prior to canning, and, consequently, the relatively high *p*H of the aqueous liquid is due to the seasonal

condition of the salmon. From reliable information it was known that these samples were packed a short time after the fish were taken from the water.

TABLE II

AVERAGES OF CERTAIN QUALITY CHARACTERISTICS OF SIX TINS OF KETA SALMON PACKED IN NOVEMBER, 1942

Character	Average of 6	Character	Average of 6
Softness (mm)	21·8	Int. of red colour (Lovibond units) ..	2·3
*p*H of aq. liquid	6·45	Int. of yellow colour (Lovibond units) ..	2·3
Vol. of free aq. liquid (ml) ..	111·5	No. of water marks	1
Vol. of free oil (ml)	0·0	Carbon dioxide value	0·058

Fourthly, it is of interest to note that analyses of putrid samples obtained by incubation of under-processed salmon show that the carbon dioxide value given by the foregoing procedure is of the order of 0·400, or greater for canned salmon in an advanced stage of decomposition. Two incubated tins of keta salmon, for example, gave the carbon dioxide values, 0·408 and >0·521, respectively. Since the carbon dioxide value, as determined by an experienced analyst, is accurate to the second decimal place, there is thus a satisfactorily wide range in this measure of spoilage from fresh to definitely putrid samples. In this connection it may be mentioned that trustworthy duplicate analyses on two different samples gave the values 0·114, 0·118 and 0·088, 0·092, respectively.

Lastly, the groups listed in Table I are not all homogeneous with respect to the variances within groups. The values of Σy^2 (carbon dioxide value) for groups 3 and 5, *e.g.*, are respectively, 0·10857 with 17 degrees of freedom and 0·000787 with 11 degrees of freedom. This, however, does not invalidate Fisher's test for straightness of regression lines, or, in this case, straightness of line of means, since Fisher's test requires only that the individual groups shall be drawn from normal populations.

TOLERANCES FOR FRESHNESS OF CANNED SALMON—Evidently, as with canned herring,[2] it is the line of means of the component groups or populations and not a line of means of arrays (regression line) that is required for practical grading purposes. From Fig. 1 it will be observed that in codes of canned salmon in which the average examiner's rating is 6, *i.e.*, stale, the average carbon dioxide value is approx. 0·200. The latter was the limit for condemned salmon originally set on the average carbon dioxide value in November, 1942, when this test was first employed in this laboratory for measuring spoilage. Owing to the rather high variance of this characteristic in some of the codes submitted for examination, however, it was subsequently found necessary to increase the stringency of the tolerance, so that the present scale for grading freshness of canned salmon in this laboratory is as follows:

Aver. carbon dioxide value	Grade	Size of sample examined
Less than 0·130	A	6
0·130 to 0·170 ..	B	12
Greater than 0·170 ..	condemned	12

SUMMARY—Conway's micro-diffusion apparatus is applied to determining the carbon dioxide evolved from the drained muscle tissue of canned salmon. Comparison of the average carbon dioxide value in samples drawn from different lots or codes with the average examiner's rating shows that the mean carbon dioxide value is directly proportional to the mean examiner's rating over the range 0·00 to 0·200 of carbon dioxide value. A scale for grading freshness of canned salmon on the basis of the carbon dioxide value is given.

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CANNED FISH INSPECTION LABORATORY
VANCOUVER, B.C.

July, 1944

Notes

COLORIMETRIC ESTIMATION OF LONG-CHAIN ALIPHATIC AMINES

We have experienced considerable difficulty in finding a satisfactory method for estimating small quantities of long-chain aliphatic tertiary amines in presence of biological material.

The amine picrate method of Richter, Lee and Hill[1] appeared to be the only one available for determination of tertiary amines in blood. In this method the amine is extracted from whole blood by shaking with light petroleum (b.p. 80–100° C.) and an excess of potassium carbonate, which liberates the free amine and at the same time has a salting-out action. (The loss of amine through combination with traces of aldehyde in the blood to form Schiff's bases is reduced by adding tyramine, which is not extracted.) The amine is transferred into a small volume of sulphuric acid saturated with sodium bromide, phospholipids remaining in the light petroleum soln., and is then taken back to light petroleum; picric acid is added to form the picrate, which gives a strong yellow colour in chloroform soln. Strong yellow colours are given by dibenzylamine, *n*-hexylamino-cyclohexane, di-*n*-hexylamino-cyclohexane, trihexylamine, 2-methylamino-octane, 2-octylamino-octane and dioctylmethylamine, as well as by the compounds mentioned by Richter *et al.*

Nevertheless, we have found that the recovery of long-chain amines, *e.g.*, dioctylmethylamine, by this method is poor, as their hydrochlorides are slightly soluble in light petroleum and therefore not completely extracted by sulphuric acid. We have been able to use this property as the basis for a simple method in which the amine hydrochloride is extracted from slightly acidified solution in water or blood directly into light petroleum. The extract is then treated with an equal volume of chloroform and the colour is developed by adding picric acid. The intensity of the colour is measured in a Spekker photoelectric absorptiometer with a No. 7 dark blue filter (Chance Bros. O.B.1). The recovery of amine is reduced if the extraction is performed in presence of either potassium carbonate or excess acid. Addition of certain solvents (*e.g.*, ethanol) increases the colour developed.

Richter *et al.* emphasised that for their method a special calibration curve is necessary for solutions in blood. We have found that with our modified procedure the same curve can be used for solns. in either blood or water. The calibration curve indicates a linear relationship for solns. containing between 50 and 200 μg of dioctylmethylamine per ml of blood. The sensitivity of this method for the determination of long-chain aliphatic amines is of the same order as we have obtained for short-chain amines, such as *iso*amylamine, by the method of Richter *et al.* Using the latter method, we were unable to reproduce the sensitivity claimed for the determination of *iso*amylamine. For satisfactory results, our method requires about 2·5 ml of a soln. containing *ca.* 20–200 μg/ml of amine. The method can be applied to the estimation of dioctylmethylamine in kidney and liver brei.

The method of Richter *et al.* and its modifications are unsatisfactory, in that yellow colours are difficult to measure colorimetrically; moreover, biological extracts themselves frequently have a slight yellow colour. We have therefore attempted to adapt for this purpose Auerbach's method[2] for the estimation of quaternary ammonium compounds. Auerbach's method depends essentially on the reaction of two mols. of the quaternary salt with one of bromophenol blue to give a blue dye, which is then extracted from the aqueous alkaline soln. with ethylene dichloride and examined colorimetrically with a No. 3 orange filter (Chance Bros. O.Y.2).

In order to facilitate the determination of quaternary ammonium compounds in biological material, we attempted to remove protein by enzymic digestion. These expts. were unsuccessful, but it was found that reasonable recoveries of the quaternary salt could be obtained by shaking horse serum and transfusion blood containing dioctyldimethylammonium iodide directly with bromophenol blue. Calibration curves have been plotted for dioctyldimethylammonium iodide in blood.

We have extended the method to alcoholic solns. containing long-chain tertiary amines. After adding a few drops of methyl iodide to the soln., to convert the amines into quaternary ammonium compounds, they were treated with bromophenol blue; the simpler amines and choline did not give colours, but excellent colours were produced by the higher tertiary amines, such as dioctylmethylamine and dioctylpropylamine.

This method is satisfactory for determining dioctylmethylamine in urine and tissue extracts, but not in blood, for which purpose our modification of the method of Richter *et al.* appears suitable.

This note is preliminary to detailed publication with the biological data elsewhere. This investigation was carried out as part of a programme of the Therapeutic Research Corporation of Great Britain, Ltd., to whom acknowledgments are now made.

B. M. C. Hopewell
J. E. Page

Glaxo Laboratories, Ltd.
Greenford, Middlesex

November, 1944

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AZO DYES AS QUANTITATIVE PRECIPITANTS OF TANNIN*

Many attempts have been made to estimate tannins by means of azo dyes,[1] but without success, because, in our opinion, the wrong dyes were used. We have found that only those azo dyes which contain *free* amino groups form with tannin ppts. which are insoluble in water and thus suitable for quantitative work. Of about 60 azo dyes that we tried, only the following six containing free amino groups gave good results—Chrysoidine,[2] Bismarck brown,[2] Congo red,[2] Trypan red,[3] Afridol blue[4] and Echtgelb.[5]

* The recent communication by F. W. Box (Analyst, 1944, **69**, 272) suggested the publication of this work begun more than 20 years ago.

Echtgelb, $HSO_3.C_6H_4.N{=}N.C_6H_3(SO_3H)NH_2$, is easily prepared in quantity in the laboratory as described by Eger,[6] and this dye, subsequently referred to as E.Y. (Eger's yellow), was used in all our later work. Our results indicate that eventually E.Y. may become a general reagent for determining the number of depsiphores[7] in any given tannin. According to Koerner and Nierenstein[8] the carbonyl group generally met with in tannins has depsiphoric properties—a view also shared by Koenig and Kostanecki.[9] The *tanned* insol. azo dye has, we find, the general formula $R.N{=}C.R_1.R_2$, and since in Eger's yellow R.N= is represented by the residue $HSO_3.C_6H_4.N{=}N.C_6H_3(HSO_3)N{=}$, we were able to check the reaction by determining carbon, hydrogen, sulphur and nitrogen in the tanned E.Y.

Method—To a molecular suspension in water of E.Y. (mol. wt. 425) add an aq. soln. of 2 mols. of potassium hydroxide, and run the soln. slowly from a burette into an aq. soln. of the tannin under investigation until no more ppt. is formed. After it has stood for 48 hr. under dust-free conditions collect the ppt. in a Gooch crucible, and wash it first with 250 ml of 5% hydrochloric acid and then with 500 ml of water. Dry at 160° C. to constant weight. The difference between the weight of the tanned E.Y. and the weight of E.Y. used gives both the tannin and the depsiphore contents.

Results—*Tannin*—The following are typical examples of the percentages of tannin found by the E.Y. method (A), as compared with the hide powder method (B), Hooper's cinchonine method[10] (C), and Spiers's permanganate method[11] (D).

	A	B	C	D
Abu-Surug (bark)	13·87	14·46	13·53	13·23
Babool (bark)	11·52	12·49	11·77	11·93
Divi-divi (pods)	13·27	13·50	12·83	13·06
Hemlock (bark)	9·41	10·76	9·89	9·99
Mimosa (bark)	29·54	30·87	29·23	29·54

Depsiphore Groups in Tannins of known Constitution—The following table shows the number of depsiphores present and found in a series of tests.

	Depsiphores present	Depsiphores found
Maclurin[12]	1	1·17
Digalloylglycol[13]	2	2·08
Trigalloyl glycerol[13]	3	3
Tetragalloyl erythritol[13]	4	4·16
Hexagalloyl mannitol[13]	6	6·08
Acertannin[14]	2	1·07
Paullinia tannin[15]	1	1·09
Hamameli-tannin[16]	2	2

Finally it should be mentioned that phenols (resorcinol, phloroglucinol, pyrogallol), aromatic acids (gallic acid, protocatechuic acid) and catechins do *not* give ppts. which resist washing with water.

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The University
Bristol

R. C. Davies
M. Nierenstein
C. W. Webster

October, 1944

THE PHOTOMETRIC ANALYSIS OF COPPER-BASE ALLOYS

II. THE DETERMINATION OF MANGANESE BY OXIDATION AT ROOM TEMPERATURE

To assist the oxidation of manganese to permanganate, heat is employed; if oxidation is effected at room temperature the method for batch work may be speeded up. In 1901 Marshall[1] found that persulphate and silver nitrate can effect the oxidation of manganese at room temp., but attempts to use this[2,3] did not find general application. Pinkus and Ramakers[4] tried similar reagents and found that at 15° C., although the oxidation was slow, it sometimes reached completion. Interference from a brownish coloration, ascribed to silver peroxide, was avoided by adding urea[5] in a method recently suggested by Osborn and Stross[6] for use with aluminium alloys.

Application of the method of Osborn and Stross[6] to copper-base alloys with similar amounts of ammonium persulphate and silver nitrate led to inaccurate absorption readings, due to the formation of

bubbles of nitrogen. It was found as the result of investigations on samples of DTD.197—a complex aluminium bronze containing about 0·4% of manganese—that smaller amounts of the above oxidants could be employed. The rate of progress of oxidation was followed by taking absorption measurements at 1-minute intervals with a Spekker Absorptiometer and the time required for complete oxidation was determined. Table I below gives a summary of the results obtained.*

TABLE I

TIME (IN MINUTES) REQUIRED FOR COMPLETE OXIDATION WITH VARYING AMOUNT OF REAGENTS

Silver nitrate	Ammonium persulphate, g									
g	0·2	0·3	0·4	0·5	0·75	1·0	1·5	2·0	2·5	3·0
0·25	29	23	17	11	8	5	–	–	–	–
0·20	–	25	18	13	10	7	5	–	–	–
0·175	–	27	20	16	11	9	6	–	–	–
0·15	–	29	22	19	14	10	7	–	–	–
0·125	–	–	23	21	15	12	10	6	–	–
0·10	–	–	35	23	17	14	12	9	6	–
0·08	–	–	–	29	21	17	14	12	8	6
0·06	–	–	–	–	30	22	17	14	12	8
0·04	–	–	–	–	–	34	25	20	17	14
0·03	–	–	–	–	–	–	33	25	20	17
0·02	–	–	–	–	–	–	–	29	27	24
0·01	–	–	–	–	–	–	–	–	75	51

* A blank space in the Table only indicates that tests were not made with that combination of reagents.

These results indicate that there is a substantial latent period before the production of permanganate colour begins, and that once this period is passed, oxidation proceeds to completion, *no matter how slow the rate*; these are not real disadvantages in the photometric analysis of batches of samples.

REVISED METHOD—The following modifications are necessary for the determination of manganese in copper-base alloys.[7] Add, where necessary, to a suitable aliquot of the initial alloy soln. in a 100-ml volumetric flask sufficient copper soln. to bring the copper content to 0·25 g. Add 10 ml of 15% filtered ammonium persulphate soln., 1 ml of 7·5% silver nitrate soln. and 1 ml of 10% filtered urea soln. Leave for at least 15 min., then make up the soln. to 100 ml with water, and measure the absorption with a Spekker Absorptiometer, using 2-cm. glass cells, Wratten No. 62 filters and a neutral filter setting of 0·15.[8]

RESULTS AND DISCUSSION—The results obtained by this method are of the same order of accuracy as for the periodate method; a selection of comparative figures is given in Table II.

TABLE II

COMPARATIVE RESULTS

Oxidation by	Sample No.															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Persulphate	0·39	0·38	0·40	0·38	0·17	0·17	0·19	0·94	1·14	1·07	1·40	1·37	1·85	1·92	2·31	2·30
Periodate	0·38	0·38	0·40	0·39	0·16	0·17	0·19	0·95	1·14	1·10	1·38	1·35	1·83	1·94	2·29	2·31

This method is more rapid than the periodate method and is of particular value where samples have a very low manganese content. The method has been confined almost exclusively to copper-base alloys, although tests on steels indicated that when chromium is present it is oxidised before the manganese.

The amounts (1·5 g of ammonium persulphate and 0·075 g of silver nitrate) were chosen as being the most economical combination for the 15-min. oxidation time, which is the most convenient in this laboratory. With other oxidation times, or with samples of different acidity or manganese content, other combinations of the reagents would be more economical.

Thanks are due to the Chief Chemist, Mr. W. T. Edwards, A.R.I.C., for advice and criticism.

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PHOTOMETRIC LABORATORY, LANGLEY ALLOYS, LIMITED.
LANGLEY, SLOUGH, BUCKS.

JOHN H. HIGH

September, 1944

MICRO DETERMINATION OF MIXED METHOXY-ETHOXY GROUPS

THE usual methods of alkoxy group determination only give information on the number of alkoxy groups present. Kunster and Maag[1] devised a method for determining mixed methoxy ethoxy compounds on 8–20 mg of the sample by absorbing the alkyl halide in alcoholic trimethyl ammonium iodide. Insoluble

tetramethyl ammonium iodide and soluble trimethyl ethyl ammonium iodide are formed and may be separated. The analysis, however, takes about 24 hours to complete. Friedrich's method[2] depends on a calculation of the number of alkoxy groups present from an ordinary alkoxy determination followed by a micro carbon estimation on the alkyl halides from a second sample. This method, although more rapid, cannot give such accurate results on the same amount of sample.

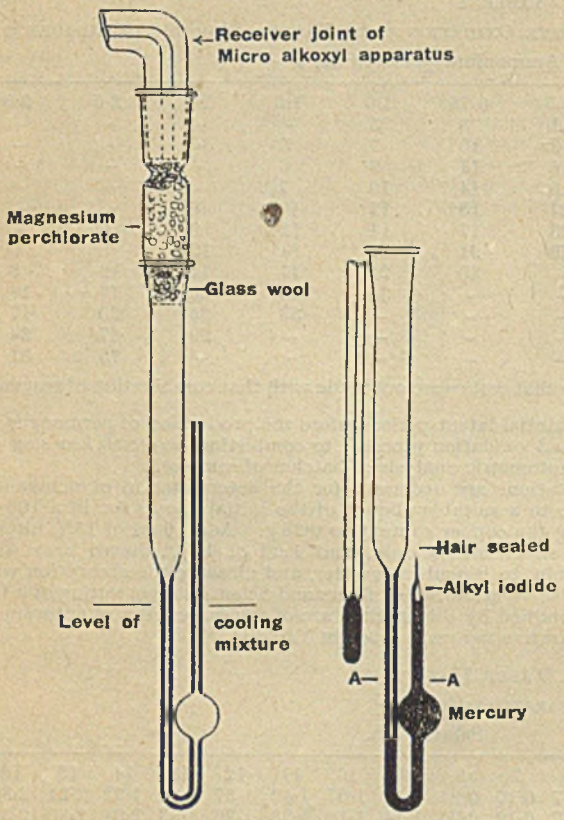

Fig. 1.

A method was required for the rapid and accurate analysis of methyl ethyl cellulose and, since the above methods were not satisfactory, I decided to investigate the possibility of quantitatively isolating the mixed alkyl halides from an arbitrary quantity of the sample and of estimating their ratio by means of physical properties. The figure obtained would then be used, in conjunction with the ordinary alkoxy figure, to calculate the exact degree of ethylation and methylation.

Experimental—Using the apparatus of Houghton and Wilson[3] with a capillary U-tube immersed in acetone and solid carbon dioxide between the trap and receiver, it was shown quantitatively that no methyl or ethyl iodide passed the U-tube. If the freezing mixture was then removed, the whole of the methyl or ethyl iodide distilled into the receiver. During these expts. it was found necessary to introduce a small magnesium perchlorate tube between the trap and the U-tube, to absorb water which otherwise was liable to block the U-tube. The modified Schleiermacher boiling-point apparatus[4,5] shown in Fig. 1 was finally designed as a receiver. The sample was introduced into the reaction flask of the microalkoxyl apparatus and distilled in the normal way in a stream of nitrogen in place of carbon dioxide. The alkyl halides were condensed in the U-tube, the gas stream escaping by means of the bulb.

The three physical constants considered are: (*a*) Critical temperature; (*b*) density; (*c*) temperature at which the combined vapour-pressures of the mixed halides reaches atmospheric, *i.e.*, boiling point.

(*a*) *Critical temperature*—This was determined by means of a capillary tube 3 cm long and about 1 mm internal and 3 mm external diameter, drawn out to a long capillary hair at one end and a short hair at the other. The tube was bent and half filled by capillarity as for the micro pyknometer.[6] It was then sealed, and the critical temperature was determined in an ordinary melting-point apparatus. Results within 1° C. were obtained on the pure halides (methyl iodide 157° C. and ethyl iodide 184° C.). On unknown mixtures, with which much slower heating was required, decomposition caused too great variations for the method to be useful.

(*b*) *Densities*—The densities of mixtures of methyl and ethyl iodides have been very accurately determined at 15° C. by Lam[7] (methyl iodide 2·2677; ethyl iodide 1·9444). He showed that no contraction occurred when the iodides were mixed and that the density/concn. relationship is a straight line. To estimate the ratio of methyl iodide to ethyl iodide to within 1% a density determination on a small quantity of material accurate to within about 0·1% is necessary. A suitable micro pyknometer requiring 5–10 cu.mm of liquid is described by Houghton.[8] This method gives the required accuracy and is rapid. The pyknometer is somewhat delicate to handle, however, and while only requiring a few minutes to construct takes something like a day to calibrate. Further, about 30 mg of the sample are required to give sufficient alkyl iodide to fill the pyknometer from the U-tube receiver. Since Lam's figures were determined at 15° C., it is necessary either to determine the densities of methyl and ethyl iodides at the temperature of the micro balance room or to cool the pyknometer to 15° C. before reading the volume.

(*c*) *Boiling-point*—In determining the b.p. of mixed alkyl iodides disconnect the U-tube receiver and allow it to warm to room temperature. Draw the capillary end off to a fine hair and pour mercury in the wide end until it stands at about the line A A with the alkyl iodide above it; mark the position of the mercury in the limbs. Tilt the receiver to force the iodide up into the hair and seal off. Heat the receiver immersed in a bath until the level of the mercury stands at the marked position in the limbs and read the temp. Since the mixed iodides will tend to be supersaturated with nitrogen, low results may be given. If, on cooling, there remains a gas space above the alkyl iodide, open the hair, force out the gas and repeat the b.p. determination. The quantity of sample needed for this method is only *ca.* 3 mg and

the time required is even less than for the density method. However, as will be seen from the figures quoted, the accuracy is not so high. Boiling-point figures for pure mixtures of the alkyl iodides are given in Table I. Also, results obtained by passing nitrogen through known mixtures of the alkyl iodides cooled

TABLE I.

BOILING POINTS OF SOLUTIONS OF ETHYL IODIDE, METHYL IODIDE MIXTURES

Saturated with nitrogen			Without nitrogen	
Et I %	B.P. °C.		Et I %	B.P. °C.
0	42·1	42·3	0	42·1
—	42·1	41·9	25·7	46·8
27·1	46·9	46·6	32·8	48·0
30·8	47·5	47·4	36·7	49·4
41·9	50·2	48·2	42·5	51·0
55·0	54·1	54·5	44·8	51·6
65·4	56·8	57·2	53·0	53·5
75·2	59·6	59·6	54·8	54·4
82·5	62·5	62·4	67·7	59·0
100	70·5	70·4	100	72·0
	70·8	70·4		

in acetone and solid carbon dioxide before determining the b.p. were plotted on a graph. Results obtained by distilling from the microalkoxyl apparatus the following known quantities of methyl and ethyl cellulose of known degree of substitution were also plotted.

Methyl cellulose (23·5% MeO) mg	Ethyl cellulose (24·2% EtO) mg	b.p. found °C.	Ethyl iodide in alkyl halide	
			calculated %	found %
11·779	2·099	43·5	12·2	8·7
6·480	4·516	49·1	35·2	37·2
4·054	5·226	50·1	50·1	44·5

The fairly wide deviation of the calculated from the found per cent. of ethyl iodide in the mixed iodides is due to variations in b.p. caused by dissolved nitrogen. Nevertheless, the accuracy is sufficient for most analyses.

SUMMARY—A micro method is described of quantitatively collecting as liquid all the alkyl iodide evolved when a compound is boiled with hydriodic acid. Attempts to determine the ratio of methyl to ethyl iodide in such a mixture by micro critical temperature measurement were unsuccessful owing to decomposition. Density determinations gave accurate results within 1% but required 30 mg of sample. Boiling-point determinations required only 3 mg of sample and gave results to within about 5%, the error being due to false boiling-points caused by dissolved gas.

I wish to acknowledge the assistance I received from Mr. H. A. B. Wilson, particularly in the many experiments leading to the boiling-point method; also to thank Messrs. I.C.I., in whose Explosives Group Research Laboratories this work was carried out, for permission to publish.

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"ROWANHURST"
BEACONSFIELD, BUCKS.

A. A. HOUGHTON

September, 1944

COLOUR MATCHING IN BLUE VALUE DETERMINATIONS

IT has been noticed by a number of observers that, when chloroform solutions of certain vitamin A preparations are treated with antimony trichloride solution in the determination of the blue value, a greenish colour develops which proves almost impossible to match if the normal technique is adopted. The colour appears to be more saturated and brighter than that of any combination of the usually available matching glasses; neutral glasses, used in the light beam traversing the test solution, do not give the required compensation.

This difficulty may readily be overcome if red glasses of low value are used in place of neutral glasses on the test side of the Lovibond instrument. A 0·5 unit or a 1·0 unit red glass allows very speedy and accurate matching where the blue glasses in the match are less than 5 units in value, and 2·0 and 4·0 unit red glasses are helpful in preliminary tests at higher blue levels. The four red glasses mentioned may conveniently be mounted in the lower opening of a standard rack in the manner normally employed for neutral glasses.

Observations made by independent observers indicate that the values of the blue glasses used in obtaining best possible matches by the proposed and the usual methods are substantially the same. For this reason it is suggested that there should be no deduction from, or addition to, the value of the blue glasses used in the match, the red glasses appearing to be without effect on the blue unit equivalent of the test solution.

6, Milner Street
London, S.W.3

K. A. Williams
November, 1944

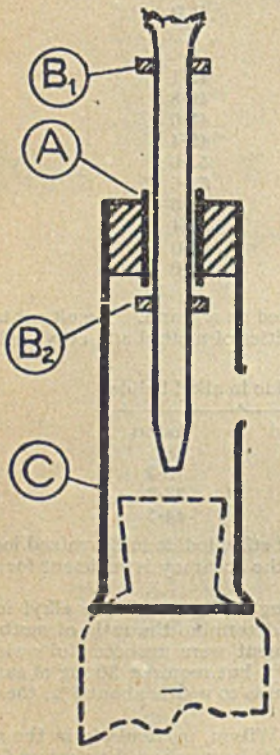

Fig 1.

AN AUTOMATIC PIPETTE FITTING FOR DELIVERING ACID INTO GERBER TUBES

In the routine determination of butter fat in milk by the Gerber method an automatic measure is frequently employed for the delivery of sulphuric acid.[1] Owing to the narrow mouth of the butyrometer tube accidental contamination of the inside of the neck with acid by the tip of the outlet of the measure may easily occur. Such contamination is likely to be transferred to the milk pipette, and thence to the sample or to a subsequent one. Should there be added water, the acidity thus caused, especially in a small sample, may prevent confirmation by the freezing-point method

An adaptor has been devised which, when fitted to the delivery of the pipette as shown in the diagram, prevents such contamination. The sleeve A, which carries a centring tube C, has a vertical travel restricted by the rubber stops B_1 and B_2. The bore of C is such that the neck of the butyrometer enters easily but without excessive play. The entry is facilitated if the lower end of C is slightly flared. When the butyrometer is raised the outlet of the measure enters it, and is constrained to remain axial. After delivery of the acid the butyrometer is lowered and removed. Not until the acid outlet has been withdrawn does the centring action cease.

Owing to the differences in the exterior diameters of the necks, it may be advisable to select a set of butyrometers for use with this device.

Reference

1. B.S.I. Specification No. 696 (1936), Parts 1 and 2.

South London Central Public Laboratory
London, S.E.11

M. A. Fill
J. T. Stock
July, 1944

Ministry of Food

STATUTORY RULES AND ORDERS

1944—**No. 1278. Order, dated November 15, 1944, amending the Fish Cakes (Maximum Prices) Order, 1944.*** Price 1d.

The principal changes made are (*a*) an increase in the minimum fish content of fish cakes from 25% to 35%, and (*b*) an increase in the minimum weight of fish cakes sold by the piece from 2½ oz. to 2¾ oz. each. *Proportionate increases in retail prices are prescribed.* Manufacturers of fish cakes are *required* to use such proportions of fresh, frozen, salted, pickled or otherwise processed fish as may from time to time be prescribed by directions.

— **No. 1280. Order, dated November 16, 1944, revoking the Ice Cream (Prohibition of Manufacture and Sales) Order, 1942.** Price 1d.

This Order permits the resumption of the manufacture and sale of ice cream as from Nov. 16, 1944.

— **No. 1281. General Licence, dated November 16, 1944, under the Use of Milk (Restriction) Order, 1941.** Price 1d.

This general licence authorises the use in the preparation or manufacture of ice cream of skimmed milk powder allocated for that purpose, while retaining the prohibition of the use of other milk or milk products imposed by the Use of Milk (Restriction) Order, 1941.

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

1944—No. 1358. **Order, dated December 6, 1944, revoking the Meat (Addition of Preservative) Order, 1941, the Margarine (Addition of Borax) Order, 1940, the Bacon (Addition of Borax) Order, 1940, and the Condensed Milk (Milk Content) Order, 1940.**
This Order revokes a number of Orders which have been replaced by Regulation 60CAA of the Defence (General) Regulations, 1939, (inserted by S.R. & O. 1944, No. 1311).*

— **No. 1447. The Labelling of Food (No. 2) Order, 1944.** Price 4d.
This Order postpones the date of operation of the Labelling of Food Order as regards labels and makes other amendments to the Order.*

Government of Bengal

CHEMICAL EXAMINER'S REPORT FOR 1942

In this, the 68th Annual Report, Dr. K. N. Bagchi states that 19,609 articles were examined, as compared with 19,631 in 1941. The decrease in the number of medico-legal cases investigated (2240 as against 2544) was largely due to transit difficulties.

Examination of Drugs—Of 68 B.P. tinctures and other preparations, 24 did not comply with B.P. requirements.

Human Poisoning Cases—Poison was detected in 364 of 759 viscera; alcohol in 168 cases and opium in 162. Next came aconite (45), oleander (39), arsenic (30) and atropine (13). There were 26 cases of abortion, and poison was found in 11; the poisons identified included madar (7), oleander (3) and aconite (1). Aconite is frequently added to *pachwai* (fermented rice gruel) by its manufacturers, with the erroneous idea of increasing its intoxicating properties.

Drugging by Chloral Hydrate—The contents of 3 bottles of foreign liquor, found beside a woman who had become unconscious after taking a drink offered by her visitors, contained alcohol and chloral. Drugging by chloral hydrate has recently been very prevalent in Calcutta and its suburbs.

Abortefacient Pill—A pill given to a woman with the object of abortion was found to contain lead oxide and madar (*Calotropis gigantea*).

Animal Poisoning Cases—The viscera of 87 horned cattle were examined, as compared with 152 in 1941. Poison was detected in 67—arsenic in 46, oleander in 15 and madar in 6. Aconite was found in the viscera of a bird.

Oleander as a Cattle Poison—The professional cattle poisoners have lately been using oleander (*Thevetia, nerifolia*) instead of white arsenic, which is easily detected even by laymen. A common recipe of the old school is a mixture of white arsenic and human hair.

Manufacture of Spurious Foreign Liquor—Samples of proprietary brands of gin and brandy, with labels, capsules, wire netting and lead seals similar to those of the genuine article, were found to have been made from methylated spirit from which the denaturants had been almost completely removed.

Chemicals used in Arson Cases—Exhibits in connection with cases of incendiarism in Calcutta contained potassium permanganate and glycerin used as a fuse for the explosion of gunpowder. Yellow phosphorus dissolved in a solvent was frequently used to cause fires in post offices and letter boxes. Other exhibits consisted of metallic sodium, potassium chlorate, and strong nitric acid. Small glass ampoules filled with strong nitric acid were thrown against passengers and drivers of tramcars.

British Standards Institution

BRITISH STANDARD CONVERSION FACTORS AND TABLES

The following Standard, B.S. 350 : 1944 has been issued.† Three ranges of tables are provided: those in considerable demand, with a range of 1–1000; those in less demand, with a range of 1–100; those in limited use, with a range of 1–9. In every instance the results of conversions are given to 5 or 6 significant figures. The adoption of the 1–1000 range for the conversions in common use ensures that, in general, results required for slide rule calculations can be read from the tables without need of summations.

Part I: Basic Tables of Units—Linear, Square and Cubic Units in British and Metric Units; Weights in British, Metric and U.S.A. Units; Miscellaneous Units; Definitions affecting Conversion.

Part II: Standard Conversion Factors—Linear Measure; Square Measure; Cubic Measure and Capacity; Weights; Speed; Stresses and Pressures; Atmospheric and Water Pressures; Weight per Unit Length; Density; Concentration; Force; Moments; Work, Heat and Energy; Power; Conversion Chart for Thermometer Scales; Wire and Sheet Metal Gauges.

Part III: Conversion Factors and Multiples.

Part IV: Selected Conversion Tables—1–100 Units.

Part V: Selected Conversion Tables—1–1000 Units.

Appendix—Four-figure Logarithms and Anti-logarithms.

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* Further particulars will be given in the February issue of The Analyst—Editor.

† British Standards Institution, 28, Victoria Street, London, S.W.1. Pp. 95. Price 3s. 6d. net, post free.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Report on the Diastatic Activity of Malt. A. D. Dickson (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 374–375)—The method of the American Society of Brewing Chemists for the determination of the diastatic power of malt, adopted as a tentative method by the A.O.A.C., involves determination of the reducing power of hydrolysed starch soln. by titration with Fehling's soln. Anderson and Sallans (*Canad. J. Res.*, 1937, C15, 70) proposed the use of the ferricyanide procedure of Blish and Sandstedt (*Cereal Chem.*, 1933, **10**, 189). The suggestion was made (*J. Assoc. Off. Agr. Chem.*, 1939, **22**, 200; 1942, **25**, 265) that the ferricyanide method should be adopted after collaborative work had been completed by the American Society of Brewing Chemists and the American Association of Cereal Chemists. The Malt Analysis Standardisation Committee of the A.A.C.C. (*Cereal Chem.*, 1939, **16**, 353) found that, in a small number of laboratories, the ferricyanide procedure showed less variation between laboratories than did the A.S.B.C. method, and a more extensive study by the same committee (Dickson, *Cereal Chem.*, 1940, **17**, 645; ANALYST, 1941, **66**, 121) showed that, although unexplained variations between laboratories were greater than was considered to be desirable, they were less than those occurring with any other method studied, and the A.A.C.C. adopted the ferricyanide procedure as a tentative method as well as the method of the A.S.B.C. A joint study of the two methods by the A.S.B.C. and the A.A.C.C. (*Cereal Chem.*, 1942, **19**, 249; *Proc. Amer. Soc. Brew. Chem.*, 1941, **90**) showed almost identical average values at the four levels of activity, and the variation between laboratories was similar. This study verified the conversion factor from ml of ferricyanide soln. into degrees Lintner suggested by Anderson and Sallans (*loc. cit.*). Both societies recommended the use of the factor 23 to convert ml of ferricyanide soln. to °Lintner when the A.S.B.C. method with 200 ml of starch soln. and a final vol. of 250 ml is employed. A third collaborative study (*Cereal Chem.*, 1943, **20**, 31) gave further indication that the two procedures give values of the same magnitude. It is therefore recommended: (1), that the ferricyanide modification for the determination of reducing power after diastasis be adopted tentatively as an alternative to the Fehling titration procedure; (2), that par. 48, "*Methods of Analysis of the A.O.A.C.*," 1940, 161, be modified according to the recommendation of Rask (*J. Assoc. Off. Agr. Chem.*, 1942, **25**, 265) by addition at the end of the second par. of the following—"Determine reducing power by (I) Fehling's modification or (II) the ferricyanide modification; (3) that values for diastatic power be reported as degrees Lintner and maltose equivalent until such time as ° Lintner can be dropped. For revised copies for both modifications see *J. Assoc. Off. Agr. Chem.*, 1944, **27**, 82. A. O. J.

Report on Colour and Turbidity in Beer and Wort. B. H. Nissen (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 389–393)—Photoelectric measurements (Nissen, *Proc. Amer. Soc. Brew. Chem.*, 1942, May, 25–27) have shown that in most colour work turbidity must be considered unless the soln. examined is brilliantly clear. At present the Lovibond Tintometer with glasses of series No. 52 and a 1/2-in. cell is the official A.S.B.C. instrument for determining the colour of beer. Extensive investigation has shown that the glasses vary considerably and inaccuracies occur both in the colour value of some glasses and in duplicate glasses, which sometimes fail to agree with one another, especially in the lower values. Iodine soln. (0·01 *N*) diluted in a series from 1 to 50 ml per 100 ml has been suggested for colour matching, but photoelectric examination of these solns. shows that concns. above 6 ml per 100 ml (*ca.* 4·25° Lovibond) match the colour of beer quite closely, but with concns. below this the colour departs increasingly from that of beer, becoming greener in shade. Even with special precautions, iodine solns. are not stable enough to serve as suitable standards. Several types of dye soln. were tried, *viz.*, the Brand dye, the American Public Health Colour Standards and the Cobalt-Iron-Copper Pharmacy Colours. Of all the colour standards, those employing inorganic salts offered the most hope, but a progressive intensity series could not be used. Each standard of the series had to be prepared separately. Recently efforts have been directed towards preparing fairly permanent standards by using the Brand dyes (*infra*). These are more stable and more easily prepared than the iodine standards. The aim, however, should be to develop some type of photoelectric colorimetric method that would register accurately the colour of beer and wort. In the investigation it was observed that measurement of turbidity is necessary. No standard method has yet been accepted as official, but the following method, in which dispersions of fullers' earth in water are arranged in series from 0 to 50 p.p.m., is recommended. Match the haziness of the beer in a wide-mouthed bottle against a set of standard bottles made up to definite turbidities, such as 0, 1, 2, 4, 6, 8, 10, 12, 15, 20, 30, 40, 50 and 100 p.p.m. of turbidity. Make the comparison by holding the standard bottle and the sample at arm's length towards a good light (preferably north daylight) and observing a dark horizontal band such as the metal frame of a window. Shake the standard bottle before matching. Immerse the bottles in water containing a suitable wetting agent which effectively removes the "sweating" that occurs if the sample is cold, and match the clouding effect against the dark band. By this method turbidities at any temp. can be read quickly. The bottles are 16-oz. wide-mouthed, square type, tall, clear, glass sample bottles $2\frac{1}{4} \times 2\frac{1}{4} \times 5\frac{1}{2}$ in. to the shoulder. The final turbidity standards are made from a standardised 100 p.p.m. stock mixture prepared as follows—Mix 1 teaspoonful of White Pptd. Fullers' Earth (A. & F. Pears, London) with 5 litres of water and stir 5 times in 24 hr. Pour off the supernatant liquid and dilute this suspension to 100 p.p.m. of turbidity, using the government candle method and equipment (Jackson turbidimeter, etc.). Make up the standard bottles from this suspension by adding to water in a 500-ml flask the required amount of stock suspension, *ca.* 5 ml of filtered Brand dye soln. and 5 ml of sat. mercuric chloride soln. and prepare aliquot dilutions for the desired standards. To prepare Brand dye soln. dissolve 0·05 g of Patent Blue V.A., 0·12 g of Resorcin Brown G, 0·20 g of Amaranth W.N. and 0·80 g of Tartrazine C. Extra (all obtainable from General Dyestuffs Corp., Chicago) in 20% alcohol and dilute to 1 litre with the same solvent. Slight

variations of the concn. of each dye may be necessary to match particular samples. The bottles should be well filled and closed with screw caps, and the standards should be renewed monthly, since they tend to become more turbid. When compared with photoelectric measurements, turbidity values estimated visually differ with the type of turbidity. With protein turbidity (by far the most usual type) agreement between visual and photoelectric measurements is good. With dextrin turbidity, occurring in frozen beer, and yeast turbidity, lower readings are obtained by the photoelectric method for the higher values, and higher readings for values of 5–10 p.p.m. as compared with the visual values. Otherwise the method has proved valuable in many tests. KWKSol Bentonite (Amer. Colloid Co.) has been used with success in place of fullers' earth and the standards appear to be more stable. This grade of Bentonite cakes less than Bentonite of the Volclay type. A. O. J.

Report on Apricot Kernel Oils [Volatile Apricot Kernel Oil] Austr. Sci. Res. Liaison, London (*Papers from Australia*, No. 348; *Bull. Imp. Inst.*, 1944, 42, 153–160)—The constants in the literature for expressed apricot kernel oil are tabulated, and a description is given of the characteristics of (*a*) volatile apricot kernel oil and (*b*) the volatile oil after removal of hydrocyanic acid. The constants were: (*a*) sp.gr. at 15°/15° C., 1·045–1·070 (a high constant indicates an exceptional proportion of HCN or an abnormal amount of benzaldehyde-cyanhydrin); optical rotation, 0° to +0° 10′; n_D^{40}, 1·5320 to 1·5450. (*b*) sp.gr. at 15°/15° C., 1·0499 to 1·055, optical rotation, 0° to +0° 10_1; n_D^{40}, 1·5420 to 1·5460; b.p., 179° C. The oil is water-soluble (1 in 300 at room temp.). It should not contain more than 1% of benzoic acid. Its main constituents are benzaldehyde, hydrocyanic acid and benzaldehyde-cyanhydrin. To detect adulteration with nitrobenzene, (1) warm with iron filings and acetic acid, distil and test the distillate with a few drops of chloride of lime soln.; a violet colour indicates the presence of aniline from reduced nitrobenzene. (2) Shake the sample with excess of sodium sulphite soln., so that the benzaldehyde is entirely combined; the coarse nitrobenzene odour will be perceptible. Volatile apricot kernel oil may be used as a flavouring essence in cooking and confectionery, and as a substitute for oil of bitter almonds in the soap industry. E. B. D.

Analysis of the Pastilles of the British Pharmaceutical Codex. N. Evers and W. Smith (*Quart. J. Pharm.*, 1944, 17, 220–225)—Variations in the weight of pastilles are discussed and figures are presented which appear to show that these may be caused by (*a*) a trend towards lighter pastilles through the run of a batch and (*b*) lack of uniformity in the amounts of base delivered to the individual moulds. Methods of analysis for 9 of the 13 pastilles of the B.P.C. are described, and it is stated that they may not be applicable to preparations other than those with glycogelatin as base. *Pastille Ammonium Chloride*; *Pastille Ammonium Chloride Compound*; *Pastille Ammonium Bromide*—Hydrolyse 10 pastilles by heating under reflux with *ca.* 100 ml of 0·1 *N* sodium hydroxide for 30 min., rinse the condenser with water, and dilute to 100 ml. Add 50 ml of 0·1 *N* silver nitrate to 10 ml of this soln., then 2 ml of nitric acid, dilute to 100 ml and filter. Titrate 50 ml of the filtrate with 0·1 *N* potassium thiocyanate, with ferric ammonium sulphate soln. as indicator; 1 ml of 0·1 *N* silver nitrate ≡ 0·00535 g of ammonium chloride or 0·009796 g of ammonium bromide. *Pastille Benzamine*—Hydrolyse 10 pastilles by heating under reflux with 100 ml of 0·1 *N* sodium hydroxide for 1 hr., transfer to a separator, acidify with dil. sulphuric acid, and extract with 5 portions of chloroform, washing each extract with the same 10 ml of water. Transfer the extracts to a tared flask, remove the solvent, dry and weigh the residue of benzoic acid. This weight, multiplied by 2·325, gives the weight of benzamine hydrochloride. The m.p. of the acid may be taken, or its equivalent determined by titration with 0·1 *N* sodium hydroxide, phenol red being used as indicator. *Pastille Cocaine Hydrochloride*; *Pastille Menthol and Cocaine*—Hydrolyse 10 pastilles by heating under reflux with 100 ml of 0·1 *N* hydrochloric acid for 2 hr., cool, transfer to a separator, extract with 2 quantities of 10 ml of chloroform, make alkaline with ammonia and extract with 5 portions of chloroform, washing each extract in turn with 2 successive portions of 2 ml of water. Transfer the chloroform extracts to a dish, remove the solvent, and dry at 100° C. for 30 min. Dissolve the residue in 5 ml of neutral alcohol, add 2·0 ml of 0·1 *N* hydrochloric acid and back-titrate with 0·1 *N* sodium hydroxide from a micro-burette, methyl red as indicator. 1 ml of 0·1 *N* hydrochloric acid ≡ 0·03396 g of cocaine hydrochloride, $C_{17}H_{21}O_4N$, HCl. *Pastille Codeine*—Hydrolyse 10 pastilles under reflux with 100 ml of 0·1 *N* hydrochloric acid, cool, transfer to a separator, make alkaline with ammonia, and extract with 3 portions of chloroform, washing each extract with the same 5 ml of water. Transfer the extracts to a tared flask, remove the solvent, dry at 100° C. and weigh the anhydrous codeine. This may be converted to the weight of codeine, $C_{18}H_{21}O_3N,H_2O$, by the factor 1·0602. *Pastille Diamorphine Hydrochloride*; *Pastille Diamorphine and Pine Compound*—The method of Allport and Jones (*Quart. J. Pharm.*, 1942, 15, 238; ANALYST, 1943, 68, 22) was found to be the most satisfactory. Hydrolyse 10 pastilles under reflux with 100 ml of 0·1 *N* hydrochloric acid for 1 hr. Add a slight excess of sodium bicarbonate to 10 ml of the soln. and extract the alkaloid with successive quantities of a mixture of 3 vol. of chloroform and 1 vol. of alcohol (90%). Wash each chloroform extract with the same 0·5 ml of water, mix the extracts, remove the solvent, and dissolve the residue in 5 ml of *N* hydrochloric acid. Transfer the soln. to a 25-ml flask, add 2 ml of 5% sodium nitrite soln. (freshly prepared), mix, add 2 ml of dil. ammonia soln., shake and dilute to 25 ml with water. Measure the colour produced in a 1-cm cell by means of a Lovibond Tintometer and correlate the yellow component of the colour with the equiv. morphine content from the table cited by Allport and Jones (*loc. cit.*, 239). This is converted into diamorphine hydrochloride by the factor 1·486. Recovery figures obtained by submitting the correct weight of medicament and of glycogelatin to the above procedures are presented in a table. J. A.

Standardisation of Assay of Penicillin. A. C. Hunter and W. A. Randall (*J. Assoc. Off. Agr. Chem.*, 1944, 27, 430–438)—In the autumn of 1943 the U.S. Food and Drug Administration was called upon to undertake regular assays of penicillin in connection with the enforcement of the Federal Food, Drug and Cosmetic Act. The difficulties of the assay soon became apparent, and inexplicably wide discrepancies were encountered between the

potency values assigned by the manufacturer and those ascertained by the Food and Drug Administration. One of the variables that had to be resolved was the reference standard. With the preparation of crystalline penicillin sodium, even on a limited research laboratory scale, it became possible to establish a master standard consisting of the chemically pure sodium salt, but, owing to present scarcity of this standard, use of a reference standard with a value determined by assay against the master standard is still necessary. Quantities of pure penicillin sodium offered to the Administration by three manufacturers were examined separately and finally mixed and assayed to form the primary standard. During this work it became apparent that it would be possible to abandon expressions of potency in terms of the Oxford unit in favour of mg of active penicillin without disturbing the clinician or confusing the results of previous work done with the Oxford unit as basis. Extensive collaborative work by eight laboratories on the primary standard established that a value of 1650 units per mg could be assigned to the pure crystalline penicillin sodium. Thus an ampoule of an impure salt of penicillin designated as containing 100,000 units will contain the antibiotic activity of 60 mg of pure penicillin sodium and may be so labelled. Actually 60 mg is 99,000 units in terms of the primary standard, but this is sufficiently close for labelling purposes and avoids the expressing of the value of the standard as a figure carried to the last digit or to the decimal. This unit, although near it in value, is not the Oxford unit, and it is suggested that this unit should be used only until routine practice has accepted measurement of potency in terms of wt. of pure penicillin. The Administration acquired a sufficient amount of penicillin calcium to provide the reference standard. The same eight laboratories, collaborating with the Administration, established the potency value of this salt against the primary standard as 370 units per mg. Referred to the primary standard, this secondary standard has the equiv. of the activity of 224 μg of pure crystalline penicillin sodium per mg. With these standards the Government assays during the period Jan.-April, 1944, and the manufacturers' declarations showed better agreement. Although numerous other factors need standardisation, *e.g.*, the test organism, the density and amount of the inoculum, the composition of the culture medium, the period and temp. of incubation and the method of computation (especially with plate cultures), the establishing of this standard of potency is considered to be a step towards more consistent assay results. A. O. J.

Biochemical

Photofluorometric Determination of Mepacrine. M. E. Auerbach and H. W. Eckert (*J. Biol. Chem.*, 1944, **154**, 597–603)—The method now described is claimed to be simpler and, if anything, slightly more sensitive than those previously reported. The improvement is due to the addition of caffeine sodium benzoate, which intensifies the fluorescence of solns. of acridine derivatives. Pipette 5 ml of oxalated whole blood into a 125-ml separating funnel containing 10 ml of 5% $Na_2HPO_4.12H_2O$ soln., add 30 ml of ether and shake for 3 min. Run off the aqueous layer and wash the ethereal layer with three 5-ml portions of 0·05 *N* sodium hydroxide and once with 5 ml of water. Shake the ethereal layer for 30 sec. with two 5-ml portions of 0·1 *N* sulphuric acid, collect the acid extract in a 25-ml graduated cylinder and wash the ether with 2 ml of water, adding the washings to the acid soln. Dilute to the 15-ml mark and add 1 ml of caffeine reagent (dissolve 10 g of caffeine sodium benzoate in 30 ml of water and 40 ml of 95% ethanol, add 20 ml of diethanolamine, dilute to 100 ml with water and mix). Mix and measure the fluorescence of the soln. in a Coleman No. 12 photofluorometer, using a soln. of 1 μg of mepacrine in 10 ml of 0·1 *N* sulphuric acid and 1 ml of caffeine reagent, diluted to 16 ml with water. Deduct from the reading the apparent fluorescence of a blank consisting of 10 ml of 0·1 *N* sulphuric acid plus 1 ml of caffeine reagent diluted to 16 ml with water. Assay urine in the same way as blood, but first dilute the sample, because of the larger quantities of mepacrine present. Mince tissue in a Waring Blendor after diluting 10 to 40-fold with water, and then pipette 10 ml of the suspension into a test-tube, add 2 ml of 40% sodium hydroxide soln. and digest the mixture for 30 min. in a water-bath at 80° C. Transfer the soln. to a separating funnel and extract with ether as in the estimation of blood. Recoveries of 95–97% of the theoretical were obtained from blood, urine, liver and other tissues. F. A. R.

Enzymatic Determination of Glutamine. R. M. Archibald (*J. Biol. Chem.*, 1944, **154**, 643–656)—A convenient method of preparing the enzyme glutaminase, which hydrolyses glutamine to glutamic acid and ammonia, has been worked out (*cf.* next abstract). The enzyme preparation can be used for estimating glutamine. *Method I—Direct Nesslerisation of digest filtrate*—Withdraw the blood under test by means of an oiled syringe to minimise haemolysis, and add 1 mg of potassium oxalate or 0·05 mg of heparin per ml in CO_2-filled centrifuge tubes. Centrifuge in a cold room and recentrifuge the separated plasma. To 1 ml of plasma or other soln., containing 0·05 to 1·2 mg of glutamine, add 0·5 ml of phosphate buffer *p*H 7·2 (1 vol. of *M* NaH_2PO_4 and 3 vols. of *M* K_2HPO_4 diluted 4-fold) and 0·5 ml of a 25% suspension of dialysed kidney. Incubate the mixture for 1 hr. at 38° C. Prepare the following three blanks: (*a*) On the reagents, using 1 ml of water and 0·5 ml of potassium cyanide soln. (add 6·5 g of potassium cyanide and 150 ml of *M* NaH_2PO_4 to 2350 ml of water) in place of the sample and enzyme. The NH_3 nitrogen in the reagent blank is B_R. (*b*) On the enzyme, using 1 ml of water to replace the sample. Incubate the mixture as in the estimation. The ammonia formed is the sum of that preformed in the enzyme and reagents plus that formed from materials in the enzyme preparation during incubation. The NH_3 nitrogen in the enzyme blank is B_E. (*c*) On the sample, using 0·5 ml of the cyanide soln. instead of 0·5 ml of enzyme suspension. Incubate the mixture as in the estimation. The ammonia formed is the sum of that preformed in the sample, plus that formed during incubation, other than by the glutaminase, plus approx. 5% of the amide nitrogen of the glutamine in the sample. The NH_3 nitrogen in the sample blank is B_S.—After incubation of the sample and the enzyme and sample blanks, add to the 4 solns. 1 ml of 10% sodium tungstate soln. and 2 ml of 0·5 *N* sulphuric acid, dilute to 10 ml and filter. Mix 0, 1, 2 and 3 ml of 0·5 *mM* ammonium sulphate with 0·5-ml portions of a cyanide-phosphate soln., prepared by mixing equal vols. of 0·04 *M* potassium cyanide and the *M* phosphate buffer, and dilute each soln. to 5 ml. In this way standard solns. containing 0, 0·014, 0·028 and 0·042 mg of NH_3 nitrogen are obtained.

To 5 ml of each tungstic acid filtrate and to each standard, add 1 ml of a soln. prepared by mixing equal vols. of 2·5% potassium persulphate soln. and 1% potassium gluconate soln.; this prevents the development of turbidity during the nesslerisation. Add 0·5 ml of Nessler's reagent (dissolve 45·5 g of mercuric iodide and 34·9 g of potassium iodide in 150 ml of water, add 112 g of potassium hydroxide and dilute to 1 litre), and, after 4–6 min., measure the absorption in a photometer at 450 $m\mu$, using a 1-cm cell. The glutamine amide nitrogen = 1·05 ($N - B_E - B_S + B_R$), where N = total NH_3 nitrogen measured in the incubated digest. *Method II—Nesslerisation of distillate from digest*—Incubate 2 ml of plasma or 1–5 ml of other soln., containing 0·05–0·6 mg of glutamine, 0·5 ml of *M* phosphate buffer and 0·5 ml of a 25% suspension of dialysed kidney in 0·04 *M* potassium cyanide for 1 hr. at 38° C. Prepare blanks for ammonia production in the enzyme (B_E), sample (B_S) and reagents (B_R), as described in Method I, and immerse all the tubes in ice-water. Transfer the contents of each tube in turn to a 50-ml centrifuge tube with a minimum of water and add 1 drop of antifoam mixture (1 vol. of sorbitan monolaurate and 3 vols. of liquid paraffin) and 2 ml of borate buffer *p*H 10 (mix 47 ml of 18 *M* sodium hydroxide, 185 g of borax and 1800 ml of water and boil for 15 min. to remove traces of ammonia, cool to 30° C. and dilute to 2 litres), and distil off ammonia under reduced pressure into 5 ml of 0·04 *N* sulphuric acid with the water-bath at 50° C. and the soln. at 35–42° C. The distillation is complete in 5 min. Transfer the distillate to a graduated flask and dilute to 10 ml. Prepare a series of standards with 1, 2, 3 or 4 ml of 0·5 *mM* ammonium sulphate, add 5 ml of 0·04 *N* sulphuric acid and dilute to 10 ml. Add to each flask 0·5 ml of Nessler's reagent and measure the colours at 450 $m\mu$ in a photometer. The glutamine amide nitrogen = 1·022 ($N - B_E - B_S + B_R$). The distillation procedure has the advantage that no glutamine is lost by pptn. of protein and, as the whole of the enzyme digest is available for analysis, smaller concns. of glutamine can be determined. No creatinine or amino acids are present to complicate the nesslerisation, no gluconate-persulphate is necessary, and cyanide need not be added to the standards. The results are more accurately reproducible and the blank values are more consistent than with direct nesslerisation, which can only be used when the concn. of interfering substances is small. Whereas Method II gave practicallr theoretical results for the recovery of glutamine added to water or dog plasma, direct nesslerisation gave a 95% recovery of glutamine added to plasma and 104–106% of glutamine added to water. F. A. R.

Preparation and Assay of Glutaminase for Glutamine Determinations. R. M. Archibald (*J. Biol. Chem.*, 1944, **154**, 657–667)—Dog kidneys give a more active and specific glutaminase preparation than beef kidneys, but the latter give a preparation that is adequate for the estimation of glutamine (*cf.* preceding abstract), and can be used if dog kidneys are not available. Remove the perirenal fat and capsules from the kidneys, emulsify with 3 parts of ice-cold 0·04 *M* potassium cyanide adjusted to *p*H 7·2 by addition of NaH_2PO_4. The emulsification is best done in a Waring Blendor when amounts of the order of 100 g are being worked up, or in a mortar with small amounts. Strain the mixture through muslin and use the filtrate within 24 hr. Emulsification and filtration are carried out at 0° C. Dialyse the filtrate in cellulose sausage casings by shaking vigorously for 2 hr. at 0° C. in a vessel containing 2·5 litres of 0·04 *M* potassium cyanide, adjusted to *p*H 7·2 by addition of 150 ml of *M* NaH_2PO_4. Dilute this 25% dialysed emulsion with 4 vols. of the 0·04 *M* potassium cyanide soln. of *p*H 7 and assay as follows. Put into each of 7 test-tubes, in the following order, 0·5 ml of *M* phosphate buffer *p*H 7·2 (for this and other reagents, see preceding abstract), 1 ml of 2·5 *mM* glutamine soln. and 0·5 ml of the 5% glutaminase soln. Incubate the tubes at 38° C. and, after 2, 5, 10, 15, 20 and 30 min., stop the enzyme action in successive tubes by adding 1 ml of 0·1% brom-sulphalein soln. or 0·01% quinone soln. Incubate a blank, containing water instead of the glutamine soln., for 15 min. After stopping the enzyme action, stopper each tube, immerse in ice-water and transfer the contents in turn with the aid of 2 ml of water to the distillation apparatus, and add 2 ml of borate buffer *p*H 10. Distil off the ammonia, nesslerise and measure against standards as described in Method II of the preceding abstract. Units of glutaminase in the 0·5 ml sample of 5% kidney emulsion $= \frac{10}{\text{min. to form 0·01 mg } NH_3 \text{ nitrogen}}$.

F. A. R.

Determination of Glucuronic Acid by the Naphthoresorcinol Reaction with the Photoelectric Absorptiometer. S. W. F. Hanson, G. T. Mills and R. T. Williams (*Biochem. J.*, 1944, **38**, 274–279)—To 2 ml of the soln., containing 10–80 μg of glucuronic acid, add 2 ml of 0·25% naphthoresorcinol soln. and 3 ml of conc. hydrochloric acid (pure). Immerse the tube in a boiling water-bath for 2 hr., cool in ice for 10 min. and add 5 ml of pure amyl alcohol. Stopper the tubes, shake vigorously for 15 secs. and pour the contents into a 50-ml separating funnel, rinsing out the tube with a further 5 ml of amyl alcohol and adding the washings to the separating funnel. Allow the aqueous layer to separate, run off the amyl alcohol soln. into a graduated tube and dilute to 11 ml with absolute ethanol. Measure the colour in a Spekker photoelectric absorptiometer (10-mm cells, Ilford 607 orange filters) and calculate the results from a calibration curve constructed with *l*-methyl glycuronide or glucurone, both of which gave the same straight line when the amounts (μg) of glucuronic acid were plotted against drum readings. The recoveries of glucuronic acid added to solns. of various glucuronides were, in general, slightly higher than the calculated values, but *l*-menthyl glucuronide could be recovered almost quantitatively from normal human urine. Mucic acid and sugars, *e.g.*, glucose, fructose and pentoses, interfere, and it has not yet been possible to find a satisfactory method of overcoming this interference.

F. A. R.

Liver Storage Test for the Estimation of Vitamin A. K. Guggenheim and W. Koch (*Biochem. J.*, 1944, **38**, 256–260)—The usual method of vitamin A bioassay, based on the curative growth test, is tedious, and a method has now been devised which gives results within 4 days. The material to be tested is fed to rats and the amount of vitamin A stored in their livers is estimated chemically. Rats weighing 35–50 g are used. The food of the mothers consists of sprouted wheat, barley, bran, seasonal vegetables and milk, but two weeks before weaning the vegetables are excluded from the diet. The young rats are fed on a vitamin

A-free diet consisting of 65% of rice flour, 13% of ethanol-extracted casein, 10% of olive oil, 8% of dried yeast, 4% of salt mixture and 100 I.U. of vitamin D per kg of food. The material to be tested is administered in 0·1 ml of olive oil on the 1st and 2nd days. On the 4th day the rats are guillotined, and the amount of vitamin A in the livers is determined by the Carr–Price method. A block comparator was used to compare the colour with that of a soln. containing 6·5% of cupric chloride and 0·3% of cobalt nitrate, but any other type of colorimeter can, of course, be used. A reference curve was obtained by plotting the amount (I.U.) of vitamin A found in the livers against the logarithm of the amount (I.U.) given per 10 g of body weight. This was a parabola, the equation of which was $x = 0{\cdot}0601\,y^{0{\cdot}755} + 0{\cdot}8175$, where x = the logarithm of the vitamin A given, expressed as I.U. per 10 g of body weight, and y = total units recovered from the livers. This reference curve holds good up to 35 I.U. per 10 g of body weight administered, whilst amounts below 6·5 I.U. per 10 g of body weight probably do not result in any liver storage at all. The method was compared with Coward's growth-rate method. Whereas the probable error of the latter is 20% and 16% in the 3- and 5-weeks' growth test respectively, using 10 male rats, the probable error in the present test, using 5 pairs of animals is only 6·5%. The main disadvantage of the test is that the materials to be tested must contain sufficient vitamin A to give appreciable storage in the liver. F. A. R.

Biological Value of Carotene from Various Sources and the Effect of Vitamin E on the Utilisation of Carotene and of Vitamin A. K. Guggenheim (*Biochem. J.*, 1944, **38**, 260–264)—Using the method of Guggenheim and Koch (*cf.* preceding abstract), the amounts of vitamin A stored in the liver when carotene from various sources was fed to rats were measured, and the biological value of the carotene was then calculated from the reference curve. It was found that the vitamin A of fish oils and the vitamin A and carotene from cow's liver were completely utilised, whereas the biological value of carotene from plant materials varied from 33 to 67, assuming the biological value of vitamin A in oil to be 100. An exception was provided by the carotene of lettuce, which was utilised as efficiently as pure vitamin A. An investigation was therefore undertaken to discover the reason for this variation in the biological value of carotene. This revealed somewhat paradoxically that the faecal excretion of carotene was higher the more efficiently the carotene was utilised, so that defective absorption could not be due to excessive faecal excretion. The factor chiefly concerned with the utilisation of vitamin A was found to be vitamin E, which has previously been shown to improve the utilisation of carotene and vitamin A in the curative growth test. Thus, when rats were given measured amounts of vitamin A or carotene in olive oil or carrots, together with varying quantities of α-tocopherol, the biological value of the vitamin A and carotene increased with the amount of tocopherol. The amount of carotene excreted also increased, thus explaining the apparent paradox previously observed. This observation also explained the differences in the utilisation of vitamin A and carotene when dissolved in different plant oils; in linseed or cottonseed oils for example, they give better growth than in olive or coconut oil. This is believed to be due to the relatively larger amounts of α-tocopherol in the former than in the latter. The complete utilisation of the carotene of lettuce is attributable to the large amounts of vitamin E in lettuce. F. A. R.

Carotene and Lycopene in Rose Hips and Other Fruits. F. C. Jacoby and F. Wokes (*Biochem. J.*, 1944, **38**, 279–282)—Dissect the flesh from the seeds, etc., of *ca.* 5 g of rose hips and divide into two portions. Weigh and grind each portion with quartz powder and a mixture (2 : 3) of acetone and light petroleum. Decant the yellow soln. into a separating funnel and grind the residue with more solvent until no further colour is extracted. Wash the combined extracts with water continuously until all the acetone is removed, and then extract the xanthophylls by shaking two or three times with 1/3 vol. of diacetone alcohol. Wash out the diacetone alcohol with water, run the petroleum extract through a column (20 × 2 cm) of activated alumina, and develop the chromatogram with light petroleum containing 1% of acetone. This removes the orange layer of carotene, and the lycopene layer is then eluted with benzene. Estimate the colours of the carotene and lycopene solns. with a Hilger-Nutting constant deviation wavelength spectrometer, measuring the density of the carotene soln. in light petroleum at 450 *mμ* and that of the lycopene soln. in benzene at 482 *mμ*. Calculate the concns. of carotene and lycopene from the $E^{1\%}_{1\,cm}$ values, which are 2500 and 2025 respectively. F. A. R.

Ultra-violet Absorption of Irradiated Vitamin A. H. Sobotka, S. Kann, W. Winternitz and E. Brand (*J. Amer. Chem. Soc.*, 1944, **66**, 1162–1164)—It has previously been found that alcoholic solns. of vitamin A esters show an increase in their greenish fluorescence when exposed to ultra-violet light for a few min.; this is followed by a decrease in the fluorescence on continued irradiation, the decrease being retarded in absence of oxygen. Information concerning the nature of this fluorescent product was obtained by studying the ultra-violet absorption spectrum of vitamin A during irradiation. The expts. were carried out with crystalline vitamin A alcohol and vitamin A acetate. Whereas vitamin A alcohol showed a slow steady decrease in fluorescence and a decrease in the peak at 328 *mμ* on continued irradiation, vitamin A acetate showed an increased fluorescence for the first 5 min. of irradiation while the absorption at 325 *mμ* dropped to less than half its original value during this time and 3 new bands made their appearance at 364, 346 and 275 *mμ*. During this period also the reaction with antimony trichloride decreased by not more than 10%. After 40 min. irradiation of vitamin A acetate the 3 peaks at 364, 346 and 325 *mμ* disappeared completely, whilst the band at 275 *mμ* increased. This band resembles that observed in deteriorated fish-oils and may be due to secondary reaction products formed by oxidation. At the same time the Carr–Price reaction was reduced to half its original value. The absorption spectrum of vitamin A acetate after irradiation for 5 min. was very similar to that of *iso*anhydro vitamin A, but the two substances differ in certain other respects. The Carr–Price reaction of the irradiated soln. cannot be accounted for by unchanged vitamin A, but is presumed to be due to oxidation products such as the compound, referred to above, possessing an absorption band at 275 *mμ*. It is not clear whether this compound is the result of oxidation of the intermediate irradiation product or is formed from vitamin A directly

by an independent reaction. It is believed that irradiation of vitamin A esters in alcoholic soln. induces the formation of a compound with more than five conjugated double bonds, which may be an excited form of *iso*anhydro vitamin A. F. A. R.

Vitamin B [Aneurine and Nicotinic Acid] in Malt Extract. J. G. Organ, E. M. James and F. Wokes (*Quart. J. Pharm.*, 1944, **17**, 183–187) —Samples of English barley have been examined for change in vitamin B_1 content on malting and brewing for the preparation of malt extract. Expts. on 11 samples, whose original vitamin B_1 content averaged 126 I.U. per 100 g of dry matter (107 I.U.–166 I.U.), indicated an average loss of 11·4% of vitamin B_1 during malting, individual results ranging from 37% loss to 1·8% gain. The actual vitamin contents of the malted grains derived from these samples ranged from 81 I.U. per 100 g of dry matter to 148 I.U. per 100 g of dry matter, av. 111 I.U. Fifteen samples of English malt of average vitamin B_1 content of 113 I.U. per 100 g of dry matter (81 I.U.–148 I.U.) showed an average gain of 15·7% during brewing, individual results ranging from 19·8% loss to 51·4% gain; 14 samples of malt extracts derived from these malts had an average vitamin B_1 content of 131 I.U. per 100 g of dry matter (96 I.U.–165 I.U.). On following the process of malting and brewing with 10 samples of English barley, an average gain of 5·0% in the vitamin B_1 content was indicated. The above percentages are calculated on dry weight basis. It is suggested that the wider variation in the vitamin B_1 content of commercial malt extracts than would be expected from the above results is due to manufacturing conditions, as is the very low B_1 and protein contents in some samples. The importance of nicotinic acid in cereal products is stressed, as the rationing of meat, etc., appeared likely to produce a deficiency of this factor in war-time diets (Kodicek, *Lancet*, 1942, **242**, 380), and a series of 15 samples of commercial malt extracts from 8 different manufacturers was examined for nicotinic acid content. This ranged from 7·5 to 13·4 mg per 100 g, and, on the whole, samples richer in aneurine and protein contained more nicotinic acid although sufficient results have not yet been obtained to determine whether there is any significant correlation between the aneurine and nicotinic acid contents. The establishment of a standard for nicotinic acid in malt extract is not recommended at present. J. A.

Destruction of Riboflavin in Milk by Sunlight. W. J. Peterson, F. M. Haig and A. O. Shaw (*J. Amer. Chem. Soc.*, 1944, **66**, 662–663)— The observation of previous workers, that exposure to light destroys riboflavin in milk, suggested that appreciable amounts of the vitamin would be lost from bottled milk exposed to sunlight. This has now been established experimentally. Fresh milk in pint bottles was exposed to direct sunlight on an open porch for various periods of time up to 210 min. The riboflavin was estimated fluorimetrically in each instance with the Coleman electronic photometer, using the method of Hand (*Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 306), except that 10 ml of acetone were added to 5 ml of milk to obtain the filtrate. Atmospheric temperatures ranged from 60 to 72° F. The observed losses after 30, 90, 120 and 210 min. were respectively 28, 50, 66 and 72%. Control samples stored at room temp. for 24 hr. or in the refrigerator for 7 days lost no riboflavin. F. A. R.

Determination of Aneurine by the Thiochrome Method after its Uptake by Yeast. R. Marcuse, T. Widhe and E. Sperber (*Nature*, 1944, **154**, 549)—It has been shown (Sperber and Renvell, *Biochem. Z.*, 1941, **310**, 160; Sperber, *id.*, 1942, **313**, 62) that baker's yeast readily absorbs aneurine, especially under aerobic conditions in presence of a substrate. Further, the aneurine in yeast occurs almost exclusively as co-carboxylase which is hydrolysed to free aneurine on boiling the yeast (*loc. cit.*; Westenbrink *et al.*, *Enzymologia*, 1940, **9**, 73). It has been found possible to determine the total aneurine content of cereals, etc., by shaking an extract of the sample with baker's yeast and assaying the extract obtained on boiling. Interfering factors in the substance under examination do not pass into the yeast. Under the conditions employed by the authors, 1 g of yeast absorbed 900–1000 μg of aneurine from a flour extract in the course of 1 hr. shaking, whence the amount of yeast usually necessary is sufficiently small for allowance to be made for the aneurine content of the yeast itself by an average value (*ca.* 4·5 μg per g). In general, 50–200 ml of a flour extract, containing 60–120 μg of aneurine, were shaken with 2 g of compressed yeast in Fernbach flasks, immersed in a water-bath at 25° C. for 90 min. Results at first were low, owing probably to inactivation of the yeast phosphatase during the shaking. The use of "Diastase Merck" proved unsatisfactory, as did the addition of fresh yeast to the shaken material. Dephosphorylation of the co-carboxylase in the shaken yeast was achieved by adding cytolysed yeast to the previously boiled extract and reboiling. Repeated analyses of the same flour gave values with a standard deviation of 2·6%. Added aneurine was recovered from a flour extract and results obtained from the assay of a co-carboxylase preparation accorded well with the results of Warburg determinations on the same material. The method is applicable to the determinations of aneurine in urine and in molasses. A detailed account of these investigations is to be published elsewhere. J. A.

Simultaneous Determination of Aneurine and Nicotinamide Methochloride. R. A. Coulson (*Nature*, 1944, **154**, 547–549)—Nicotinamide methochloride, the principal urinary derivative of nicotinic acid, nicotinamide and nikethamide, can be estimated in urine by adsorption on "Decalso," elution with potassium chloride solution and extraction of the eluate with alkaline *iso*butanol (Coulson, Ellinger and Holden, *Biochem. J.*, 1944, **38**, 150), whence it is converted into a whitish fluorescent derivative, F_2, which may be estimated fluorimetrically. If the potassium chloride eluate is treated with alkaline potassium ferricyanide before the extraction with *iso*butanol, the methochloride is converted into a deep blue fluorescent derivative, F_3, similar to thiochrome, which interferes with the determination of aneurine in urine, in which both the latter and nicotinamide methochloride are normally present. No procedure has been discovered for separating the two blue fluorescent pigments and no adequate blank correction is possible, since omission of the potassium ferricyanide results in the formation of the derivative F_2. It has been found that the "aneurine equivalent" of the blue fluorescent pigment, F_3, can be determined by visual comparison with thiochrome standards, and a method for the assay of both aneurine and nicotinamide methochloride in urine and foods is described.

Wash 1-g portions of "Decalso" into thistle funnels, with an internal bore of 4 mm, and activate according to the procedure of Hennessy and Cerecedo (*J. Amer. Chem. Soc.*, 1939, **61**, 179). Adjust the urine or food extracts to *p*H 4·0 and filter through these columns; wash each with 20 ml of 1% acetic acid and elute with 8 ml of 25% potassium chloride soln. Treat half of each eluate successively with 0·25 ml of 0·5% potassium ferricyanide, 1 ml of 20% sodium hydroxide soln. and 5 ml of *iso*butanol and shake for 1 min. Compare the blue fluorescence (due to the thiochrome and the derivative F_3) with thiochrome standards prepared from aneurine solns. treated in the same manner. To the other half of each eluate add 1 ml of 20% sodium hydroxide and 5 ml of *iso*butanol and shake for 5 min. Compare the whitish fluorescence with standards prepared from pure nicotinamide methochloride solns. treated in the same manner, and from the results of this assay compute the fluorescence due to F_3 and subtract it from the total blue fluorescence due to F_3 and thiochrome. It is stated that the "Decalso" obtainable in Great Britain will not completely adsorb aneurine or nicotinamide methochloride from concentrated urines if the vol. exceeds 10 ml; urine should be diluted to a vol. equivalent to an excretion of 150 ml per hr. and 1–10 ml of this diluted sample taken for assay. The results of determinations on three different vols. of urine should be averaged. No interference by any of the fluorescent pigments normally present in urine has been observed; the potassium chloride eluates are invariably non-fluorescent. Taking the above precautions and those suggested by Wang and Harris (*Biochem. J.*, 1939, **33**, 1356) for the thiochrome assay of aneurine, it is claimed that this method gives results for aneurine to an accuracy of ±10%, and for nicotinamide methochloride to ±5%, and it is felt that some workers have claimed too high a degree of precision for the thiochrome technique.

Nicotinamide methochloride has been detected in meat, liver and milk of animals which methylate nicotinamide, while it appears to be absent from cereals. An assay of a complete diet, assuming complete extraction of the methochloride by the methods recommended for aneurine, has shown a total intake of *ca.* 7 mg per day, which concn. is sufficient to cause an appreciable error in the aneurine assay if no correction for the presence of F_3 is made. It would seem that the determination of nicotinic acid in animal products gives results that are too high, since alkali hydrolysed extracts of the physiologically inactive methochloride give a positive cyanogen bromide reaction. J. A.

Bacteriological

Determination of the Relative Toxicities of Disinfectants. J. W. Appling and J. F. McCoy (*Paper Trade J.*, 1944, **119**, 14th Sept., *T.A.P.P.I. Sect.*, 112–114)—The method is an adaptation of the Petri plate method and is intended for the testing of disinfectants used to prevent slime in paper manufacture. Make a stock solution of known strength of the sample in water and pipette suitable aliquots (*e.g.*, 0–0·5 ml) into a series of sterile Petri dishes. Melt some nutrient agar (Bacto), cool it to 55° C., and pour 10 ml into each plate. Mix the contents of each plate, and leave for 2 hr. at room temp. Meanwhile, prepare a suspension of the organism concerned (*e.g.*, *Aerobacter aerogenes*, an important organism in paper mill slime) from a 24-hr. bottle slant grown at 37° C.; this is made by slanting *ca.* 20 ml of nutrient agar in an 8-oz. narrow-mouthed, square bottle, and the entire growth is aseptically washed from the slant into a dilution bottle containing sterile water, and diluted to 100 ml. Divide each plate into halves, streak these separately with a 3-mm loopful of the suspension, and incubate at 37° C. for 24 hr. or, with the lower concns. of sample, long enough to obtain a good growth. Note the inhibiting concn. (*C*) as % by wt., and multiply by 10,000 to obtain the relative toxicity. Results are tabulated comparing the effects of 10 commercial disinfectants on the above organism. J. G.

Organic

Use of Bromine in the Estimation of Cellulose in Plant Materials. J. G. Shrikhande (*Biochem. J.*, 1944, **38**, 207–209)—The use of chlorine, as suggested by Cross and Bevan, or sodium hypochlorite, as suggested by later workers, has disadvantages when used for removal of lignin in the estimation of cellulose in the tropics, and sodium hypobromite is recommended as an alternative in these circumstances. This produces "lignone bromide" which, under acid conditions, is yellow, like "lignone chloride," and produces a purple colour in cold sodium sulphite soln. The original method has been slightly modified. Powder the material to a uniform state of subdivision (64–80 mesh) and stir 1–2 g in 100 ml of 3% sodium sulphite soln. Heat to boiling, filter and transfer the residue back to the beaker with 100 ml of water. Add 10 ml of a 10% soln. of potassium bromide containing 2·5 g of potassium bromate per 100 ml, and 5 ml of 10% w/v sulphuric acid. Leave for 15 min. with occasional stirring, filter under suction on a cloth filter and, after washing the residue with water, transfer back to the beaker with 50 ml of water and add 50 ml of 6% sodium sulphite soln. Heat to boiling and leave in a boiling water-bath for 20 min. Repeat the bromination 5 or 6 times until the purple colour due to "lignone bromide" in cold sulphite soln. is very faint. Then suspend the cellulose product in 100 ml of water, add 10 ml of bromide-bromate mixture and 10 ml of sulphuric acid followed by about 10 ml of 5% sodium hydroxide soln. until the liquid is colourless. After 15 min. filter, wash and extract as before with 100 ml of 3% sodium sulphite soln. After one further acid treatment to ensure that the material is completely de-lignified, boil the final cellulose product in 250 ml of water for 2 mins. Wash on the filter, transfer to a weighed Gooch crucible with a cloth disc, dry at 100° C. and weigh. The method has the disadvantage over the chlorine method that it takes longer, but has the advantage that there is less danger of tissues being damaged by excessive halogenation. The method gave results in very close agreement with those obtained by the hypochlorite method. F. A. R.

Determination of Alkali Solubility of Cellulose. Anon. (*Svensk Papperstidn.*, 1944, **47**, 190–194)—The following (Swedish Standard) method gives results somewhat lower than those obtained by the usual α-cellulose determination, but the new method is more rapid and is independent of temp. variations and of the moisture content of the sample. Cut the sample into 1-sq. cm. pieces, condition it for 2 hr., and determine the loss in wt. at 105° C. To 5 ± 0·01 g of air-dry pulp, add 50 ml of 18 ± 0·01% (wt./wt.) sodium hydroxide soln. (containing

not more than 0·1% of sodium carbonate) at 20° C. After 1 min. mash with a rounded glass rod, and exactly 3 min. after the first addition add a further 50 ml of the alkali, and mix thoroughly. After exactly 45 min., filter on a Buchner funnel (a special filter is described) pouring the filtrate back until it runs clear. Filtration should be complete in 5 min., and no air should be sucked through. To a mixture of 5 ml of filtrate, 10 ml of 0·5 *N* potassium dichromate and 45 ml of water, add 100 ml of lead-free sulphuric acid (sp.gr. 1·84), with rotation, set aside for 10 min. and cool the mixture to *ca.* 20° C. The volumes of filtrate and dichromate may be adjusted according to circumstances, but the total vol. before adding the acid must be 60 ml, and there must be at least 2·5 ml of dichromate soln. in excess. Add 200 ml of water and back-titrate with standard 0·1 *N* ferrous ammonium sulphate (containing 10 ml of conc. sulphuric acid per litre), using 2 drops of 0·025 *M* ferroin sulphate soln. as indicator; the end-point is the formation of a red colour. Alternatively, dilute the cooled liquid with 500 ml of water, add 20 ml of 5·0% potassium iodide soln., and titrate with 0·1 *N* sodium thiosulphate, with starch as indicator. Allow for a blank titration (*b* ml), using half quantities of all the above reagents as described. Then 1 ml of 0·1 *N* dichromate soln. ± 0·000675 g of wood polyoses. If *G*g is the oven-dry wt. of sample taken, and *a* ml the vol. of 0·1 *N* ferrous salt or thiosulphate consumed, then, the alkali solubility (*S*) is 1·35 $(2b–a)/G$% (oven-dry), and the α-cellulose is (100–*S*)%. Duplicate results should agree to within ±0·1%. J. G.

Australian Tea Tree [Ti-tree] Oils. Austr. Res. Liaison, London (*Papers from Australia*, No. 409; *Bull. Imp. Inst.*, 1944, **42**, 161–164)—Bull. No. 14 of the Sydney Technological Museum describes the essential oils obtained by steam distillation from three varieties of Australian tea trees—*Melaleuca linariifolia* (A), *Melaleuca alternifolia* (B) and *Leptospermum citratum* (C). (A) occurs abundantly in Coast Districts of New South Wales and Queensland((B) is found in North Coast District of New South Wales. Both trees follow water courses and flourish in swampy regions. (C) grows sparsely in certain districts in Queensland. (A) and (B) have a nutmeg odour; (C) has a lemon odour modified by citronellal. All are of a pale lemon colour. Yields: (A) 1·5 to 2%; (B) *ca.* 1·8%; (C) 1·0 to 1·5%. Characteristics are:

	(A)	(B)	(C)
Sp.gr. at 15/15° C.	0·8927–0·8992	0·8950–0·9050	0·8792–0·8856
Optical rotation	+3·3° to +6·8°	+6·8° to +9·8°	+3·5° to +5°
n_D^{20}	1·4752–1·4780	1·4760–1·4810	1·4688–1·4757
Ester val.	1·3 to 2·7	2 to 7	—
Ester val. after acetylation ..	58 to 82	80 to 90	—
Solubility	—	0·6–0·8 vol. of 8% alcohol	1–1·2 vol. of 70% alcohol.

The principal constituents of (A) are α- and γ-terpinene, cymene, cineole (16–20%), Δ′-terpinenol-4, sesquiterpenes, etc. (B) contains *d*-α-pinene, α- and γ-terpinene, cymene (cineole *ca.* 8%), Δ′-terpinenol-4, sesquiterpenes, etc. (C) contains from 75 to 85% of total aldehydes (mainly citral, 45–50%; citronellal, *ca.* 35%); the balance consists of geraniol and citronellol and their formic and acetic acid esters, sesquiterpenes and sesquiterpene alcohols, and citronellol and their formic and acetic acid paratively, and (B) a very high germicidal value when tested against pure carbolic acid, using *B. typhosus* as test organism. (A) is used for soaps and perfumery and (B) for surgical and dental purposes; the latter oil has the proprietary names of "Ti-Trol" (oil) and "Melasol" (the water-sol. form). (C) is used in the manufacture of citral, citronellal and citronellol. Citral from the crude oil is free from citronellal and is superior to citral prepared from any other source except, possibly, *Backhousia citriodora* oil. E. B. D.

Licuri [Ouricoury] Wax. (*Bull. Imp. Inst.*, 1944, **42**, 165–167)—Licuri wax is obtained from the Brazilian palm *Cocos coronata* Mart. (*Syagrus coronata* Mart.). It was formerly marketed as Ourici, Urucury or Bricuri. Constants determined by the Institute of Chemistry of the Brazilian Ministry of Agriculture were: m.p. 84·8° C., sap. val., 78·8; iodine val., 8·6; acid val., 5·5; total fatty acids, 47·54%; sol. in hot alcohol and in ether; sp.gr. at 15° C., 1·010 (M. Silva, Inst. Nacional de Tecnologia, Rio de Janeiro, Bull. No. 67, 1940). These constants closely resemble those of carnauba wax, for which it may be a cheaper substitute; it is claimed that its solubility in the common commercial solvents is greater than that of carnauba wax. The two waxes can be distinguished microscopically (in the powdered form as separated from the leaves) and by shaking vigorously 3 g of the sample with 10 ml of 10–20 % sodium hydroxide soln.; licuri yields a yellowish-orange, and carnauba wax a colourless solution. (*cf.* ANALYST, 1941, **66**, 341.) E. B. D.

Qualitative Analysis of Proteins. A Partition Chromatographic Method using Paper. R. Consden, A. H. Gordon and A. J. P. Martin (*Biochem. J.*, 1944, **38**, 224–232)—A variant of the silica gel partition chromatogram previously described (*Biochem. J.*, 1943, **37**, 79; ANALYST, 1943, **68**, 283) has been devised, in which filter-paper is used instead of silica gel. It is a useful qualitative method when only very small amouuts of protein, *e.g.*, of the order of 200 μg, are available. Draw a pencil line 5 cm from one end of a strip of Whatman No. 1 filter-paper, 1·5 × 20–56 cm, and by means of a capillary tube apply 2–4 μl of the soln. to be analysed, containing 5–15 μg of each amino acid, along the centre portion of the pencil line. Immerse this end of the strip in a trough containing the water-saturated solvent, holding it in position with a microscope slide. Pass the paper over a glass rod to avoid capillary siphoning between the outside of the trough and the paper, and let the rest of the strip hang freely. Place the unit in an air-tight chamber in which an atmosphere saturated with water and the solvent is maintained. When the solvent has run a convenient distance down the filter-paper strip, remove the paper and mark the position of the solvent front. After drying, spray the paper with a 0·1% soln. of ninhydrin in *n*-butanol and again dry the paper. Heat at 80° C. for 5 min. and mark the bands which develop in pencil, as the colour fades after a few days. In the original paper a table is given showing the rate of movement of each amino acid in different

solvents with and without the addition of acids, bases and salts. The most suitable solvent for a particular purpose can be selected by consulting this table. Owing to the fact that some amino acids have the same partition coefficient between water and a particular solvent, it is not possible to separate them when this solvent is used and a band is then obtained which contains two or more amino acids. These can be separated by carrying out a two-dimensional chromatogram, using a second solvent as follows. Put 6–12 μl of the soln., containing 200–400 μg of protein, near one corner, 6 cm from either edge, of a sheet of filter-paper, 18 × 22 in. Immerse one edge of this paper in a trough similar to, but much longer than, that previously described and allow the sheet to hang over a glass rod as before. The sheet must hang in an air-tight chamber saturated with solvent and water vapour. Allow the chromatogram to develop for 24–72 hr. and dry and return the paper to the trough, immersing the adjacent edge in the second solvent. Leave for a further 24–48 hr., dry as before, spray with ninhydrin soln. and heat at 80° C. for 5 min. This second treatment results in the separation into its components of any band formed by two or more amino-acids in the first treatment. Paper strips must be handled with forceps and not with the hand, as finger-marks show after heating with ninhydrin. The most useful solvents were found to be phenol, collidine, *n*-butanol, tertiary amyl alcohol, benzyl alcohol, *o*-, *m*- and *p*-cresol and *iso*butyric acid. In deciding which pair of solvents to use in the two-dimensional chromatogram it is helpful to plot the rates of movement of the amino acids in one solvent against those in the second solvent. In this way the possibility of separating individual amino acids from one another is demonstrated graphically.

F. A. R.

Determination of Small Amounts of Anthracene in Tar and Tar Oil Fractions. F. R. Cropper and N. Strafford (*J. Soc. Chem. Ind.*, 1944, **63**, 268–272)—The existing Höchst test for anthracene (*Standard Methods of Testing Tar and its Products*, 2nd Ed., 1938, p. 299; S.T.P.T. Committee) when applied to tar and its distillation fractions is inconvenient and gives a measure only of the "available" anthracene under the conditions of the test. Studies of the ultra-violet absorption characteristics of anthracene and other components of low grade tar oils, tar and pitch have been made with a view to devising a rapid and reasonably accurate method of determining the actual anthracene content. Comparison between the absorption spectra of purified anthracene and of a number of anthracene oils, in the region 340 $m\mu$ to 390 $m\mu$, showed that other components in the oils caused distortion of the normal anthracene curve. Direct ultra-violet absorption measurements on the oils are therefore not satisfactory. It was found, however, that the anthracene could be separated chromatographically from the other components and subsequently determined from ultra-violet absorption measurements on the anthracene fraction recovered from the chromatogram. The anthracene zone in the chromatograph column was detected by its fluorescence when observed under a normal ultra-violet lamp. Tests with purified anthracene showed that the amount recovered from the chromatogram was about 95% of that added to the column; a similar figure was allowed for in estimating the unknown samples. The precision of the determination depends on the particular type of sample; it is about ±25% of the figure for pitch, and ±4% to ±7% of the figures for light and heavy anthracene oils. This is considered satisfactory, as the test was developed only for application to tar products containing up to 6% of anthracene.

B. S. C.

Inorganic

Iodimetric Determination of Tin. T. B. McDow, K. D. Furbee and F. B. Clardy (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 555–556)—*Brass, bronze, etc.* (Cu > 2%)—Dissolve 1–10 g in 10–30 ml of nitric acid (1 : 1) in a 300-ml conical flask, boil off nitrous oxides and dilute to 100 ml. Add 3–5 ml of 10% aluminium nitrate soln. and ammonia (10 ml excess), boil, and filter on Whatman No. 42 paper. Wash the ppt. twice with 5% ammonium nitrate soln., return the filter to the flask and treat it with 10 ml of sulphuric, 5 ml of perchloric, and a few drops of nitric, acids (all strong). Heat gently, adding nitric acid, drop by drop, to prevent darkening of the liquid. Evaporate to evolution of strong white fumes. *Solder, etc.* (Cu < 2%)—Weigh an amount containing 0·1–0·2 g of tin into a 300-ml conical flask, heat with 10 ml of sulphuric acid and 5 g of potassium sulphate until lead sulphate, if present, becomes white. *Titration*—Cautiously dilute either soln. to *ca.* 100 ml, add 75 ml of strong hydrochloric acid and 10 g of 10-mesh nickel shot, and close the flask with a one-hole stopper holding a doubly-bent glass tube, both ends of which are cut short; the exit end is fitted with a rubber tube dipping into a beaker filled with water. Boil gently for 30 min., dipping the rubber tube into 10% sodium bicarbonate soln. towards the end of the reduction. Cool the flask in water, maintaining the supply of bicarbonate. When the liquid is cold remove the stopper, rapidly add ice and solid carbon dioxide or pellets of bicarbonate, and titrate at once with iodine as usual.

W. R. S.

Modified Volumetric Persulphate Method for Manganese. H. D. Hillson (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 560–562)—Decomposition of the permanganic acid is retarded by means of sodium phosphate; no filtration is required (*cf.* bismuthate method). Dissolve 0·5–1 g of high-grade manganese ore in *aqua regia*, filter, and ignite and fuse the residue with sodium carbonate. If the melt is green, dissolve it in 18 ml of sulphuric acid diluted with water, which is added to the original filtrate. Evaporate until heavy fumes are evolved, cool, dilute, and transfer to a graduated 250-ml flask. Pipette 25-ml portions (0·03–0·05 g of Mn) into flasks. Treat with 3–5 g of disodium phosphate, 10 ml of syrupy phosphoric acid, 10 ml of freshly-prepared 20% ammonium persulphate soln., and 5 ml of 0·1 *N* silver nitrate. Place the flask in a boiling water-bath for 14–20 min., or boil gently over a small flame for nearly 2 min., cool at once in water to 20° C. and add 25 ml of 6 *N* sulphuric acid. Add a few drops of osmium tetroxide catalyst (1 g dissolved in 390 ml of 0·1 *N* sulphuric acid) and titrate with 0·1 *N* sodium arsenite soln.

W. R. S.

Colorimetric Estimation of [Small Amounts of] Nickel in Steel. C. G. Hummon (*Steel*, 1944, **114**, 97; *Nickel Bulletin*, 1944, **17**, 158)—This absorptiometric method is intended particularly for rapid and accurate (±0·002%) analysis for the control of scrap. Dissolve 0·5 g of the sample in 15 ml of nitric acid (1 : 2) and boil out nitrous gases. Add

10 ml of 20% ammonium persulphate soln. and boil gently for 15 sec. Add 1 ml of methyl alcohol, cool slightly, add 10 ml of water and 25 ml of conc. ammonia soln., and boil gently for one min. Cool, add 10 ml of ammonia soln., mix, and add 10 ml of ammonium persulphate soln. Add 5 ml of a 1% soln. of dimethylglyoxime in *iso*propyl alcohol and shake for 30 sec. Dilute to 200 ml, mix, allow to settle for 5 min., and filter enough of the soln. (discarding the first 5–10 ml) to make the absorptiometer measurement. The importance of the choice of colour filter is stressed. A green filter, as used for manganese determination, together with a yellow lantern filter is recommended. L. A. D.

Tannin Separation of Beryllium and Aluminium. G. W. Sears and H. Gung (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 598)—Nichols and Schempf's method hinges on the accurate control of *p*H (*Id.*, 1939, **11**, 278). In place of a *p*H meter the authors use a mixture of 0·1% solns. of methyl red and bromocresol green (1 : 6). To the assay soln. (500 ml) containing the buffers add a drop of indicator and then ammonia (1 : 1) until the soln. is blue-green (*p*H > 5). Cautiously add 6 *N* sulphuric acid while stirring; the soln. becomes blue purple, reddish-purple, and finally red. The first appearance of reddish-purple indicates *p*H 4·6, at which the separation is effected. The colour change is readily distinguished. W. R. S.

Micro-determination of Fluorine in Coal. H. E. Crossley (*J. Soc. Chem. Ind.*, 1944, **63**, 280–288)—Fluorine may occur in coal in quantities of the order of 100 p.p.m. and a method adaptable to the determination on 1–5-g samples was required. An experimental survey of methods for determining small amounts of fluorine of the order of up to 100 μg was made. The method found most suitable was developed from the work of Barr and Thorogood (ANALYST, 1934, **59**, 378) and Sanchis (*Ind. Eng. Chem., Anal. Ed.*, 1934, **6**, 134), based on the bleaching action of fluoride on a zirconium-alizarin lake in a medium of dil. sulphuric and hydrochloric acids. Owing to variations in the colour with different supplies of zirconium salt and alizarin S, permanent colour standards were not satisfactory, and visual matching in Nessler tubes of the test soln. with a standard soln. prepared at the same time and with the same indicator, was necessary. The methods of separation used were (*a*) incineration of the coal with sodium carbonate and distillation of hydrofluosilicic acid from the residue; this gave a tendency to low results which could be overcome by fusing after incineration, and removing silicates with zinc compounds before distillation; (*b*) combustion in a calorimetric bomb, with distillation as before; a by-product, nitrosylsulphuric acid, discoloured the lake, but could be removed by reduction with a zinc-copper couple prior to distillation. The two methods gave results in good agreement, fluorine being determined to the nearest 5 p.p.m. on 1 g of coal by the bomb method, or to the nearest 1 p.p.m. on 5 g of coal by the incineration method. *Bomb method*—Burn a 1-g sample of coal in a calorimetric bomb in the usual way, with 5 ml of water in the bomb. Cool for 30 min. and slowly exhaust the oxygen. Rinse out the bomb, collecting the liquid, filter, dilute to about 100 ml, and add 5 ml of 20% copper sulphate soln. and 3 g of zinc dust. Add 10 ml of 50% sodium hydroxide soln. (stored in waxed vessel) and boil for 90 min. Cool, filter and collect the filtrate in a 250-ml Claisen distilling flask; the flask is fitted with a dropping funnel, Liebig condenser and thermometer with the bulb immersed in the liquid. Add 35 ml of conc. sulphuric acid through the tap funnel. Distil and return the first 20 ml of distillate to the tap funnel. Resume distillation, and when the b.p. has reached 135° C., add the contents of the tap funnel, drop by drop, followed by water, to maintain a boiling temperature of 135° ± 5° C. Collect 200 ml of distillate and concentrate it by evaporation to 90 ml, adding 5 ml of 0·75% sodium carbonate soln. and a pointed glass rod, to promote uniform boiling. For the determination, prepare standards for the range 0–180 μg of fluorine, in steps of 10 μg, by diluting the required volume of standard fluorine soln. to 96 ml (the standard soln., containing 1 μg of fluorine in 0·01 ml, is prepared by distilling 0·221 g of pure sodium fluoride with dilute sulphuric acid and collecting 1 litre of distillate boiling at 135° ± 5° C.). The indicator solution is prepared 30 min. before required for use, by first diluting a stock zirconium-alizarin soln. in the proportion of 2 vol. of stock soln. and 3 vol. of water, and then adding this soln. to four times its vol. of a mixture of 1 part of 3 *N* sulphuric acid to 3 parts of 3 *N* hydrochloric acid. Add 5 ml of this acid indicator to each standard soln. and the test soln. Boil for a few sec., and set aside to cool for at least 4 hours, preferably overnight. Transfer the liquids to 100-ml Nessler glasses and compare colorimetrically. [The zirconium-alizarin stock soln. is prepared by adding 100 ml of 0·17% alizarin S soln. to 100 ml of 0·87% crystalline zirconium nitrate soln., slowly and with vigorous shaking, and keeping for at least 24 hr. before use.] *Fusion method*—Mix a 1–2-g sample of coal with 3 g of sodium carbonate in a porcelain crucible, and cover the mixture with a further 3 g of sodium carbonate. Heat at 475° ± 25° C. until the carbon has gone (18 hr. or more is necessary). Transfer to a platinum crucible and heat at 1000° C. for 15 min. Extract in 200 ml of water, boil, and add 10 ml of 17·7% crystalline zinc sulphate soln. Boil for 2–3 min. with stirring, cool and filter. To the filtrate add a few drops of methyl red indicator, and then a slight excess of 50% sulphuric acid followed immediately by 2·5 ml of ammoniacal zinc carbonate soln. (60 ml of conc. ammonia, 25 g of zinc oxide, 50 g of ammonium carbonate and 110 ml of water). Boil in a covered beaker until the odour of ammonia is not perceptible and not more than 50 ml of liquid remain. Cool, filter and distil as for the bomb method. In both methods the test liquids at all stages should not be kept in contact with glass for more than a few hours; waxed vessels should be used. *Corrections*—Blank determinations are advised. A correction is necessary with amounts of fluorine greater than 80 μg due to a loss in the distillation process probably caused by absorption of hydrofluosilicic acid by silica gel deposited on the inside of the distilling flask, as follows:

Amount of fluorine in distillate	Fluorine lost, to be added	Amount of fluorine in distillate	Fluorine lost, to be added
μg	μg	μg	μg
Less than 80	nil	155–180	20
85	5	185–210	25
90–120	10	360	50
125–150	15		

Another correction may be necessary for the amount of fluorine not recovered from the ash in the bomb crucible. This can be determined by the fusion method, but it can be taken as 5 μg of

fluorine with coals containing less than 60 p.p.m. and 10 μg with coals containing more fluorine.
S. G. C.

Further Studies of the Molybdenum Blue Reaction [for Phosphorus]. R. E. Kitson and M. G. Mellon (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 466–469)—A spectrophotometric study of the reaction has included investigation of the effects of reagent concn., phosphorus concn., *p*H, buffer composition, temperature, order of addition of reagents and presence of other ions. A modification of the A.O.A.C. procedure is proposed which gives coloured solutions of improved stability. *Method*—Take a suitable amount of soln., neutral to litmus, free from interfering ions and containing the phosphorus as orthophosphate, in a 100-ml volumetric flask (0·002 to 0·2 mg of P for visual comparison in Nessler tubes, 0·1 to 1·5 mg for photometric methods using a 1-cm cell). Dilute to 30 ml to prevent pptn. of molybdiphosphoric acid and add 5 ml of 0·8 *M* boric acid if fluorine is present. (If more than 1000 p.p.m. of fluorine are present remove by fuming with sulphuric acid). Add in order, mixing continuously 10 ml of a 5% soln. of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, in *N* sulphuric acid, 10 ml of 0·5% hydroquinone soln. and 10 ml of sodium sulphite soln. (110 g.p. litre), and dilute to 100 ml. The *p*H should be between 3·0 and 4·7; if it is not, adjust the amount of molybdate or sulphite reagent. Leave for 30 min. and compare the colour intensity with standards. Use a red filter with an absorptiometer or a wavelength band in the range 600 to 700 mμ with a spectrophotometer. The transmission at λ=650 mμ obeys Beer's law at phosphorus concns. from 1 to 15 p.p.m. Notes on the effects of 60 ions are given. Thus, strong oxidising or reducing agents, silicates and several metals must be absent; arsenate (100 p.p.m.) and arsenite (50 p.p.m.) do not interfere in the determination of 2·5 p.p.m. of phosphorus.
L. A. D.

Borax as a Standard Buffer Solution. A. D. E. Laughlan (*Nature*, 1944, **154**, 577)—In the investigation of small errors in glass electrodes, it has been found that 0·05 *M* solns. of borax invariably have a reaction of *p*H 0·05 below the accepted values (9·23 at 20° C.—Britton, "*Hydrogen Ions*"). This discrepancy is not due to differences in methods of preparation of the material, as solns. prepared under the same conditions from different samples give uniformly low results, nor to errors of hydration, a 0·05 *M* soln. made with dehydrated borax giving a *p*H value of $9{\cdot}17_2$ at 20° C. A sample of borax was recrystallised from boiling water containing sodium hydroxide and then recrystallised twice from distilled water, and the product was dried between filter-paper in the air. A 0·05 *M* soln. was prepared, distilled water previously boiled in a "Pyrex" flask and cooled being used and carbon dioxide being excluded by means of a soda-lime tube. The *p*H of this soln. was measured with hydrogen and calomel electrodes which had been kept at constant temperature for 24 hr.

Hydrogen electrode °C.	Calomel electrode °C.	E.M.F. mV	*p*H	*p*H reduced to 20° C.
20·2	20·5	782·6	$9{\cdot}18_2$	$9{\cdot}18_0$
20·8	21·2	782·6	$9{\cdot}17_0$	$9{\cdot}17_6$
21·0	21·2	782·8	$9{\cdot}17_7$	$9{\cdot}17_5$

Under the above conditions the calomel cell gave potentials in agreement with those quoted by Michaelis ("*Die Wasserstoffionenkonzentration*"), and a check with 0·05 *M* potassium hydrogen phthalate (*p*H 3·97) indicated that the calomel cell had a potential correct to within 0·2 mV of Michaelis' values. From a long series of tests it has been found that the temperature coefficient of 0·05 *M* borax solns. as given by Walbum (Clark, "*The Determination of Hydrogen Ions*") is quite correct, and the above *p*H values at 20° C. are computed on the basis of this figure and rounded off to the nearest *p*H 0·01. The value of 9·18 at 20° C. for a 0·05 *M* soln. of $Na_2B_4O_7.10H_2O$ made up with carbon dioxide-free water would therefore seem to represent a more correct figure than 9·23 usually given.
J. A.

Microchemical

Quantitative Microdetermination of Glucose and Maltose in Mixtures. D. L. Morris (*J. Biol. Chem.*, 1944, **154**, 561–567)—Although several different reagents are reduced by both glucose and maltose, the ratio of the reducing powers of glucose and maltose vary with each reagent. Thus, by reducing a mixture of the sugars with two such reagents, it should be possible, by solving a pair of simultaneous equations, to calculate the concn. of each sugar. To obtain satisfactory results, the equations resulting from the two reactions must be linear and their accuracy must be very high. Moreover, the total reduction of the mixture of glucose and maltose must be exactly equal to the sum of the reductions of the sugars alone. A method of estimation based on this principle has been worked out, using the Hagedorn–Jensen ferricyanide reagent and a modification of Somogyi's "high alkalinity" copper reagent (*J. Biol. Chem.*, 1939, **119**, 741). To 5 ml of the sugar soln., add 5 ml of the Hagedorn–Jensen ferricyanide-carbonate soln., stopper the tube with a glass bulb and heat for 15 min. in a boiling water-bath. Cool and add 5 ml of the soln. of zinc sulphate and potassium iodide and 3 ml of 5% acetic acid. Titrate the excess iodine with 0·005 *N* sodium thiosulphate and subtract the titre from that obtained in a blank determination. The glass bulbs should be left in place until the beginning of the titration. Trouble was encountered with the Somogyi reagent, because although the maltose reduction is linear, the g ucose reduction is not linear for the first few mg. The difficulty was overcome by adding the equivalent of 0·4–0·6 mg of glucose to the reagent itself, so that the titration falls on that part of the curve in which the reduction is linear. The additional glucose, being also present in the blank, is automatically corrected for when the blank is subtracted from the titres of the soln. under examination. Add 80–100 mg of glucose to each litre of Somogyi's "high alkalinity" reagent and enough extra potassium iodate (2·5–3·5 ml of *N* soln.) to bring the blank up to the usual value. This reagent is not quite stable, but does not change appreciably in the course of a day. If the blank titration increases by more than 1 ml, it can be brought back to normal by addition of glucose. Estimate the sugars as described by Somogyi (*loc. cit.*) using the same quantity of glucose-maltose soln. as in the Hagedorn–Jensen method. If R_{Cu} and R_{Fe} represent the reductions for the copper and ferricyanide reagents respectively and *G* and *M* are the amounts in mg of glucose and maltose, $R_{Fe} = G + 0{\cdot}811\,M$ and $R_{Cu} = G + 0{\cdot}578\,M$., whence $M = 4{\cdot}29\,(R_{Fe} - R_{Cu})$. The factors in these two equations were obtained experimentally,

the reduction of maltose being 0·811 times that of glucose with the ferricyanide reagent and 0·578 times that of glucose with the copper reagent. The method is not directly applicable if dextrins are present, but satisfactory values can be obtained by carrying out two estimations with each reagent, one before and the other after the glucose and maltose have been destroyed by fermentation. From the differences in the results the amounts of glucose and maltose can be calculated. The results obtained with mixtures of glucose and maltose were in very close agreement with the calculated values. F. A. R.

Physical Methods, Apparatus, etc.

Spectrochemical Analysis of Solutions, using Spark Excitation. H. A. Sloviter and A. Sitkin (*J. Opt. Soc. Amer.*, 1944, **34**, 400–405)—The investigation was carried out to study the various factors in the solution method of Scheibe (*Z. angew. Chem.*, 1936, **49**, 443) which affect reproducibility of excitation, and to adapt the method for the routine spectrochemical examination of steel. Scheibe's method of preparing each electrode by sparking for 1 min. before impregnation was found to be unsatisfactory. The most uniform and rapidly absorbent electrodes were obtained by heating spectroscopically pure graphite rods at 900°–1000° F. in a muffle furnace for 1 hr. When cool, these were cut on a rotating alundum wheel into suitable lengths with perfectly smooth and flat tops. If stored in closed containers, these electrodes retain their absorbent properties for a long time. Another point is that electrodes should be used soon after impregnation; prior ageing produces a diminution in the intensity of the spectrum lines obtained. Impregnation with a single drop resulted in almost all the impregnated material remaining in the top ¼-in. of a 5/16 in. diam. electrode. Further tests showed that there is no preferential absorption of the metallic impurities in steel, but the electrode acts as a filter for silica, retaining it in the uppermost portions. With electrodes impregnated with one drop of soln., almost all the material volatilises in a 45 to 60 sec. sparking period. A scheme has been devised for the analysis of solns. of steels based on the above findings. The impurities determined are manganese, chromium, nickel, vanadium, molybdenum and copper. B. S. C.

Reviews

MAGNETO-CHEMISTRY. By Professor PIERCE W. SELWOOD. Pp. ix + 287. New York: Interscience Publishers, Inc.; London: Imperia Book Co. 1943. Price $5.

The face of chemistry has changed almost out of all recognition since those far-away days when John Dalton sagely remarked to a pupil "Thou knowest, thou canst not split an atom." In an era when the atom lived up to its name it was easy enough to define physicists as men of science interested in the properties of molecules, whereas chemists were more deeply concerned with the properties of atoms. To-day, when the atom is recognised as a complex structure, whose description demands a technical knowledge surpassing that of an engineer bent on the explanation of the "particular go" of a steam-engine, the boundary between the two sciences has become fuzzy and ill-defined. Still, the concept of valence, and problems concerning molecular constitution and structure remain as part of the field of knowledge tilled by the chemist, and in the development of that field many physical properties are requisitioned.

Among those physical properties, magnetic properties are by no means the least important, and there are many magnetic properties, a knowledge of whose variation in a series of related compounds would throw much light on problems of structure. So many, indeed, are these effects, that a complete study of them would demand not one, but maybe a dozen volumes of the size of that under review. The author has, therefore, restricted rather severely the field of his work, and has defined magneto-chemistry as "*the application of magnetic susceptibilities and of closely related quantities to the solution of chemical problems.*" Within these well-defined limits, the author's treatment of his subject is scholarly and thorough. The book opens with a section dealing with methods of measurement of susceptibility which, considered as a concise and lucid account of the principal methods, leaves nothing to be desired. Then follow sections dealing with atomic and molecular diamagnetism, atomic and molecular paramagnetism, complex compounds, metallic dia- and paramagnetism, and ferromagnetism. The book closes with a section on applied magnetometric analysis, which contains some exceedingly interesting studies, *inter alia*, of magnetism and adsorption, the structure of alloys, and magnetism and catalysis.

The book is non-mathematical; well constructed and clear diagrams present the argument to the eye; the chemical interest is always present, and the bibliographical references are full and precise. The book can be warmly recommended, alike to the specialist looking for a lucid survey, and to the student who desires to adventure into a new and fascinating field of research. ALLAN FERGUSON

ANNUAL REPORTS OF THE PROGRESS OF CHEMISTRY FOR 1943. Vol. XL. Pp. 254. Published by The Chemical Society, London. 1944. Price 15s. (to Fellows 8s. 6d.).

This volume comprises the Reports on General and Physical Chemistry, Inorganic Chemistry, Crystallography, Organic Chemistry, Biochemistry and those held over from last year, *viz.*, the Reports on Radioactivity and Sub-atomic Phenomena and Analytical Chemistry.

The Analytical Chemistry Report consists of a review of recent methods for the Analysis of Steel and a review of recent developments in Fractional Distillation. As the author of the first-named review points out, one third of all the elements are to be found in various steels and, consequently, details of the methods used in steel analysis cannot fail to interest all analysts. Fractional distillation finds increasing application in analytical problems, and the author of this review deals largely with laboratory columns and accessories.

The attention of readers of THE ANALYST may be drawn to Dr. H. T. S. Britton's survey of the Application of Electrometric Methods to the Study of some Ionic Reactions. The discussion of the conditions governing the composition of precipitated metallic hydroxides, the action of alkali on solutions of mercuric salts in presence or absence of alkali salts, and the use of mercuric oxide for standardising acid solutions are subjects of particular interest.

In the Biochemistry Report the more significant findings in recent work on the influence of ascorbic acid on the repair of wounds are summarised, and the Report by Drs. Chain and Florey on Antibacterial Substances produced by Bacteria and Fungi is particularly welcome, as also is the Report on Viruses.

In the Inorganic Chemistry Report analysts will be interested in Dr. A. J. E. Welch's section on the preparation of the solid elements in a pure state.

Advantage has been taken of the relatively stationary state of nuclear physics to present an interesting account of the special research tools used in this study.

A glance at the terminal subject index will convince the reader that it behoves him not to rely upon picking out his own requirements from these reports, but, with judicious skipping, to read the volume through. He will be rewarded with the solution of some of his own problems, increase of his general knowledge of chemical science, and unification of much of the uncorrelated information that he encounters in his general reading. A. O. JONES

THE ANALYTICAL CHEMISTRY OF INDUSTRIAL POISONS, HAZARDS AND SOLVENTS. Second Edition. By M. B. JACOBS, Ph.D. Pp. 661. New York: Interscience Publishers, Inc. London: Imperia Book Co. 1944. Price $7.00.

This volume is a second revised reprint of the 1941 edition noticed in THE ANALYST, 1941, **66**, 477. The author, who is senior chemist in the Department of Health, New York City, very aptly dedicates his work,

> ". . . . . to those who toil,
> within the factory, the shop or mine
> . . . . . .
> To those whom industry may cause to ail
> To all of those whom dust and fumes despoil."

It is first of a series of projected monographs on analytical chemistry and its application. It is in no way a text book on toxicology, but it certainly covers a very useful, indeed a unique field. Details of its contents have already been mentioned in this Journal (*loc. cit.*) and are not materially changed in this edition. But since the first issue time has allowed of the proving of the volume by practical use, both as to its analytical methods and the presentation of data. We have found it very useful and reliable. It is a great convenience to have all the needful information, including a host of references, in one volume; and the range of compounds dealt with helps to bring to mind the possibilities in those not infrequent cases in which some toxic action is suspected but the analyst has to consider for himself to what it may be due, and devise methods accordingly. Here he will find fruitful suggestions and methods for putting them to the test.

In the reviewer's opinion the book is particularly welcome. The question of the physiological action, if any, of this or that chemical arises with ever increasing frequency. In general, it is a very difficult one to answer by reason of lack of information. What there is is widely scattered and not always very critical. It so often happens that some untoward symptoms arise where some compound has been used, and it subsequently appears on record as a fact that such an action results. The proof is no more than "post hoc, propter hoc"; so there is real need of collected data from carefully sifted sources. Dr. Jacobs's work goes some way towards supplying this need. It is to be hoped he will keep it up to date and that it will run to many editions—new editions are better than reprints. H. E. Cox

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