ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs Analysis

Detection of Pasteurisation, and Detection of Raw Milk in Pasteurised Milk. M. F. Bengen. (*Z. Unters. Lebensm.*, 1933, **66**, 126–136.)—Certain parts of the albumins of milk coagulate at 63° to 65° C., *i.e.* at the temperature of pasteurisation. Addition of a definite amount of ammonium sulphate to milk yields a "limiting serum" which will contain such readily coagulable albumins if the milk treated were raw, but will be devoid of them if the milk had been previously heated to 63° to 65° C. even for a few minutes. Hence, in the former case the serum becomes turbid when carefully heated to 65° C., whereas in the latter it remains clear up to 70° C. and shows turbidity only at 71° C.

The "limiting serum" is prepared as follows: 10 grms. of finely powdered ammonium sulphate are dissolved in 50 c.c. of the milk as rapidly as possible by shaking. The serum separates immediately, and may be filtered at once through a close filter-paper, such as Schleicher and Schüll's extra hard No. 605 (12·5 cm. diameter). Two serums are thus prepared, one from the milk as received, and the other from the same milk previously heated to 63° to 65° C. for 5 minutes. The two serums are then examined together to ascertain if they become turbid at the same temperature when heated or if any "turbidity-difference" exists between them.

For this purpose two test-tubes containing the serums (5 c.c.) are suspended in a roomy beaker containing water at 65° C., which is then heated so that the temperature rises about 1° C. per minute; an accurate thermometer is essential. The tubes are examined without being removed from the bath. It is advisable to use, in a third test-tube, a standard turbid solution, which renders the determination of the turbidity point more exact and is prepared as follows: 0·3 grm. of raw potato starch is mixed with cold water, the mixture being then made up to about 50 c.c., and kept boiling gently for 10 minutes; after addition of 15 c.c. of glycerin, the boiling is continued for 10 minutes. The liquid is then quickly cooled, mixed with 25 c.c. of alcohol, and made up to 100 c.c. In stoppered vessels it keeps well. Five c.c. of this solution, covered with a layer of liquid paraffin, is heated for 10 minutes in a water-bath at 70° C., and then has the slight turbidity suitable for comparison with the heated serums.

The method described does not indicate whether the pasteurised milk has been heated for the prescribed 30 minutes, but with a number of the milks examined it gave definite indications where the results of Schardinger's aldehydo-reductase test were inconclusive.

When many samples of milk have to be examined, a preliminary sorting test may be made by placing 5-c.c. samples of the different serums (prepared as described above) in test-tubes, and immersing these for 10 minutes in a water-bath at exactly 70° C. The serums of pasteurised milk then remain clear, whilst those of milks which

have been insufficiently heated or have been subsequently mixed with raw milk become more or less turbid. When raw milk is present the degree of turbidity is fairly closely proportional to the amount of the raw milk. T. H. P.

Determination of Sulphate in Wine by the Benzidine Method. E. Lobstein and M. Ancel. (*Ann. Chim. anal.*, 1933, **15**, 389–397.)—Determinations of sulphate in a number of red and white wines as barium sulphate by the French official method gave results from 1 to 14 per cent. lower than similar determinations made after the wines were ashed. Calculated as grms. of potassium sulphate per litre of wine, the mean difference is 0·04 to 0·05, so that the permitted quantity, 2 grms. per litre (determined by the official method), is actually about 2·05 grms. per litre.

Tests made with sulphuric acid and various sulphates show that the barium sulphate and volumetric benzidine methods yield results agreeing within 1 per cent.

Two procedures for the application of the benzidine method to wines are as follows: (1) Fifty c.c. of the wine (carefully neutralised with 0·1 *N* sodium hydroxide), 5 c.c. of 0·1 *N* hydrochloric acid, and 30 c.c. of 4 per cent. benzidine hydrochloride solution (1 per cent. solution is strong enough for white wines) are mixed in a conical flask. After 20 minutes, the liquid is filtered through a small plain filter, the flask being washed out with a neutral mixture of alcohol and ether (1 : 1), and the precipitate washed with the same mixture until free from hydrochloric acid. The filter and precipitate are shaken in a 250-c.c. conical flask with 50 to 100 c.c. of strictly neutral distilled water, and the whole is titrated with 0·1 *N* sodium hydroxide in presence of phenolphthalein, with shaking after each addition of alkali. Dissociation of the benzidine sulphate is facilitated by boiling the liquid, but under these conditions an alkali solution, standardised by titration, when hot, with standard sulphuric acid, must be used. The results thus obtained differed by 1·7 per cent. at the most from those of determinations as barium sulphate after calcination of the wine. Results accurate to within 1 per cent. may be obtained as follows:

(2) Fifty c.c. of the wine are evaporated in a 250-c.c. Pyrex flask, and the residue is calcined until white. When cold, the ash is extracted with four 20-c.c. quantities of water, which are filtered into a conical flask. The liquid is boiled for some minutes with a few drops of dilute hydrochloric acid (1 : 10) to expel carbon dioxide, and is then neutralised with 0·1 *N* sodium hydroxide solution. It is then shaken with 2 drops of dilute hydrochloric acid (1 : 10) and 100 c.c. of benzidine hydrochloride solution (0·4 per cent.), and left for 20 minutes. The subsequent operations are as described under (1). T. H. P.

Distinction of Wine Vinegar from other Vinegars. A. Patzauer. (*Chem.-Zeit.*, 1933, **57**, 735.)—The method described is based on the different forms of the crystals of calcium tartrate, etc., obtained under various conditions. Two solutions are employed: (*a*) 20 per cent. aqueous potassium acetate solution containing 0·5 c.c. of formaldehyde solution per 100 c.c. to prevent mould-growth; (*b*) 0·2 grm of *l*-tartaric acid and 0·4 grm. of crystallised sodium acetate are dissolved in 20 grms. of water, and the solution treated with 0·3 c.c. of solution (*a*), left

overnight, and then filtered without disturbing the sediment; the filtrate is made up to 100 c.c. and treated with 0·5 c.c. of formaldehyde solution.

In making the test, 10 c.c. of the clear filtered vinegar are shaken in a test-tube with 1 c.c. of (*a*), 0·5 c.c. of (*b*) being then added, and the liquid again shaken, and left either overnight or for some hours. If the liquid remains clear and free from deposit, the vinegar contains no wine. In presence of tartaric acid, that is, if the vinegar is derived from wine, the liquid becomes turbid within a few minutes and gives a deposit, of characteristic appearance under the microscope, after some hours. Pure wine, and also vinegar to which wine has been added, show crystals of calcium tartrate or racemate; for the latter X-shaped crystals are characteristic. Poorly fermented wine may show tufts of calcium malate. If the wine has been properly and completely fermented to acetic acid, it shows only S-shaped crystals, but if such fermentation is incomplete, oblong crystals of calcium racemate are also formed. T. H. P.

Analytical Classification of Fish Liver Oils. N. Evers and W. Smith. (*Pharm. J.*, 1933, **131**, 128–129.)—II. *Analytical results on Fish-liver Oils.*—Further details are given of analyses reported on in Part I (ANALYST, 1932, **57**, 735), particularly the amount of unsaponifiable matter and its iodine value, and acid phthalic esters obtained from the soluble and insoluble in petroleum spirit, the unsaponifiable matter for six *Gadidae* fish, six Elasmobranch, 2 flat fish and the monk fish.

III. *The spectrographic examination of Fish-liver Oils.*—Twenty-nine cod-liver oils were examined for vitamin *A* by measuring the intensity of absorption at a wave-length of 328$m\mu$, and the results were compared with the "blue values" obtained by the antimony trichloride colour test, which latter are shown to be only very approximate measures of the vitamin *A* value of the oil (328$m\mu$ band). The correlation between blue value and intensity of absorption at 328$m\mu$ for halibut-liver oils was found not so good as was believed to be the case by Haines and Drummond (ANALYST, 1933, **58**, 356). D. G. H.

Determination of Chlorogenic and Caffeic Acids. W. Plücker and W. Keilholz. (*Z. Unters. Lebensm.*, 1933, **66**, 200–238.)—The results of a number of experiments show that chlorogenic and caffeic acids may be titrated potentiometrically, with quinhydrone as indicator electrode. Chlorogenic acid in aqueous solution (50 c.c.) may be determined by treatment with 30 per cent. potassium hydroxide solution (12 c.c.) for one hour at the ordinary temperature. In this way the chlorogenic acid is converted into caffeic acid, which may then be determined by acidifying with phosphoric acid, extracting with ether, and either titrating with 0·1 *N* sodium hydroxide solution or determining the dry residue of the extract.

A method is worked out for the determination of chlorogenic and caffeic acids in mixtures of the two with quinic acid. The caffeic acid is precipitated as the lead compound from the ethereal solution, and the chlorogenic and quinic acids as lead salts from the aqueous solution, separate electrometric titrations being afterwards carried out. The scheme has been applied to caffeine-potassium chlorogenate, which has a known chlorogenic acid-content, in order to ascertain the

factors required for calculating the results. The method gives results which are reproducible, and, with mixtures of the two acids, accurate; quinic acid does not interfere. Unreliable results were, however, obtained from an aqueous raw coffee extract, and these were traced to the presence of citric and phosphoric acids; after these acids had been removed by precipitation with barium acetate in alcoholic solution, the method proved satisfactory. In a number of roasted coffees examined, only traces of caffeic acid could be found, and raw coffee showed no dioxystyrol.

Chlorogenic acid is an unstable compound, and is decomposed by prolonged boiling of its aqueous solution. It occurs in two different forms, having the respective specific rotations, $[\alpha]_D$, $-35{\cdot}45°$ and $-47{\cdot}90°$. T. H. P.

Chlorogenic Acid Content of Coffee. W. Hoepfner. (*Z. Unters. Lebensm.*, 1933, **66**, 238–251.)—In the determination of chlorogenic acid in coffee by the lead precipitation method, the preliminary extraction of this acid by 95 per cent. alcohol is far less complete than its extraction by water. The extent to which the lead compound is precipitated varies with the experimental conditions, and the composition of the precipitate is also variable. As a result of further work on the author's colorimetric method (ANALYST, 1933, 100), certain details in procedure are elaborated (*cf.* also Griebel, ANALYST, 1933, 621). The content of chlorogenic acid in raw coffees is found to lie between 6·3 and 7·7 per cent. (on dry matter), and that in roasted coffees varies, with the degree of roasting and the origin of the coffee, from 3·2 to 4·5 per cent.; it is possible that these limits require slight extension in both directions. In the raw beans the chlorogenic acid is present as potassium-caffeine and potassium salts; the potassium salt is the first to undergo destruction during the roasting. T. H. P.

Gravimetric Determination of the Antiseptic Constituents of Hops. T. K. Walker and J. J. H. Hastings. (*J. Inst. Brewing*, 1933, **39**, 509–512.)—The authors' simplified modification (*id.*, 1929, **35**, 229) of Ford and Tait's gravimetric method (*id.*, 1932, **38**, 351) frequently gives high values for the α-resin, owing to the slow precipitation with it of the lead salt of a non-antiseptic substance. This may be present in the hop in amounts varying from a trace to 3 per cent., and is an acidic, almost colourless, viscous liquid, which is soluble in water or methyl alcohol, but almost insoluble in ether or petroleum spirit. The following procedure (which takes 3½ hours) is, therefore, proposed:—About 50 grms. from the centre of the bulk sample are freshly minced to a fine meal, and 10 grms. are extracted with 100 c.c. of methyl alcohol containing no acetone or water (b.pt. 64·6° C. at 760 mm.), in the apparatus described (*loc. cit.*), or by shaking, without rotation, at intervals of 3 minutes for 20 minutes in a 300-c.c. glass-stoppered bottle with an internal diameter of not more than 5 cm. Commercial grades of methyl alcohol are unsatisfactory. When the mixture has stood for 10 minutes the liquid is poured through a fluted paper, the residual meal being pressed with a glass plunger, if necessary, in order to obtain 60 c.c. of clear filtrate. Of this, exactly 50 c.c. are mixed in a separating funnel by gentle swirling with 100 c.c. of a 1 per cent. solution of sodium chloride and 50 c.c. of petroleum spirit, and the mixture is then shaken vigorously. After separation, the petroleum layer is

filtered into a 200-c.c. flask, and the residual liquid is re-extracted with three 40-c.c. portions of petroleum spirit; the last extract should be colourless. Any tarry clots which form at the beginning of these operations should be re-dissolved in methyl alcohol separately and returned to the liquor. The paper is washed, the filtrate is diluted to 200 c.c. with petroleum spirit, and exactly 50 c.c. of the extract are evaporated in a tared flask and weighed after 1½ hours at 100° C., when the factor 80·8 gives the percentage of total soft resin (T). Of the remaining petroleum extract, 100 c.c. are evaporated (the last 5 c.c. being removed rapidly under reduced pressure), and a solution of the residue in not more than 20 c.c. of methyl alcohol at 45 to 50° C. is precipitated at 60° C. by the addition of a 1 per cent. solution of lead acetate in methyl alcohol containing 1 c.c. of glacial acetic acid per litre, this temperature (60° C.) being maintained for 5 minutes (*cf.* Ford and Tait, *loc. cit.*). The volume of lead acetate solution to be added is determined by a trial titration of the residue from 50 c.c. of the extract. That the necessary amount has been added is shown by the formation of a distinct brown colour when a drop of the supernatant liquid (taken immediately after the precipitate has settled) is placed on one side of a filter-paper moistened on the other with a 10 per cent. solution of sodium sulphide. After 30 minutes at room temperature the mixture is filtered on a tared sintered glass crucible, and the precipitate is washed with methyl alcohol, dried for 1 hour at 100° C., and weighed. The percentage of α-resin is given by the factor 25·45, whilst β-resin $= (T - \alpha)$; whence the preservative value $(P) = 10(\alpha + \beta/3)$. The above factors include corrections for the effect, on the determination, of the water present in the hops, although the result gives the percentage on the weight of hops as received, and not on the bone-dry weight. Results for 50 samples of English, American and Continental (1928–1929), and old cold-stored hops showed that Ford and Tait's method gave average results for α- and β-resins, and P higher by 0·17, 0·16 and 1·9 per cent., respectively, than the new modification. J. G.

Precipitation of Alkaloids by Tannins and the Use of Antipyrine in the Detection of Tannins. A. H. Ware and V. Smith. (*Pharm. J.*, 1933, **131**, 148–149.)—Although only 6 out of 26 alkaloidal hydrochlorides gave precipitates with gallotannin, according to Fear (ANALYST, 1929, **54**, 316), it has been found that, with adjustment of the p_H value or on addition of a suitable electrolyte, probably all alkaloids may be precipitated. If 2·5 c.c. of a 1 per cent. solution of gallotannin are added to 5 c.c. of a 1 per cent. solution of the alkaloidal salt, and no precipitation occurs, 5 c.c. of a 0·1 per cent. solution of sodium bicarbonate are added, when the mixture will usually have a p_H value of 7 to 7·5, and will form precipitates with the hydrochlorides of amylocaine, apomorphine, arecolin, diamorphine, emetine, ephedrine, cocaine, morphine, and procaine; also with benzamine lactate, homatropine hydrobromide, physostigmine sulphate, and pilocarpine nitrate. Quinine acid hydrochloride gave a slight opalescence, increased by addition of more bicarbonate. Another method by which all the foregoing alkaloids, etc., were precipitated, and, in addition, acriflavine, theobromine sodium salicylate, and theophylline sodium acetate, consists in dissolving a little of the substance in 3 c.c. of a 10 per cent. solution of sodium acid phosphate, warming, if necessary,

and adding to the cold solution 3 c.c. of a 2 per cent. solution of gallotannin, and then sufficient, but not more than 3 c.c., of a mixed phosphate solution, which contains 5 per cent. of disodium phosphate and 10 per cent. of acid sodium phosphate. The mixture is well shaken and filtered. This test can be used for the detection of alkaloids in mixtures, vegetable powders and extractives, the substance (if solid) is moistened with a strong solution of basic lead acetate, or (if a concentrated extract) it is rubbed out with powdered wood fibre, and is then extracted with a mixture of 1 part of amyl alcohol, 1 part of carbon tetrachloride and 3 parts of chloroform. After filtration the solvent is extracted with 5 c.c. of a warm solution of sodium acid phosphate, and the test completed as above. If antipyrine is used as a routine reagent for tannins, 5 c.c. of freshly prepared aqueous extractive are treated with 0·5 grm. of acid sodium phosphate, the mixture is filtered, and the filtrate poured into 5 c.c. of a 2 per cent. solution of antipyrine. If only a slight precipitate is obtained, the test is repeated with 0·01 grm. of sodium bicarbonate instead of phosphate, and if no precipitate then forms tannin is almost certainly not present. If precipitation occurs, the mixture is evaporated to expel alcohol, filtered, adjusted to the original volume, and the filtrate is tested as above. Antipyrine is not very suitable for quantitative work, as tannin may be lost during the process of washing out the sodium phosphate. D. G. H.

Chemical Tests for *Strophanthus*. E. M. Smelt. (*Pharm. J.*, **1933, 131**, 150–151.)—In order to distinguish the various species of *Strophanthus* the six following tests should be applied to the residues obtained after crushing 0·25 grm. of seeds and digesting with 10 ml. of alcohol (70 per cent.) at about 60° C. for 5 minutes, filtering the cooled mixture through cotton wool, evaporating the filtrate, and removing fat from the residue by washing twice with petroleum spirit. In each case approximately 1 mgrm. of residue is mixed with the reagent and allowed to stand:—(i) One drop of 75 per cent. v/v sulphuric acid; (ii) 1 drop of test solution of ferric chloride B.P. and 1 drop of sulphuric acid; (iii) 5 ml. of hydrochloric acid containing 1 per cent. w/v of phenol, warmed to 50°–60° C.; (iv) 1 drop of phenoldisulphonic acid (B.P. 1914 formula); (v) 0·1 ml. of a 1 per cent. v/v solution of furfural dissolved in 95 per cent. alcohol and 0·5 ml. of 75 per cent. v/v sulphuric acid added; (vi) 5 ml. of hydrochloric acid containing 0·1 per cent. w/v of resorcinol, heated to 60° C. for about 5 minutes. By these tests (*a*) *Strophanthus Kombé* (the only species recognised in the B.P. 1932) is readily distinguished from (*b*) *Strophanthus Emini* by test (i) (*a*) green, (*b*) brown turning to violet colour; (iii) (*a*) red-brown tinge, (*b*) violet colour; (v) (*a*) greenish-grey in 5 minutes, indigo blue in 15 minutes, and (*b*) deep violet; (vi) (*a*) red-orange, and (*b*) purple colours. The same tests distinguish (*c*) *Strophanthus gratus* and (*d*) *Strophanthus sarmentosus*, (i) (*c*) orange-pink, (*d*) pink in 5 minutes; (iii) (*c*) violet, (*d*) almost colourless; (v) (*c*) deep violet, (*d*) pink; (vi) (*c*) purple, (*d*) pale red-orange. The colours obtained with (*e*) *Strophanthus Nicholsonii* might be confused with those given by *Strophanthus Emini*, but the former are usually decidedly paler, particularly in test (v). Characteristic colours were not obtained with (*f*) *Strophanthus hispidus* or with (*g*) *Strophanthus Courmonti*, although it should be possible to distinguish them. Test (iv) gave in all cases the same colours as test (i).

Colours obtained with test (ii) were: After 5 minutes, (*a*) green, (*b*) dark blue, green in 5 minutes, (*c*) pale brown, (*d*) brownish-pink, (*e*) green, (*f*) red-brown, and (*g*) yellowish-green. D. G. H.

Relative Activity of Ergotoxine and Ergotamine, with special Reference to the Assay of Ergot Preparations. E. Lozinski, G. W. Holden and G. R. Diver. (*Pharm. J.*, 1933, **131**, 137.)—Fluid extracts of ergot were assayed colorimetrically by Allport and Cocking's modification (*Quart. J. Pharm.*, 1932, **5**, 341) of Smith's technique, and biologically by the Broom and Clark technique (*J. Pharm. Exp. Ther.*, 1932, **22**, 59), using ergotoxine ethanesulphonate and ergotamine tartrate as standards. Ergotoxine and ergotamine were found to be practically equivalent by the colour assay, but by the biological method ergotamine possesses only 60 to 65 per cent. of the activity of ergotoxine, and experiments with the extracts confirmed these figures. It is suggested, therefore, that, in the colorimetric assay of ergot, a correction (60 per cent. of observed readings) should be introduced to compensate for the lower activity of the ergotamine. D. G. H.

Determination of Acriflavine and Related Medicinal Dyes. A. D. Powell and G. F. Hall. (*Pharm. J.*, 1933, **131**, 136–137.)—The method used for the examination of flavine dyes depends on the insolubility of the ferricyanides, which may be almost completely precipitated from aqueous solution on addition of excess of standard potassium ferricyanide solution. Two grms. of the sample are dissolved in 250 ml. of water, and *N* hydrochloric acid is added until the solution is faintly red to Congo-red paper. Five grms. of sodium acetate solution are then added, followed by 50 ml. of 0·1 *M* potassium ferricyanide solution, added with constant stirring. After 10 minutes the precipitate is filtered off, washed with three portions of 50 ml. each of water. Ten ml. of hydrochloric acid (sp.gr. 1·16), 10 grms. of sodium chloride, 1 grm. of potassium iodide, and 3 grms. of zinc sulphate in 10 ml. of water are then added to the combined filtrate and washings, with mixing after each addition. After 3 minutes the liberated iodine is titrated with 0·1 *N* sodium thiosulphate solution, the solution being allowed to stand for another 3 minutes before the final completion of the titration. A blank determination is made on 25 ml. of the ferricyanide solution (1 ml. of 0·1 *M* potassium ferricyanide solution is equivalent to 0·08883 grm. of acriflavine or 0·07788 grm. of euflavine). D. G. H.

Determination of Free Sulphur [in Ointments, etc.]. N. L. Allport. (*Pharm. J.*, 1933, **131**, 141–142.)—The method is based on Castiglioni's process of converting free sulphur into thiocyanate by treatment with potassium cyanide, the thiocyanate being titrated against silver nitrate after the addition of formaldehyde to remove the excess of potassium cyanide (*Z. anal. Chem.*, 1932, **91**, 32). Fifty c.c. of a reagent made up of 40 grms. of potassium cyanide A.R., 90 c.c. of triethanolamine, and water to 1 litre, are added to a weighed amount of the material under examination, containing about 0·1 grm. of sulphur. Unless the material is an ointment with paraffin base, 1 grm. of simple ointment or soft paraffin is added and a little pumice, and the mixture is heated for 30 minutes

under a reflux condenser. After cooling, 10 c.c. of formaldehyde solution are added, the mixture is acidified with dilute nitric acid, and exactly 50 c.c. of 0·1 *N* silver nitrate solution are added. After the addition of 0·5 grm. of decolorising charcoal, if necessary (*e.g.* with Pulv. Glycrrh. Co.), the liquid is filtered through asbestos, the residue washed with water, and the filtrate titrated with 0·1 *N* ammonium thiocyanate solution (1 c.c. of *N*/10 $AgNO_3$ = 0·0032 grm. of sulphur). The value of the blank is found by mixing 50 c.c. of the reagent with 10 c.c. of formaldehyde acidified with nitric acid, and titrating. The results agreed well with those obtained by the oxidation process, and the method is a rapid one for sulphur ointments and compound liquorice powder. D. G. H.

Colour Reaction of Bismuth. A. D. Powell. (*Pharm. J.*, 1933, **131**, 150.)—Bismuth may be detected in about 10 ml. of an aqueous solution of the drug or chemical by adding 2 ml. of dilute hydrochloric acid and about 0·5 grm. of potassium iodide, mixing, and adding 5 ml. of industrial spirit or of acetone, and 5 to 10 ml. of ethyl acetate, when a red colour in the upper layer, which separates after shaking, indicates the presence of bismuth. For a quantitative test in the absence of lead, the material is extracted twice with ether, and the bismuth is re-extracted from the ethereal solution by shaking with 5 ml. of strong ammonium chloride solution, followed by two washings with 25 and 5 ml. of water made slightly acid with hydrochloric acid. The combined extracts are neutralised with ammonia, and excess (1 or 2 drops) of dilute hydrochloric acid is added. The ether is removed by warming, the volume of the cooled solution adjusted to 50 ml., and 1 ml. of a dilute solution of sodium sulphide is added. A control test with a definite amount of bismuth present is made and the colours compared. In the presence of copper or mercury the solution must be rendered alkaline, and potassium cyanide added. If the amount of bismuth is very small and traces of lead are present, the test solution should be concentrated to 1 to 5 ml., and placed in a narrow, stoppered cylinder; hydrochloric acid to give a concentration of 0·5 *N* is added, and then about 0·2 grm. of potassium iodide. After the addition of 5 drops, or less, of ethyl acetate, the mixture is shaken, and the colour of the upper layer is matched against a control. Bismuth may be rapidly determined by the first method in 25 ml. of urine. Coloured substances may be extracted from the urine with ether, and samples of precipitated substances should be acidified with hydrochloric acid, and an amount taken equivalent to the original 25 ml. of urine. The second method is suitable for samples of tissues and organs from animals that have been injected with bismuth. D. G. H.

Determination of Thyroxine in the Thyroid Gland. N. F. Blau. (*J. Biol. Chem.*, 1933, **102**, 269–278.)—A method is presented for the quantitative extraction of thyroxine from alkaline hydrolysates of thyroid substance, which depends upon the insolubility of thyroxine in an acid solution and upon the pronounced solubility of the acid salt of the product in butyl alcohol. Distribution ratios for thyroxine, diiodotyrosine and potassium iodide between butyl alcohol and an aqueous phase, acidified with sulphuric acid, were first determined at room temperature in the presence of the products of hydrolysis of muscle proteins and of the salt that would form upon addition of the acid to 2 *N* sodium hydroxide

solution; on the basis of the data obtained, the following procedure for the determination of thyroxine in thyroid substance was devised:—In a Kjeldahl flask 1·25 grm. of the dried, defatted gland is refluxed for 18 hours with 100 c.c. of 2 *N* sodium hydroxide solution, as recommended by Leland and Foster (*J. Biol. Chem.*, 1932, **95**, 165), then the alkaline hydrolysate is cooled, transferred to a 300-c.c. separating funnel, and cooled in running water to below room temperature. From 0·5 to 2·0 c.c., or more, of brom-cresol green indicator (prepared according to Kolthoff, *The Colorimetric and Potentiometric Determination of* p_H, New York, 1931, 30), are added until the solution assumes a greenish tint, and a 1 : 1 solution of sulphuric acid is run in from a burette fairly rapidly, and with vigorous shaking of the funnel, to prevent the formation of a heavy flocculent precipitate of the products of protein cleavage, until the colour just changes to yellow; about 0·2 c.c. more is added, and the volume of acid used is noted. A test drop is removed from the funnel with a glass rod and brought into contact with a small drop of bromphenol blue indicator. A p_H of 3·5 to 3·1 will usually be found. The total volume of solution in the funnel is calculated. To the Kjeldahl flask 1 or 2 drops of brom-cresol green are added, and just enough acid (2 to 5 drops) to adjust the reaction to about p_H 3. A volume of butyl alcohol, equal to that of the total volume of the acid solution to be extracted, is measured out, used in small portions to rinse the Kjeldahl flask, and then poured into the funnel until all has been added. The whole is shaken, then left aside to cool to room temperature for from 60 to 90 minutes. Next, the light straw-coloured aqueous layer is drawn off, leaving any slight amount of tar or interfacial precipitate which may be formed in the butyl alcohol, and a volume of 4 *N* sodium hydroxide solution, containing 5 per cent. anhydrous sodium carbonate, equal to that of the volume of butyl alcohol taken, is added, shaken for a few minutes, and left to stand for an hour or 90 minutes. The dark brown alkaline solution is drawn off to the last drop. The butyl alcohol remaining in the funnel is shaken with a volume of the sodium hydroxide-carbonate mixture equal to one-half the volume of butyl alcohol originally taken. After an hour or longer, it is well separated, the end of the funnel below the stop-cock is washed with distilled water, and the butyl alcohol is run into a distillation flask of suitable size. The funnel is rinsed with several small portions of butyl alcohol which are added to the flask. A few drops of concentrated sodium hydroxide are added, and the butyl alcohol is distilled off from a water-bath at a low pressure; the distillation is continued nearly to dryness. The residue is washed with distilled water and dilute sodium hydroxide into a 250-c.c. nickel crucible. The contents of the crucible are then evaporated over a sand-bath or hot plate to dryness and ashed, and the iodine is determined as in the method of Kendall (*J. Biol. Chem.*, 1915, **20**, 501).* P. H. P.