ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Amino-acids, Acetous Perchloric Acid Titration of. P. Ekeblad. (*Svensk. farm. Tidskr.*, 1953, **57**, 185.) The acetous perchloric acid titration of amino-acids has been tested on glycine and asparagine. It was found that glycine as well as *l*-asparagine monohydrate can be dissolved directly in acetic acid but that anhydrous asparagine requires an excess of perchloric acid. In all cases heating was necessary to obtain complete solution within a reasonable time. Heating without perchloric acid caused low and irregular results; apparently the amino-acids are partially acetylated. For glycine the acetylation is avoided if excess of perchloric acid is added. Heating of asparagine with excess of perchloric acid causes high results; probably the amido linkage in the asparagine molecule is broken down. The use of formic acid permits rapid dissolving of amino-acids without heating. The formic acid should contain as little water as possible and the final solution should contain not more than 2 per cent. of formic acid. The following method is proposed for the titration of amino-acids not soluble in acetic acid at room temperature. 1 milli-equivalent of the finely divided amino-acid is dissolved in 0·5 ml. of formic acid (97 to 100 per cent.) and the solution diluted with 25 ml. of anhydrous acetic acid. After the addition of Blue-BZL solution, the solution is titrated with 0·1N acetous perchloric acid to a red colour. S. L. W.

Ammonia in the Presence of Hydrazine, Determination of. W. Puch and W. K. Heyns. (*Analyst*, 1953, **78**, 177.) Procedures are described for the determination of ammonia in mixtures of ammonium and hydrazine salts. Known weights of hydrazine hydrochloride were added to known volumes of the standard ammonium salt solutions and, after oxidation of the hydrazine, the ammonia was distilled. For the oxidation 3 procedures using iodic acid, bromine, and alkaline permanganate were used. Equally good results were obtained with all 3 methods and a distillation time of 9 to 10 minutes was sufficient. R. E. S.

Antimony in Aromatic Organic Compounds, Determination of. N. T. Wilkinson. (*Analyst*, 1953, **78**, 165.) Methods were investigated for determining antimony in organic compounds that also contain nitrogen and chlorine. Decomposition of the organic compound by fusion with sodium peroxide, cane sugar and potassium nitrate in a Parr bomb was not satisfactory, as an antimony compound was formed that was insoluble in dilute nitric acid or dilute hydrochloric acid. In the recommended process the organic matter was destroyed by wet digestion with nitric and sulphuric acids, and the antimony compound formed was dissolved in a mixture of hydrobromic acid and bromine; the antimony was then reduced by sulphurous acid, the excess of sulphur dioxide being removed by boiling, and was determined volumetrically by titration with standard potassium bromate solution. In an alternative procedure the antimony was precipitated as the sulphide, separated by filtration, brought into solution and then titrated with potassium bromate solution. Satisfactory antimony recovery experiments are recorded in which aliquots of potassium

antimony tartrate solution were taken and known weights of *p*-chloraniline, aniline hydrochloride, and pyridine hydrochloride were added. The chlorostibinate of diphenylamine was also analysed by this method. R. E. S.

Cadmium, Microdetermination of. B. E. Saltzman. (*Analyt. Chem.*, 1953, **25**, 493.) A procedure applicable to the determination of micro quantities of cadmium in samples containing as much as 5 to 10 mg. of the common interfering metals is described. An improved dithizone separation is used with cyanide as a suppressing agent in two extractions from strongly alkaline solution, and using tartaric acid as the stripping medium. Stable colours are obtained and losses due to decomposition are controlled by using hydroxylamine in the extractions and by reducing the time of contact of the chloroform with the alkali. Working details of the method are given by which cadmium can be determined spectrophotometrically in a volume of 15 ml. with a sensitivity of 0·05 μg. The separation of thallium is made possible by the development of a special procedure which transposes the dithizonate of thallium with cobalt. Satisfactory results for the recovery of cadmium from urine, water, and spelter are quoted. R. E. S.

Carbonyl Compounds, Determination of. A. J. Feuell and J. H. Skellon. (*Analyst*, 1953, **78**, 135.) A volumetric method of determining aldehydes and ketones with semi-carbazide has been devised. The carbonyl compound was dissolved in water and the semi-carbazide reagent added; after filtering the precipitate and washing, hydrochloric acid and potassium cyanide were added to the filtrate which was then titrated with standard potassium iodate solution. Experiments with salicylaldehyde and benzaldehyde showed that useful results could be attained with a precipitation time of only 10 minutes and a 20 to 60 per cent. excess of reagent. The application of the semi-carbazide method to oxidised oils possessing marked reducing properties was disappointing, as semi-carbazones could not be precipitated and the method consequently failed. For estimating the carbonyl groups in the products of oxidation of fatty acids and esters by gaseous oxygen, the method of Maltby and Primavesi (*Analyst*, 1949, **74**, 498) was modified by the use of two reagent solutions instead of a single one, so that interference from colour inherent in the sample was eliminated and variations from neutrality in the sample were simultaneously compensated; the matching procedure was also adapted to daylight or artificial light. The method has been found useful for studies of the carbonyl content of the various fractions obtained in the separation of the complex end-products of oxidised fatty-acid esters. It has also been used to follow the steady decrease in carbonyl content occurring during the thermal catalytic autoxidation of ketohydroxystearic acid. R. E. S.

Copper, New Colorimetric Reagent for. G. F. Smith and D. H. Wilkins. (*Analyt. Chem.*, 1953, **25**, 510.) A new complexing reagent specific for copper, 2:9-dimethyl-4:7-diphenyl-1:10-phenanthroline, is proposed which has the highest molecular extinction coefficient in its application to the cuproine reaction of any previously known reactant in this category of the phenanthroline-type products. Spectrophotometric curves for a series of tests are shown, the absorption bands having sharp peaks with small absorption over the 500 to 700 mμ wavelength range. Beer's law applies over the range of copper concentrations 1 to 10 p.p.m. The copper complex cations are not entirely free from the effects of air oxidation (0.05 per cent. per hour under ordinary laboratory conditions). Commonly occurring ions such as chloride, nitrate, perchlorate, and phosphate as well as sulphate and citrate do not interfere. Metal

ions should not interfere but the application of the reagent to the determination of copper in biological, medicinal and food products would require further investigation. R. E. S.

Cyanide and Thiocyanate, Colorimetric Determination of. J. M. Kruse and M. G. Mellon. (*Analyt. Chem.*, 1953, **25**, 446.) A study was made of methods available for the determination of free and combined cyanide and thiocyanate in industrial wastes. Two procedures were examined based on the use of a pyridine-benzidine or pyridine-pyrazolone reagent to react with cyanogen halide formed by treating the sample with bromine or chloramine-T. A comparison of the two methods showed that the pyrididine-pyrazolone reagent formed a more stable colour with cyanide and this was finally chosen because of its greater precision and sensitivity. The main problem was the treatment of the sample necessary to permit the application of the reagent and 3 separate procedures were developed for the determination of free cyanide, total cyanide, and cyanide in the presence of thiocyanate. Work is outlined which led to the publication (*Sewage and Ind. Wastes*, 1951, **23**, 1402) of step-by-step directions for the separation and subsequent colour development for sewage analysis. Tests on sewage containing known amounts of various metals and the desired constituents showed the procedures to have satisfactory workability, sensitivity, and precision for the industrial application intended. R. E. S.

3-5-Diiodothyronine, Colorimetric Determination of. W. H. C. Shaw. (*Analyst*, 1953, **78**, 253.) An examination was made of the application of Millon's reagent to the determination of 3:5 diiodo-L-thyronine occurring as an impurity in L-thyroxine, since the reagent gives reddish colours with tyrosine and mono-iodotyrosine, but not with 2:6-substituted phenols, such as diiodotyrosine and thyroxine. The procedure of Roche and Michel (*Biochim. Biophys. Acta*, 1947, **1**, 335) was not directly applicable to the determination, since solutions were invariably turbid and the heat treatment prescribed (30 minutes at 60° C.) was insufficient to develop more than a trace of colour, this being adsorbed on the precipitate. The addition of the surface active agents sodium lauryl sulphate and trimethylcetylammonium bromide at concentrations of about 0·02 per cent. w/v proved effective, although the former was slightly better. In conjunction with 0·7 N sulphuric acid, sodium lauryl sulphate successfully prevented precipitation with up to 1 mg. of diiodothyronine. With larger quantities, opalescence sufficient to cause marked deviations from linear colour response developed. Increases in the concentration of surface-active agents failed to secure any improvement with more than 1 mg. R. E. S.

Fatty Acids, Determination of, by Potentiometric Titration. B. W. Grunbaum, F. L. Schaffer and P. L. Kiri. (*Analyt. Chem.*, 1953, 25, 480.) A titration assembly is described which provides isolation from atmospheric carbon dioxide and uses commercially available electrodes and *p*H meter. Using a rotating magnetic stirrer, ethanolic solutions of fatty acids were titrated with carbonate-free aqueous potassium hydroxide of constant normality provided by an ion exchange column. The method was applied to pure fatty acids in quantities of 0·005 to 0·025 microequivalent and to the fatty acids from liver digests. The *p*H readings of the meter were used empirically, the end-point with fatty acids being approximately 9·0. Replicate titrations were carried out with pure stearic and palmitic acids and with a mixture of stearic, palmitic, myristic, and lauric acids with satisfactory results. R. E. S.

Gamma Benzene Hexachloride, Determination of. I. Hornstein and W. N. Sullivan. (*Analyt. Chem.*, 1953, **25**, 496.) The procedure used involves the dechlorination of gamma benzene hexachloride to benzene and its subsequent nitration to *m*-dinitrobenzene, which after extraction is allowed to react with methyl ethyl ketone in the presence of strong alkali; the violet-red colour that develops is measured photometrically. In the estimation of gamma benzene hexachloride in air the air sample is drawn through gas-washing bottles containing acetic acid, and the amount absorbed in the acetic acid is determined; as an alternative the air sample is taken through an alumina adsorption column, the adsorbed gamma benzene hexachloride being washed off the column with acetic acid. The vapour is readily adsorbed, especially by rubber, and no material other than glass should precede the adsorbents. As a check on the methods, analyses were made in a closed cabinet, in which the walls had been treated with gamma benzene hexachloride and equilibrium attained in the enclosed air space; using both acetic acid and alumina adsorption, results corresponding to a "relative lindane saturation' of about 91 per cent. based on the theoretical value calculated from vapour pressure measurements obtained. The precision of the method appeared to be about ± 2 per cent., the results obtained showing good agreement with theoretical values calculated from known vapour pressure measurements. Details of procedure are given. R. E. S.

Glycerol in Fermentation Solutions, Chromatographic Determination of. A. F. Williams. (*Nature, Lond.*, 1953, **171**, 655.) A rapid and simple chromatographic procedure has been developed for the determination of glycerol in solutions obtained by the sulphite fermentation process of Cuban blackstrap molasses. The method involves the preparation of a column of coarse-grade cellulose powder (2·5 g.) which acts as a support for chromatographic alumina (5 g.). Sufficient of the sample is taken to give up to about 0·5 g. of glycerol and containing about 3 ml. of water, its volume being 5 ml. (approximately). After addition of sodium sulphite (0·5 g.), sodium acetate (1 g.) and acetic acid (0·1 ml.) to the sample, the resulting solution is mixed with alumina (15 g.) and the mixture then transferred to the prepared column. The glycerol is eluted with 250 ml. of solvent (acetone containing 5 per cent. v/v of water and 0·05 per cent. v/v of glacial acetic acid). Results obtained from recovery experiments are given. R. E. S.

Iodine Titration with Thiosulphate, End-point in. G. Knowles and G. F. Lowden. (*Analyst*, 1953, **78**, 159.) 5 methods were tried to determine the most suitable method for the detection of the end-point in the titration of iodine with thiosulphate; 2 depended on a colour change, the indicators being starch and sodium starch glycollate, and 3 were electrical: an amperometric method, the dead-stop end-point method, and a derivative polarographic method. The sensitivity of sodium starch glycollate appeared to be rather less than that of starch. Soluble starch indicator could lead to errors, on the low side, of the order of 20 to 40 μg. of iodine in volumes of 50 to 200 ml., and sodium starch glycollate was even less accurate. Comparisons of the derivative polarographic titration showed that although not so precise as the amperometric, it was capable of giving an end-point with a possible error of ± 0·01 ml. of 0·0025 N iodine solution (± 3 μg. of iodine). Results obtained with the dead-stop end-point method varied with the applied potential difference; the method also gave a slightly less sensitive indication of the presence of

iodine than the amperometric method. Of the 5 methods tried, the amperometric is the most satisfactory. The simple circuit described, incorporating a pointer micro-ammeter, detects one μg. of iodine in 40 ml. of solution.

R. E. S.

Magnesium, Colorimetric Determination of. A. E. Harvey, J. M. Komarmy and G. M. Wyatt. (*Analyt. Chem.*, 1953, **25**, 498.) A spectrophotometric method is given for the determination of magnesium using the reagent Eriochrome Black T, 1-(1-hydroxy-2-naphthylazo)-2-hydroxy-5-nitro-4-naphthalenesulphonic acid; at *p*H values from 7 to 10 this reagent forms a slightly dissociated soluble red complex with magnesium. Absorption curves are given for the dye, the magnesium, and the calcium complexes at a number of *p*H values; the light absorption of both magnesium and calcium complexes decreases as the *p*H decreases, but only the calcium complex absorption curve becomes identical with the reagent absorption curve at *p*H 7·75, suggesting the possibility of determining magnesium in the presence of calcium by arithmetical calculation using absorption values over a range of *p*H. Owing to the likelihood of errors, however, it is recommended that calcium is removed by precipitation as sulphate from a 90 per cent. methanol solution. Analyses of water samples by the proposed method agreed with gravimetric determinations within the usual error encountered in water analysis. R. E. S.

ESSENTIAL OILS

***Eucalyptus eudesmioides* Bark, Essential Oil of.** A. Blumann, M. Michael and D. E. White. (*J. chem Soc.*, 1953, 788.) An essential oil was obtained by the extraction of the finely-ground bark of *Eucalyptus eudesmioides* F. Muell. with light petroleum and ether followed by steam distillation of this extract. The volatile oil was then fractionated and yielded cineole, *d*-borneol (isolated as the 3:5-dinitrobenzoate), an uncharacterised hydrocarbon fraction, and globulol (isolated as the 3:5-dinitrobenzoate), a sesquiterpene alcohol previously obtained from *E. globulus* oil. A. H. B.

ORGANIC CHEMISTRY

Phenol, Purification of, for Chromatography. P. H. Mars. (*Pharm. Weekbl.*, 1953, **88**, 319.) Crystalline phenol (50 g.) is melted and dissolved in 100 ml. of light petroleum (40° to 60° C.) by warming to 50° to 55° C. Water (1 to 2 ml.) is added, the mixture is shaken thoroughly, and allowed to separate. The light petroleum layer is then poured off and allowed to crystallise. The light petroleum may be used again, while the phenol is dried in a current of air, and finally in a desiccator. To avoid drying, the phenol may be dissolved by the addition of a little water, the concentration of phenol determined by taking the refractive index, and the product diluted to the required strength.

G. M.

Steroid Sapogenins, Infra-red Absorption Spectra of. R. N. Jones, E. Katzenellenbogen and K. Dobriner. (*J. Amer. chem. Soc.*, 1953, **75**, 158.) The infra-red absorption spectra of 35 steroid sapogenins and derivatives were investigated and the band intensities compared on a molecular extinction coefficient basis. The steroid sapogenins are of considerable interest because they are the starting materials for the bulk synthesis of steroid hormones. Many absorption spectra are recorded and the position of the bands and their correlations with molecular structure are discussed. A. H. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Amino-acid Constituents of Normal Urine, Chromatographic Investigation of. W. H. Stein. (*J. biol. Chem.*, 1953, **201**, 45.) A separation of amino-acid constituents of normal urine has been effected using columns of the ion exchange resin Dowex 50 and a range of phosphate buffer solutions of varying *p*H. Substances responsible for various peaks in the effluent are identified by the addition of known amino-acids to the urine, the designated peak rising without loss of symmetry. Special colour tests and paper chromatography were also used as supplementary aids to identification. The absence in freshly voided urine of significant quantities of aspartic acid, citrulline, proline, glucosamine, hydroxylysine and ornithine has been indicated in this way. The presence of appreciable quantities of glutamine, reported by Archibald, could not be confirmed, as the substance decomposes during chromatography at *p*H 3. The presence of a number of unidentified substances has also been revealed; many of these substances are labile to acid hydrolysis. Asparagine, not hitherto reported as a constituent of normal human urine, was identified as an assymmetry on the trailing edge of the serine peak. It is much more readily detected in the urine of patients suffering from Wilson's disease. Quantitative data on a number of amino-acids has been made available for the first time by this method. It is also revealed that only about 10 mg. of cystine is excreted as such per day, whereas previous microbiological and polarographic methods had indicated that this figure was between 70 and 100 mg. It is suggested that these latter methods may actually determine peptides, such as a glutathione as cystine, giving rise to erroneous results. The glutamic acid content increases on standing, indicating that much of the conjugated glutamic acid is present in a labile form. The quantity of almost all the amino-acids rises after acid hydrolysis and gives an indication of the extent to which they are present as conjugates. Relatively large amounts of glycine, glutamic acid and aspartic acid are liberated by acid hydrolysis; only a small portion of the conjugated form of these amino-acids can be accounted for as hippuric acid, glutamine and asparagine. The amount of proline, cystine, serine, threonine, valine and tyrosine is also markedly increased by hydrolysis. Little taurine, leucine, methylhistidine or arginine is excreted in the conjugated form. J. B. S.

Insulin, Reversible Dissociation of. F. Fredericq. (*Nature, Lond.*, 1953, **171**, 570.) The sedimentation and diffusion constants of insulin in moderately alkaline solutions were determined respectively by a "Spinco" analytical ultracentrifuge and by the Lamm's scale method. A figure is given showing the effect of *p*H on the sedimentation constant of insulin (corrected to 20° C. and water) at a constant concentration of 0·25 per cent. of protein and ionic strength 0·1. Another figure shows the effect of protein concentration on the sedimentation constant of insulin at *p*H 10·1 and ionic strength 0·1 and 0·2 (buffer potassium chloride-glycine). The sedimentation constant rises when the concentration decreases from 1 to 0·7 per cent. in accordance with the ordinary charge effect, but goes down below this point because the dissociation becomes the overwhelming factor. At both buffer concentrations, the values tend to become identical and point towards a minimum at around 1·2. Similar conclusions can be drawn from diffusion data. The extrapolation of sedimentation and diffusion constants at zero protein concentration gives a molecular weight around 6000 for the insulin submolecule. In neutral or alkaline media, the dissociation is not influenced by the nature of the anions present. A. H. B.

Suxamethonium (Succinylcholine) Iodide. L. E. Tammelin. (*Acta chem. Scand.*, 1953, **7**, 185.) The synthesis of phenylsuccinylcholine from phenylsuccinic acid is described. The latter substance is converted to the corresponding acid chloride which is condensed in dioxan solution with choline chloride to yield phenylsuccinylcholine chloride; the latter is then converted to the crystalline iodide, m.pt. 250° C. (decomp.) by treatment with potassium iodide. Succinylcholine has a relatively short-lived curare-like action, and like *d*-tubocurarine it paralyses the skeletal muscles, the effect with both substances starting with the eye and pharyngeal muscles and ending with the diaphragm. Phenylsuccinylcholine is considerably less powerful than succinylcholine though it is able to produce a severe paralysis of the extremities. The hydrolysis of both succinylcholine and phenylsuccinylcholine has been investigated under varying *p*H and temperature conditions using Hestrin's method for the photometric estimation of choline ester. The hydrolysis is catalysed by the esterase in cobra venom. The hydrolysis of the two substances by enzyme preparations from various organs of the rabbit has also been studied. The results indicate that succinylcholine is hydrolysed more slowly than acetylcholine, decomposition occurring at much the same rate in blood, liver, voluntary and smooth muscle. Experiments with the electric organ of the electric ray *Torpedo*, which can be regarded as a huge neuromuscular end-plate, suggest that the cholinesterases in end-plates strongly affect the rate of succinylcholine hydrolysis. The relationship between succinyl choline hydrolysis and the number of equivalents of acid liberated in the reaction and the rôle of cholinesterases in changing the clinical picture after injection of succinylcholine is discussed. J. B. S.

Vitamin E in Cod-liver and Other Fish-liver Oils. F. Brown. (*Nature, Lond.*, 1953, **171**, 790.) The tocopherol content of samples of cod-liver oil was determined by the Emmerie-Engel method. Since this is not specific for tocopherols, the following check was carried out. Unsaponifiable material obtained after alkaline hydrolysis in the presence of pyrogallol was freed from carotenoids, vitamin A and sterols and chromatographed on "Vaseline" coated or silicone-impregnated paper using a variety of developing solvents. On spraying with $\alpha\alpha'$-dipyridyl and ferric chloride, a substance having the same R_F value as α-tocopherol was found, and no other substance reducing ferric chloride was detected. The following quantities were found in fish-liver oils: cod, 25·6, 30·2, 32·2, angler fish, 31·9, blue skate, 25·3, haddock, 18·0, ling, 27·2 and turbot, 45·0 mg./100 g. The typical signs of vitamin E deficiency in animals whose diet includes the oils may be due to the presence of the highly unsaturated acids of the oil. G. B.

BIOCHEMICAL ANALYSIS

Adrenaline and Noradrenaline in Suprarenals of Guinea-pigs, Colorimetric and Biological Estimation of. U. S. Von Euler and B. Hökfelt. (*Brit. J. Pharmacol.*, 1953, **8**, 66.) Biological assays of adrenaline and noradrenaline have been compared with the results obtained by the colorimetric method of Euler and Hamburg. The catechol amines were extracted from guinea-pig suprarenals and estimated biologically on the cat's blood pressure and fowl's rectal cæcum. The results obtained by the colorimetric method were in good agreement with the biological estimations, as distinct from the results of Shepherd and West, who previously had reported that the colorimetric method gave erroneous results. G. F. S.

Amino-acids, Chromatography of. A. L. Levy and D. Chung. (*Analyt. Chem.*, 1953, **25**, 396.) A new two-dimensional system for the qualitative analysis of amino-acid mixtures is recorded. Butanol-acetic acid-water (4:1:5) proved to be the most generally satisfactory solvent for one-dimensional papers; however chromatograms showed that the pairs threonine-glutamic acid, methionine-valine, *iso*leucine-phenylalanine, and to a lesser extent glycine-serine, were inseparable in butanol-acetic acid. To separate these four pairs of amino-acids the aqueous buffer procedure of McFarren (*Analyt. Chem.*, 1951, **23**, 168) was modified by the omission of potassium chloride and by the use of suitable mixtures of phenol and *m*-cresol. The most satisfactory two-dimensional system was found to be butanol-acetic acid-water, followed by 1:1 *m*-cresol-phenol, *p*H 9·3 borate buffer, run on Whatman No. 52 paper (an acid-washed paper, with considerable wet strength). The experimental procedures for protein hydrolysis and for the preparation of the two-dimensional chromatogram are given. R. E. S.

Caffeine, Fate in Man and Evaluation in Biological Material. J. Axelrod and J. Reichenthal. (*J. Pharmacol.*, 1953, **107**, 519.) A simple and sensitive method is described for the estimation of caffeine in biological materials and the results of a study of its absorption, excretion, distribution and rate of biotransformation in man. The estimation is based on the method of Ishler *et al.* 1 to 5 ml. of plasma is shaken with benzene and sodium chloride and an aliquot quantity of the benzene phase removed and shaken with 5N hydrochloric acid. An aliquot quantity of the acid extract is transferred to a quartz cuvet and the optical density at 273 mμ determined in a spectrophotometer. Comparisons are made with standard solutions of caffeine. Urine and tissue homogenates may also be extracted by a slightly modified technique. Experiments in man showed that caffeine was rapidly and completely absorbed from the gastro-intestinal tract, but only 1 per cent. was excreted in the urine, indicating almost complete biotransformation. The rate of biotransformation was 15 per cent. per hour. In dogs, caffeine was distributed in various tissues in proportion to their water content. There was a considerable accumulation of caffeine in the body after repeated coffee drinking. G. F. S.

PHARMACY

DISPENSING

Invert Sugar Solution for Injection, Preparation of. J. C. de Jong and W. A. Moeys. (*Pharm. Weekbl.*, 1953, **88**, 317.) As a source of energy, invert sugar given by injection is much more satisfactory than glucose. It is quicker in action, there is less danger of thrombophlebitis, and the excretion in the urine is much lower. A suitable, and colourless, solution may be prepared as follows. Dissolve 950 g. of sucrose in 4·5 l. of freshly distilled water and add 5 ml. of N hydrochloric acid. Heat for 1 hour at 100° C., cool off and adjust to a *p*H of about 6. Shake with asbestos, filter, fill into infusion bottles, and sterilise for 1 hour at 100° C. G. M.

GALENICAL PHARMACY

Liquorice; Effect of Trace-metal Content on Colour of Liquid Extract. S. Collett. (*Mfg. Chem.*, 1953, **24**, 124.) 3 samples of liquid extract of liquorice, all from the same batch, and each having a volume of about 3 fl. oz. were taken. One was kept as a control sample; a second was kept in contact

with a few fragments of granulated zinc for 2 months, the container being shaken once a day and the cork momentarily removed; and the third kept for the same period in contact with a few pieces of metallic lead, the container being shaken daily. The samples were then centrifuged and each portion examined with a view to determining if any metal had been taken up, if any change had occurred in the colour value owing to the presence of the metal, and what effect the metal had on the glycyrrhizin content of the extract. A 1–20 dilution of each sample was prepared, using 25 per cent. ethanol as diluent, and the colour assessed by determining absorption in the E.E.L. photoelectric colorimeter. It was shown that contamination with lead results in an increase and with zinc a decrease in the colour value of liquid extract of liquorice; contamination with lead, moreover, accentuates the red component. It seems probable that the metals exert their effect by combining with the glycyrrhizin; lead, by forming a highly-coloured salt, and zinc by precipitating the glycyrrhizin as the zinc salt. S. L. W.

NOTES AND FORMULÆ

Bacitracin, Stability of Solutions of. V. Würtzen. (*Farm. Tid. Kbh.*, 1953, **63**, 280.) A solution of bacitracin of 2000 units/ml. retains a satisfactory activity after 3 weeks storage at ordinary temperature, and is reduced to half strength in about 50 days. It may be kept for 3 months in a refrigerator. The addition of a buffer salt (pH = 6·0) decreases the stability. G. M.

Disulfiram (Antabuse). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1953, **151**, 1408.) Disulfiram is bis(diethylthiocarbamyl) disulphide, and occurs as a white to light grey, odourless, almost tasteless powder, m.pt. 72° to 73° C., soluble in about 25 parts of ethanol, 14 parts of ether, and 5000 parts of water. When dissolved in methanol and treated with a methanolic solution of cupric chloride, a green colour develops, which changes rapidly from yellow-green to deep green. It contains not more than 400 p.p.m. of heavy metals and yields nor more than 0·5 per cent of ash. The loss in weight on drying in a vacuum oven at 60° C. for 4 hours does not exceed 0·5 per cent. Disulfiram contains 9·21 to 9·68 per cent. of nitrogen (determined by semimicro Kjeldahl), and 42·2 to 44·3 per cent. of sulphur. The sulphur is determined by fusing the disulfiram in a Parr bomb with sodium peroxide, potassium nitrate, sucrose, and potassium chlorate, and estimating the resulting sulphate by precipitation with barium chloride. Disulfiram is used in the treatment of alcoholism.

G. R. K.

Metharbital (Gemonil). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1953, **151**, 1000.) Metharbital is 5:5-diethyl-l-methylbarbituric acid and occurs as a white, crystalline, faintly aromatic powder, m.pt. 151° to 155° C., soluble in about 23 parts of ethanol, 38 parts of ether, and 830 parts of water; a saturated solution has pH 5·6 to 5·7. A solution in sodium hydroxide yields a white precipitate, soluble in ammonia, with silver nitrate and with mercuric chloride. A 0·001 per cent. solution in 0·1 N sodium hydroxide exhibits an ultra-violet absorption maximum at about 244 mμ ($E_{1\text{ cm.}}^{1\text{ per cent.}}$, abour 440). Metharbital contains not more than 400 p.p.m. of sulphate and yields not more than 0·1 per cent. of ash. The loss in weight on drying at 105° C. for 4 hours does not exceed 1·0 per cent. It contains 13·85 to 14·42 per cent. of nitrogen, determined by semimicro Kjeldahl, and 95·0 to 105·0 per cent. of metharbital when assayed by measuring the absorption at 244 mμ of a 0·001 per cent. solution in 0·1 N sodium hydroxide. Metharbital is used in the treatment of epilepsy. G. R. K.

Sulfisoxazole (Gantrisin). (*New and Nonofficial Remedies, J. Amer. med. Ass.*, 1953, **151**, 739.) Sulfisoxazole is N^1-3:4-dimethyl-5-isoxazolylsulphanilamide and occurs as a white, odourless, tasteless, crystalline powder, m.pt. 192° to 195° C., freely soluble in diluted hydrochloric acid and soluble in ethanol. A solution in sodium hydroxide becomes green and yields a greenish-blue precipitate on the addition of copper sulphate (distinction from sulphathiazole). A cold solution in diluted hydrochloric acid becomes yellow on the addition of sodium nitrite (distinction from sulphanilamide) and yields an orange-red precipitate on the subsequent addition of β-naphthol in alkaline solution. Sulfisoxazole loses not more than 0·5 per cent. of its weight when dried at 105° C. for 4 hours, and yields not more than 0·1 per cent. of sulphated ash; it complies with limit tests for chloride and heavy metals. It contains 95·0 to 105·0 per cent. of sulfisoxazole when assayed by solution in excess sodium hydroxide and titration with sulphuric acid, and 97·0 to 103·0 per cent. when assayed by measuring the absorption at 5400 Å. of a solution prepared by dissolving the sample in sodium hydroxide, diazotising, and treating with ammonium sulphamate and *N*-(1-naphthyl)ethylenediamine dihydrochloride.

G. R. K.

PHARMACOGNOSY

***Datura innoxia*, Alkaloids of.** W. C. Evans and M. W. Partridge. (*Nature, Lond.*, 1953, **171**, 656.) 3 samples of *Datura innoxia* Miller, one from the 1950 crop and the others from the 1952 crop, were analysed by the method described previously (*J. Pharm. Pharmacol.*, 1949, **1**, 593; 1952, **4**, 769), and the presence of hyoscine (0·24, 0·30 and 0·37 per cent.) was confirmed. The fraction at first considered to be hyoscyamine (0·035, 0·062 and 0·073 per cent.) afforded a picrate of melting point considerably below that of hyoscyamine picrate; fractional crystallisation of this picrate from water gave two picrates, one which was hyoscyamine picrate and the other which proved to be metaloidine picrate. The simplest conclusions with respect to *D. innoxia* are that the main site of alkaloidal syntheses are the roots for hyoscine and the aerial parts for hyoscyamine.

R. E. S.

PHARMACOLOGY AND THERAPEUTICS

Antibiotics, Actions on Intestinal Absorption. R. Ferrando, J. Bost and Denise Brenot. (*C.R. Acad. Sci. Paris.*, 1953, **232**, 1618.) A comparison of the absorption of casein hydrolysates in isolated segments of the ileum of the anæsthetised rat *in vivo* has shown that aureomycin and procaine penicillin facilitated their absorption. Dilutions of the hydrolysates containing known amounts of nitrogen were introduced for 15 to 25 minutes into the intestinal segment and then removed and the nitrogen depletion measured. Solutions with and without the addition of the antibiotics could be compared in the same segment of ileum.

G. F. S.

***Aspidosperma oblongum*, A.DC., Pharmacology of the Alkaloids of.** J. J. Banerjee and J. J. Lewis. (*Nature, Lond.*, 1953, **171**, 802.) Experiments with a 6 per cent. solution of the alkaloids of *Aspidosperma oblongum* A.DC. in isotonic saline solution at *p*H 6·8 indicate the presence of a potent cardioactive substance which antagonises acetylcholine or is associated with anti-acetylcholinergic substances. The alkaloids antagonise the action of acetylcholine and barium chloride in the isolated rat or rabbit ileum and guinea-pig,

rat or rabbit uterus. They inhibit the action of histamine and acetylcholine in the guinea-pig ileum and adrenaline on the isolated rabbit uterus. Inhibition of the action of acetylcholine in the frog rectus abdominis, curare-like neuromuscular blocking in the frog gastrocnemius-sciatic preparation and graded local anæsthetic action by the frog plexus method have been observed. A small dose (0·06 mg.) reduces the rate and amplitude of contractions in the isolated and *in situ* frog heart, and 0·6 mg. causes irreversible stoppage. In Langendorff heart preparations of the cat, rabbit and guinea-pig, the alkaloids cause reduction in rate and amplitude, followed by reversible auriculo-ventricular block, unmodified by previous atropinisation. The effects of lethal and sub-lethal doses in frogs and mice are described. G. B.

Benzoyltropine and Benzoyl-ψ-tropine, Quaternary Derivatives of, with Anticholinergic and Local Anæsthetic Properties. L. Gyermek. (*Nature, Lond.*, 1953, **171**, 788.) The methyl, ethyl, propyl, butyl and benzyl quaternary derivatives were prepared from benzoyltropine and benzoyl-ψ-tropine and examined for antimuscarine effect (isolated intestine of rabbit and guinea-pig), ganglion-blocking action (nictitating membrane of cat), curare-like effect in frog, infiltration anæsthesia (skin of rat abdomen) and conduction anæsthesia (frog plexus). Special study was given to the relationship between stereochemical structure and pharmacological effect. The aralkyl and higher alkyl quaternary derivatives showed activities similar to the tertiary compounds but the methyl derivatives were relatively inactive. Therefore the generally accepted view that the local anæsthetic action of amines is confined to secondary or tertiary compounds is not valid. G. B.

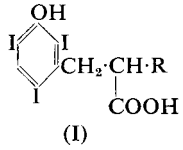
Benzylcholine, Methacholine and Acetylcholine, Correlation of Pharmacological Responses to Activity of Cholinesterases. T. Koppanyi, A. G. Karczmar and G. C. Sheatz. (*J. Pharmacol.*, 1953, **107**, 482.) This paper reports the pharmacological responses to cholinergic agents before and after the administration of anticholinesterase drugs in rabbits and dogs. They were initiated to elucidate the rôle of "effector" and "transport" cholinesterases in controlling the responses to cholinergic agents. Advantage was taken of the different pharmacological properties of acetylcholine, methacholine and benzoylcholine in studying this problem. Methacholine is hydrolysed *in vitro* only by true or acetylcholinesterase while benzylcholine is only affected by pseudocholinesterase. *In vivo* benzoylcholine causes only limited vasodepression and stimulation of gastro-intestinal motility and with doses larger than 1 mg. nicotinic actions entirely predominate, vasopressor effects due to stimulation of the sympathetic ganglia, and fasciculations and paralysis of voluntary muscles. The vasopressor effects were abolished by ganglionic blocking agents but not by adrenalectomy. Methacholine had only vasodepressor effects, largely due to vasodilation, which were blocked by atropine. It had no nicotinic actions. Acetylcholine showed predominantly muscarinic effects and atropinisation and employment of large doses were necessary for nicotinic effects. Di*iso*propyl phosphorofluoridate and other cholinesterase inhibitors converted the muscarinic effects of small doses of benzoylcholine to nicotinic effects and potentiated the nicotinic effects of larger doses. The muscarinic effects of methacholine were not potentiated by di*iso*propyl phosphorofluoridate. The degree of benzoylcholine potentiation by di*iso*propyl phosphorofluoridate followed closely the inhibition of cholinesterase activity in the blood and maximum potentiation was obtained with doses of di*iso*propyl phosphorofluoridate which completely inhibited cholinesterase but not acetylcholinesterase,

while responses to acetylcholine were first potentiated by doses of di*iso*propyl phosphorofluoridate which inhibited cholinesterase and acetylcholinesterase. Blood cholinesterase was shown to be the factor controlling the fate and the intensity of the pharmacological responses of benzoylcholine. This investigation has shown the importance of cholinesterase and acetylcholinesterase outside the neuroeffectors ("transport" cholinesterases) in controlling the fate of certain cholinergic agents prior to their reaching the neuro-effectors.

G. F. S.

***dl*-, *l*-, and *d*-Berbine, Determination of the Sympatholytic Activity of.** R. Hamet. (*C.R.Acad. Sci. Paris*, 1953, **256**, 1616.) Berbine, a synthetic compound which differs from yohimbine by the absence of both the OH group and the carboxymethoxyl from the pyrrol nucleus, has a sympatholytic action in the dog. A comparison of the *dl*-, *l*- and *d*-isomers showed the hypertensive effects of adrenaline to be abolished and ultimately reversed by 4·44 mg./kg. of the *dl*-, 3·24 mg./kg. of the *l*-, and 37·86 mg./kg. of the *d*- isomers, compared with 4·52 mg./kg. of the natural alkaloid corynanthine. 1 to 2 mg./kg. of berbine reduced the arterial pressure, while 2 to 4 mg./kg. increased a little the frequency and amplitude of the respiratory movements. G. F. S.

Cholecystographic Agents. D. Papa, H. F. Ginsberg, I. Lederman and V. DeCamp. (*J. Amer. chem. Soc.*, 1953, **75**, 1107.) The preparation and preliminary pharmacological evaluation of a new series of iodinated compounds of general formula (I) in which R = H, $-CH_3$, $-C_2H_5$, n-C_3H_7, n-C_4H_9, is described. Also compounds were prepared in which the phenolic –OH of compound I was replaced by –H or –I and R = $-C_2H_5$ (compounds II and III respectively). The compounds (I, R = C_2H_5 and R = n-C_3H_7) were outstanding in the quality of gall-bladder contrast and complete absence of side effects and residual medium in the colon. Compounds II and III were ineffective.



(I)

A. H. B.

Hydantoins as Anticonvulsants; 5-R-5-(2-Thienyl)-hydantoins. J. J. Spurlock. (*J. Amer. chem. Soc.*, 1953, **75**, 1115.) The synthesis of nineteen 5-substituted-5-(2-thienyl)-hydantoins and nine 3-alkyl- or 1:3-dialkyl-5-substituted-5-(2-thienyl)-hydantoins is described. The results of the anticonvulsant tests using the electroshock method with cats, with 5:5-diphenylhydantoin as standard are recorded. Only a few of the compounds were as active as 5:5-diphenylhydantoin.

A. H. B.

Isoniazid, Absorption, Distribution and Excretion of. B. Rubin and J. C. Burke. (*J. Pharmacol.*, 1953, **107**, 219.) After single or repeated oral doses of isoniazid in dogs it was found that peak plasma levels of the drug usually occurred within 30 minutes and only occasionally as late as 2 hours. The average 30-minute plasma concentrations were strictly proportional to and, when expressed as μg./ml., nearly identical with the oral dose in mg./kg. of body weight. The average plasma disappearance rate of the drug was 19 per cent. per hour. There was found to be relatively uniform distribution of isoniazid in plasma, spinal fluid, stomach, brain, liver, lung, spleen and intestine after the attainment of peak plasma levels; highest drug concentrations were obtained in the kidney after the first few hours, and lowest concentrations in the heart and omental fat. There was no chemically detectable cumulation of the drug in body

fluids or tissues with chronic doses tolerated for 2 to 8 months. Nearly one-half to four-fifths of an oral dose is excreted in the urine in 24 hours as unchanged drug plus *iso*nicotinic acid. With the evidence available to date it appears that absorption, distribution and excretion characteristics in the dog are similar to those in man. S. L. W.

Liquorice and Cortisone in Addison's and Simmond's Disease. J. G. G. Borst, S. P. ten Holt, L. A. Vries and J. A. Molhuysen. (*Lancet*, 1953, **264**, 657.) A synergistic effect between liquorice and cortisone was found in the treatment of 3 cases of Addison's disease. Liquorice alone did not exhibit the deoxycortone-like effect seen in normal people, but one of the patients, with a short period of suprarenal insufficiency before treatment, reacted well to liquorice at first. The effect of liquorice on the mineral metabolism was partially restored by giving 2·5 mg. of cortisone daily, and completely restored by 10 mg. daily. 2 of the patients were also treated with liquorice and adrenocorticotrophic hormone, but failed to respond, while a patient with Simmonds-Sheehan disease reacted favourably to liquorice and cortisone, liquorice and adrenocorticotrophic hormone and liquorice alone. It is considered possible that the favourable response of some Addisonian patients to liquorice is due to the presence of remnants of funtioning suprarenal tissue. The explanation of the activity of liquorice is discussed. J. R. F.

Liquorice and Glycyrrhetinic Acid, Effects of, on Salt and Water Metabolism. W. I. Card, W. Mitchell, J. A. Strong, N. R. W. Taylor, S. L. Tompsett and J. M. G. Wilson. (*Lancet*, 1953, **264**, 663.) The effects of liquorice and its derivatives on salt and water balance have been investigated. The preparation from crude extract of liquorice, block juice, was found to be capable of producing water, sodium and chloride retention in normal individuals. Similar results were also obtained by using glycyrrhetinic acid, which was also shown to have effects on the weight and electrolytes of an Addisonian patient, resembling those of deoxycortone and cortisone. The survival time of adrenalectomised rats, which is prolonged by deoxycortone, was not, however, lengthened by liquorice and its derivative. The mechanism of action of the liquorice is obscure, but it is suggested that the results are most readily explained on the assumption that its action on the kidney is similar to that of adrenal cortical hormones. J. R. F.

Menadione Sodium Bisulphite and Vitamin K_1, Comparative Effects on the Hypoprothrombinæmia Induced by Dicoumarol. W. G. Bannon, C. A. Owen and N. W. Barker. (*J. Lab. clin. Med.*, 1953, **41**, 393.) The counteracting effects of menadione sodium bisulphite and vitamin K_1 in prothrombin deficiency induced by dicoumarol have been compared in patients. Prothrombin times were determined by the method of Quick *et al.* Comparisons were made within the same patients, in paired patients and in other patients with varying degrees of hypothrombinæmia and dicoumarol administration. Vitamin K_1 was definitely more effective than menadione sodium bisulphite and 250 mg. of vitamin K_1 by mouth nearly always restored the prothrombin time to normal within 24 hours, whatever the height of the prothrombin time or the duration of the dicoumarol therapy. There was no refractoriness to renewed administration of dicoumarol afterwards. G. F. S.

Phenylbutazone, Toxic Effects of. J. C. Leonard. (*Brit. med.* J., 1953, **1**, 1311.) In 1526 patients treated with phenylbutazone, 22 per cent. showed toxic reactions. The most frequent complications were œdema, nausea, vomiting and diarrhœa, although more serious effects such as reactivation of peptic ulcer, hæmatemesis and melæna were observed. Skin rashes were fairly common and jaundice was noted in 6 patients. The effects on the blood included depression of any or all of the cellular elements. There were 11 cases of agranulocytosis and several of granulocytopenia. The incidence of reactions does not seem to be related to dosage, reactions occurring commonly with small doses. A fatal case of aplastic anæmia and one of agranulocytosis are described described in detail. G. B.

***iso*Propylnoradrenaline, Pharmacological Action of.** A. Lindner and C. Stumpf. (*Scientia Pharm.*, 1953, **21**, 1.) A comparative examination of the pharmacological actions of the *laevo* and *dextro* isomers of *iso*propylnoradrenaline showed that the toxicity of the former, for mice, is about 1·3 times that of the latter, when administered perorally or intravenously. With subcutaneous application this proportion is increased to 2·2. In pharmacological activity the *lævo* form is always the more active, the ratio of the activities of the two forms being as follows: broncholytic action, 5·4; blood pressure effect, 11·8; action on heart frequency, 8·7; spasmolytic effect, 7·9. The effect of *l-iso*propylnoradrenaline in lowering blood pressure is especially noticeable, as it is of the same order as that of acetylcholine: 4 μg. produces a mean drop in blood pressure, with chloralosed cats, of about 50 mm. of mercury. G. M.

Quaternary Compounds possessing Lasting Local Anæsthetic Action. K. Nádor, F. Herr, G. Pataky and J. Borsy. (*Nature, Lond.*, 1953, **171**, 788). When tertiary nitrogen compounds with local anæsthetic action are converted into alkyl or aralkyl quaternary derivatives, the resulting compounds show a delayed local anæsthetic effect of long duration. This effect usually develops after 30 minutes but frequently it is delayed 12 to 16 hours. The bromobenzylate of procaine was obtained by reaction of procaine with benzyl bromide in benzene or acetone, care being taken to exclude even traces of humidity. Similarly, amethocaine bromobenzylate was prepared in methanol-ether and cinchocaine bromobenzylate in ethanol-ether. The methyl quaternary derivatives were made in the same way. Generally the methyl derivatives were less active, but some of the benzyl quaternary derivatives were more effective local anæsthetics than the tertiary compounds. G. B.

Suxamethonium, Pharmacology of. G. F. Somers. (*Brit. J. Pharmacol.*, 1953, **8**, 19.) The actions of suxamethonium, a short-acting neuromuscular relaxant, have been studied in the cat. Suxamethonium acted like decamethonium first potentiating the twitches of the soleus and tibialis anticus muscles before producing a brief period of paralysis. Neostigmine potentiated and prolonged the paralysing action. Although closely related chemically to acetylcholine, suxamethonium showed no muscarine-like actions on the parasympathetic nervous system. In large doses it showed nicotine-like actions causing a rise in blood pressure through stimulation of the sympathetic ganglia which was prevented by ganglionic blocking drugs. G. F. S.

(ABSTRACTS *continued on p. 656.*)

HALE-WHITE'S MATERIA MEDICA, PHARMACOLOGY AND THERAPEUTICS, 29th Edition by A. H. Douthwaite. Pp. viii + 512 (including Index). J. and A. Churchill, Ltd. 1952. 20s.

The first edition of this book was published 60 years ago and the present author has been responsible for the 10 editions which have appeared during the past 21 years. It is no mean task to undertake revision of a textbook on a subject which frequently changes and rapidly extends its frontiers. The continued success of this enterprise is well reflected in the appearance of the 29th edition—a fitting tribute to the popularity and usefulness of this type of textbook. In previous editions much information was given about the character and nature of drugs, but in the new edition, this part of the text has been considerably revised and condensed to permit more extensive discussion of the actions and uses of drugs. This pharmacological bias has added greatly to the value of the book, which now includes an account of most of the recent work on the subject. The contents are arranged in three major sections. The first deals with definitions, pharmaceutical matters and general principles of prescribing; the second with substances which are used chiefly for their local action, and in the third section are the substances used chiefly for their general action. This arrangement of the subject-matter is convenient for describing the materia medica but is less well adapted for pharmacological description. For example, it might be anticipated that the analgesics drugs would be described in one section but the only reference in the index leads to page 286 where the antipyretic drugs, acetanilide, phenazone, phenacetin and amidopyrin are discussed, and these are separated from the salicylates by a chapter on cinchona and the drugs used in the treatment of malaria, whilst the more potent analgesics are discussed in the section on opiates on pages 199 to 211. The disadvantages of this arrangement are also apparent in the description of veratrine under local anæsthetic drugs; *iso*nicotinyl hydrazide in the vitamin chapter is pharmacologically isolated from the other tuberculostatic drugs described under antibiotics. A method of presenting the subject in a completely logical fashion however has not yet been described, and this is therefore only a minor criticism of a textbook which continues to maintain its reputation for its clarity of exposition.

ANDREW WILSON.

(ABSTRACTS *continued from p. 653.*)

Tetanus Toxoid, Rapid Specific Preventive Action of. M. Raynaud and E. A. Wright. (*Nature, Lond.*, 1953, **171**, 797.) The injection of 500 Lf doses of tetanus toxoid into mice completely prevented local tetanus and death from 1 MLD of tetanus toxin given subcutaneously 24 hours later. Toxoid from another laboratory was used, administered intravenously to ensure that the protection was not a local effect or dependent on the toxin and toxoid being made from the same strain of organism. Protection lasted only a short time, no protection being observed 6 days after injection of the toxoid. Since no antibodies have been detected in the first days after the administration of toxoid, the most probable explanation is that the toxoid acts either by prior blocking of the hypothetical receptor substance in the central nervous system or by competitive inhibition of the action of the toxin. G. B.