ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Acetylsalicylic Acid, Phenacetin, Caffeine and Bromvaletone in Admixture, Determination of. M. Langejan and J. A. C. van Pinxteren. (*Pharm. Weekbl.*, 1951, **86**, 508.) 1·5 g. of the mixture is dissolved in 5 ml. of ethanol and titrated with 0·5N sodium hydroxide, with phenolphthalein as indicator. This gives the content of acetylsalicylic acid. The titrated liquid is shaken out with 3 × 50 ml. of chloroform, the aqueous phase being rejected. The chloroformic solution, after washing with a little water, is evaporated, and the residue is evaporated with 5 ml. of ethanol. The residue is refluxed for 30 minutes with 10 ml. of 4 N sulphuric acid, cooled, neutralised with sodium bicarbonate (litmus paper) and shaken out with 3 × 25 ml. of chloroform, the chloroform extracts being washed with a little water. The aqueous solution is warmed to remove chloroform, treated with 10 ml. of 4 N sodium hydroxide and boiled down to about 25 ml. After cooling 35 ml. of water, 10 ml. of N potassium cyanide solution and 40 ml. of hydrochloric acid (38 per cent.) are added; 0·1N potassium bromate solution is added drop by drop until there is no yellow colour, followed immediately by a mixture of 20 ml. of phenol solution (8 per cent.), 10 ml. of N potassium bromide solution and 1 g. of potassium iodide. The iodine is then titrated with 0·1N thiosulphate. The number of ml. used × $\frac{22.3}{3}$ represents mg. of bromvaletone. The chloroformic solution is concentrated to 10 ml. and shaken out with 2 × 5 ml. of 4 N sulphuric acid, then with 5 ml. of water. The chloroformic solution is evaporated, the residue being re-evaporated twice with a little ether. The residue is dried at 100° C., and represents the caffeine (anhydrous). The aqueous solution from the last chloroform extraction is diluted with 500 ml. of water and treated with 30 ml. of concentrated hydrochloric acid and 25 ml. of 25 per cent. solution of potassium bromide. The mixture is cooled to about 4° C. and titrated with 0·1M sodium nitrite until, after standing for 5 minutes, the mixture turns starch iodide paper blue. The rate of titration should be, for a titration of up to 4 ml., 2 ml. per minute; for 4 to 8 ml., 1 ml. per minute; and for 8 to 14 ml., 0·5 ml. per minute. The sodium nitrite is standardised against pure phenacetin (1 ml. = 17·9 mg. of phenacetin). G. M.

Alcohols, Paper Partition Chromatography of, using the Potassium Xanthogenates. Tatsuo Kariyone and Yohei Hashimoto. (*Nature, Lond.* 1951, **168**, 511.) Alcohols are converted to the corresponding xanthogenates by treatment with pure carbon disulphide and pure powdered potassium hydroxide and the residual solid product dissolved in water. The aqueous xanthogenate solution is submitted to paper partition chromatography by an ascending method using alkaline butanol as the developing solvent. The xanthogenate spots are detected either by their dark brown luminescence under ultra-violet light or by spraying with Grote's reagent. With solid alcohols the xanthogenates are dissolved in formamide to give the corresponding ammonium xanthogenate. The method has been successfully applied to the detection of small quantities of methanol (0·1 per cent.) in samples of ethanol. Both spots can be identified under ultra-violet light and give colours with Grote's reagent, both of which are

yellow initially, though that due to ethanol becomes brown within half an hour. R_F values for the following alcohol xanthogenates are given:—methanol 0·23, ethanol 0·35, *iso*propanol 0·44, *iso*butanol 0·54, *n*-butanol 0·55, *iso*amyl alcohol 0·62, benzyl alcohol 0·45, octyl alcohol 0·15, *cyclo*hexanol 0·04, ethylene-chlorhydrin 0·91. J. B. S.

Alkaloids in Tissues, Colorimetric Determination by Methyl Orange. A. O. Gettler and I. Sunshine. (*Anal. Chem.*, 1951, **23**, 779.) The authors have modified the methyl orange reaction developed by Brodie and Udenfriend (*J. biol. Chem.*, **158**, 705) to apply it to the quantitative determination of alkaloids in human organs. The main steps in the method were the extraction of alkaloids from tissue with boiling acidified water; the extraction of these alkaloids from the filtered aqueous solution by means of chloroform; and the formation of a chloroform-soluble coloured compound of the alkaloids with methyl orange. Experimental details are given and results are quoted for the analysis of tissues containing known amounts of various alkaloids. The recoveries of most of the alkaloids and other organic compounds including antihistamines were good. Low recoveries were experienced with cocaine probably due to decomposition during the steam distillation, since aqueous solutions of cocaine that were not heated gave good recoveries. Pontocaine and nupercaine were not satisfactorily estimated by the process and experiments with morphine gave poor results. R. E. S.

Alkaloids, Precipitation of, in Ethereal Solution. H. Wachsmuth. (*J. Pharm. Belg.*, 1951, **6**, 86.) The precipitation of alkaloids by silicotungstic acid in aqueous solution is a reaction of low sensitivity, and the composition of the products is variable. On the other hand, by precipitation in ether the sensitivity is high and the precipitate has a constant composition corresponding to 1 molecule of the acid to 3 of alkaloid. The alkaloid, if a base, is dissolved in ether and precipitated with an ethereal or ether-ethanolic solution of the acid. Alkaloidal salts are dissolved first in ethanol. In any case the final concentration of ethanol should be about 12 per cent. The precipitate is washed with ether containing 20 per cent. of ethanol, under slight suction, and dried at 50° C. The results are in general slightly high. As an alternative the alkaloid may be determined by difference iodimetrically, a portion of the filtrate being evaporated to dryness, taken up in water, and treated with iodide-iodate, the free iodine being then titrated with thiosulphate. Results obtained by this latter method are somewhat higher. The sensitivity of the precipitation is high, opalescence being visible at a dilution of 1 in 2 millions. G. M.

***p*-Aminosalicylic Acid, Detection of Impurities in.** O. E. Neufeld. (*Med. J. Austral.*, 1951, **1**, 727.) Several tests are given for the detection of impurities in commercial samples of *p*-aminosalicylic acid. Readily carbonisable substances are tested for by dissolving 10 mg. sample in 2 ml. cold sulphuric acid when no more than a light yellow colour is produced without a bluish fluorescence. The colour produced by a 3 per cent. solution with 5 per cent. ferric ammonium sulphate solution is used for comparison purposes. Ether-soluble matter should not exceed 0·05 per cent. The melting-point is regarded as unreliable, but a limit test for *m*-aminophenol (less than 0·1 per cent.) is given, based on the fact that *p*-aminosalicylic acid as an acid forms an ether-insoluble salt with sodium bicarbonate, whereas meta-aminophenol at this *p*H (7·5 to 7·8) remains the free phenol and can be extracted by an organic solvent like ether. R. E. S.

Antihistamine Preparations, Spectrophotometric Assay of. D. Banes. (*J. Assoc. off. agric. Chem. Wash.*, 1951, **34**, 703.) The antihistaminic compounds studied included thonzylamine, pyranisamine, tripelennamine, and methapyrilene of the ethylenediamine group. Substances examined as being likely to interfere with the estimation were ephedrine sulphate, amphetamine sulphate, naphazoline hydrochloride, benzocaine, butacaine sulphate, dibucaine hydrochloride, procaine hydrochloride, aminophylline, caffeine, phenacetin, and acetylsalicylic acid. The ultra-violet absorption spectra of the various antihistaminics were determined in 0·1N sulphuric acid; the results are given in the table.

Antihistamine salt	Distinctive $\lambda_{max.}$ mμ	Extinction coefficient $E^{1\ per\ cent.}_{1\ cm.}$
Thonzylamine hydrochloride	313	104
Methapyrilene hydrochloride	315	269
Pyranisamine maleate	314	196
Tripelennamine hydrochloride	314	274
Prophenpyridamine maleate	265	212
Chloroprophenpyridamine maleate	264	219
Doxylamine succinate	262	227
Diphenhydramine hydrochloride	258	16·5

Various procedures are given for the isolation of the antihistamines prior to spectrophotometric estimation, involving four variations of immiscible solvent extraction. In addition to solvent extraction, chromatographic methods were necessary for some of the compounds examined; a 1·0M solution of monobasic potassium phosphate adsorbed on celite was found to constitute a convenient immobile phase for the isolation of prophenpyridamine, chloroprophenpyridamine, doxylamine, or pyranisamine, when chloroform was used as the mobile solvent. Diphenhydramine was quantitatively split into a neutral fragment and an amine fragment by heating with moderately strong acid, the neutral product being isolated from basic substances and compared spectrophotometrically with standards for assay purposes; the procedure permitted an accurate estimation of diphenhydramine in the presence of ephedrine, amphetamine, naphazoline, aminophylline, caffeine, phenacetin, acetylsalicylic acid, benzocaine, or dibucaine. Recoveries using the suggested procedures ranged from 96 to 103 per cent. R. E. S.

Ascaridol, Determination of. H. Bohme and K. van Emster. (*Arch. Pharm. Berl.*, 1951, **284**, 171.) The reagent is the leuco base of 2·6-dichlorphenolindophenol, prepared by reduction of the latter with ascorbic acid. The base should give a practically colourless solution in alcohol. For the determination, 10 to 12 mg. of the sample is mixed with 1·00 ml. of pure toluene: 0·3 to 0·5 ml. of this solution is treated with 0·2 ml. of 1·5 per cent. solution of the leuco base in absolute alcohol, and 5 ml. of toluene containing 5 per cent. of acetic acid, and heated on the water-bath for 20 minutes by the side of a blank test. After cooling, the volumes are made up to 10 ml. with toluene-acetic acid and the extinction is determined using filter S53. It is important that the solvents should be pure—the extinction coefficient of the blank should not be greater than 0·1. G. M.

Barbiturates, Cobalt Reaction for Identification of. H. Gomahr and H. Kresbach. (*Scientia Pharm.*, 1951, **3**, 148.) Cobalt compounds of barbitone and narconumal were prepared in the crystalline form: in both cases the compounds contained 2 molecules of barbituric acid to 1 of cobalt. Both of these cobalt compounds gave a compound with pyridine. The authors conclude that

the compound is a complex in which the barbituric acid is bound to the cobalt by main valencies, and the basic component is linked to the first co-ordination sphere. The exchange of these bonds is the cause of the change of colour observed when using different bases. The necessity for using an optimum proportion for the reaction mixture is explained by an excess of inorganic base giving basic cobalt salts, while excess of organic base forms compounds of the type of dipyridine cobaltochloride. It is thus necessary to use a reaction mixture corresponding to the stoichiometric composition of the complex, i.e., cobalt (base)n (barbiturate)$_2$. The difference in the behaviour of N- and C-substituted derivatives is that in the latter two co-ordinative valencies of the cobalt are already saturated on the imino groups of the barbituric acid in the form of an inner complex salt. These views do not agree with those expressed by other workers. It is concluded that, in spite of the value of the method for identification purposes, it is for structural reasons unsatisfactory as a quantitative test, especially with N-substituted compounds, while qualitatively it is not reliable for very small quantities of the di-imide barbiturates. G. M.

Barbiturates, Copper Reaction for Detection of. H. Gomarh and H. Kresbach. (*Scientia Pharm.*, 1951, **19**, 154.) The reagents used are a 1 per cent. aqueous solution of copper sulphate, and a mixture of 1 volume of pyridine with 9 volumes of chloroform. A few mg. of the substance is dissolved in 1 ml. of the solvent and treated with 1 ml. of copper sulphate solution; barbiturates give a violet colour in the chloroform solution; thiobarbiturates a green one. The colour is stable for several hours. The barbituric acid may be recovered by adding a little ammonia and shaking out into the aqueous layer. The reaction may be applied directly to sodium salts, and to tablets. Hydantoins give a blue colour under these conditions, while carbamides, urethanes, sulphones remain colourless. While caffeine and theobromine give no colour, blue is given by caffeine sodium benzoate, and green by caffeine sodium salicylate, theobromine sodium salicylate, theophylline sodium salicylate, methyl- and propylthiouracil, and phenylcinchoninic acid. G. M.

Barbiturates, Identification of, by X-ray Analysis. T. Y. Huang. (*Acta Pharm. Internat.*, 1951, **2**, 443.) X-ray diffraction patterns may be used for the micro-identification of crystalline barbiturates. Data are recorded for allobarbitone, hexobarbitone, and hexemal, and for 4 different modifications of phenobarbitone. G. M.

Benzocaine, Cocaine and other Local Anaesthetics, Chromatographic Separation of. F. Jaminet. (*J. Pharm. Belg.*, 1951, **6**, 81.) Benzocaine, amylocaine, cocaine, amethocaine and procaine may be separated by paper chromatography. About 20 to 50 µg. of the substance, in the form of hydrochloride, is used, and the solvent is prepared by shaking 50 ml. of *iso*butanol with 7·5 ml. of concentrated hydrochloric acid and 13·5 ml. of distilled water. After separation into two phases, the upper one is used as solvent and the lower one for saturating the atmosphere. The chromatogram may be observed in screened ultra-violet light (procaine and benzocaine), or developed with Dragendorff's reagent (with which benzocaine does not react), or by spraying the paper first with a solution of sodium nitrite in acetic acid, then with an ammoniacal solution of α-naphthol. G. M.

Cardiac Glycosides, Colour Reactions with Antimony Trichloride. F. Jaminet. (*J. pharm. Belg.*, 1951, **6**, 90.) Colour reactions with antimony trichloride may be used for the determination of various heterosides and their genins. *Characterisation of digitoxin and gitoxin.* About 3 mg. of the material is treated with

1 ml. of a solution of 50 per cent. of antimony chloride in acetic anhydride. After solution is complete, the mixture is warmed for exactly 3 minutes at 75° C., then cooled quickly. The resulting solution shows two maxima at 490 and 590 mμ due respectively to digitoxin and gitoxin. *Determination of ouabain and k-strophanthoside.* From 0·5 to 2 mg. of the material is treated as above, the solution being finally diluted with 5 ml. of acetic anhydride. The absorption is determined at 490 mμ in a 1 cm. cell. It is important to adhere exactly to the prescribed conditions in both cases. It may be noted that the colour obtained with ouabain and strophanthoside increases with the time of heating, that with gitoxin decreases. *Fluorimetric determination of ouabain.* The sample (0·1 to 1·4 mg.) is treated as before, the final solution being diluted with 10 ml. of acetic anhydride. The fluorescence is compared against a standard prepared with 2 mg. of ouabain, using filters U.V. and P.C. and a Coleman spectrophotometer. G. M.

Cinchona Alkaloids, Separation by Paper Partition Chromatography. D. J. Lussman, E. R. Kirch and G. L. Webster. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 368.) A method for the separation of the major alkaloids of cinchona by means of paper partition chromatography, using *cyclo*hexanol or *cyclo*hexanone with hydrochloric acid as the solvent mixture is described. The position of the individual alkaloids upon the chromatogram was identified by means of R_F values determined with pure samples, coupled with the observation of fluorescence in the cases of quinine and quinidine and the development of a coloured compound by cinchonidine and cinchonine when the chromatogram was treated with a potassium iodoplatinate reagent. The most efficient separations were obtained using *cyclo*hexanol saturated with distilled water as solvent, but more reproducible results were obtained when the *cyclo*hexanol was saturated with dilute acid. *cyclo*Hexanone gave a quicker development of the chromatogram than *cyclo*hexanol, but the efficiency of the separation was greater using the latter solvent. Using this method as little as 3 μg. of quinine or quinidine, 25 μg. of cinchonidine, and 100 μg. of cinchonine may be detected. A. H. B.

Digitalis, Chemical Assay of. M. Langejan. (*Pharm. Weekbl.*, 1951, **86**, 593.) As a preliminary to working out a chemical process for the assay of digitalis, a study was made of digitoxin. This gives on complete hydrolysis 1 molecule of genin and 3 of digitoxose. A semi-micro titrimetric method is given for the latter, and also a colorimetric one based on the reaction of Bial. By modifying this reaction it has been made more specific for digitoxose: 1 ml. of the digitoxose solution is mixed with 2 ml. of Bial's reagent, and the mixture is heated for exactly 1 minute in a water-bath, and cooled quickly. The liquid is transferred to a 25-ml. measuring flask, washed in with 5 ml. of 25 per cent. hydrochloric acid, then made up to the mark with water. The extinction is measured at 620 mμ. This reaction can be carried out without interference from relatively large quantities of other sugars, while digitoxose in glycosides may be determined without previous hydrolysis. Free and combined digitoxose may be distinguished by shaking the latter into chloroform. Digitoxin is not easily hydrolysed, and it was not found possible to hydrolyse it completely without decomposition of the sugar. The aglycone content (free and combined) may be determined colorimetrically using 3 : 5-dinitrobenzoic acid by the method of Kedde. The extinction coefficient in this reaction is increased after boiling with acid. Treatment of the glycosides or aglycones with alkali decreases this extinction, indicating the production of alterations in the molecule. G. M.

Formaldehyde, Acidimetric Determination of. W. Krause. (*Pharm. Zentralh.*, 1951, **90**, 218.) About 2 g. of formaldehyde solution is diluted with 50 ml. of water and treated with 4 drops of a mixture of equal parts of 0·1 per cent. ethanolic solution of dimethyl yellow and 0·1 per cent. ethanolic solution of methylene blue. The mixture is neutralised to a grey colour, and a neutralised solution of 2·5 g. of hydroxylamine hydrochloride in 50 ml. of water is added. After standing in a closed flask for 1 hour, the mixture is titrated with N potassium hydroxide, 1 ml. of which corresponds to 30·03 mg. of formaldehyde. For smaller amounts of formaldehyde, 1 g. of hydroxylamine hydrochloride and 0·1N alkali may be used. G. M.

Glacial Acetic Acid and other Non-Aqueous Solvents, Titrations in. M. E. Auerbach. (*Drug Standards*, 1951, **19**, 127.) The methods used for the titration of weak bases and weak acids in non-aqueous solvents are described, special mention being made of the titration of weakly basic substances by dissolving in glacial acetic acid and titrating with perchloric acid—the so called "acetous-perchloric" titration. A general method for this determination is given. A standard solution which is 0·1N in respect to perchloric acid is prepared by mixing the requisite amount of 70 per cent. perchloric acid with glacial acetic acid, and adding enough acetic anhydride to react with the water necessarily added with the perchloric acid. This solution is standardised by such primary standards as sodium acetate, sodium carbonate, guanidine carbonate, potassium acid phthalate, or sodium salicylate. The standard, or the sample, is dissolved in 25 ml. of acetic acid, 5 drops of 0·1 per cent. crystal violet indicator (in glacial acetic acid) are added, and the solution titrated with the standard acetous-perchloric acid. Crystal violet shows a series of colour changes in the vicinity of *p*H 0 to 1. Before the end-point, the colour becomes blue, at the end-point a definite clear green, and further addition of perchloric acid produces a yellow colour. Amino acids, alkaloids, pyridine, β-naphthylamine, salts such as sodium potassium tartrate, etc., and salts of organic bases such as picrates, citrates, oxalates, etc., could be titrated directly by the above method. Apparently any basic substance stronger than anthranilic acid can be titrated in this way using crystal violet as indicator. The method is particularly useful where the advantages of a good organic solvent, and the ability to titrate a really weak base can be combined. For example, benzocaine ointment may be assayed by dissolving in chloroform, diluting with acetic acid, and titrating directly. A. H. B.

Morphine, New Colour Reaction of. R. Castagnou and C. Paoletti. (*Bull. Soc. Pharm. Bordeaux*, 1951, **89**, 91.) By warming 5 ml. of morphine solution with 0·5 ml. of formaldehyde and 5 drops of ammonia for 30 minutes on the water-bath, a yellow colour is produced. The sensitivity is 1 mg. The reaction is given also by diacetylmorphine, but not by ethylmorphine. G. M.

Nitrites in the Presence of Nitric Acid, Iodopermanganate Determination of. R. C. Brasted. (*Anal. Chem.*, 1951, **23**, 980.) The iodopermangante method consists of adding an aliquot portion of a nitrite solution to a known volume of standard 0·1N permanganate solution acidified with 5N sulphuric acid, and after the completion of the relatively slow reaction between the nitrite and permanganate ions, reducing the excess of permanganate with iodide and titrating the liberated iodine with standard 0·1N thiosulphate. The present investigation studies the effect on nitrite determination when the permanganate solution is acidified with varying amounts of nitric acid instead of sulphuric acid, the effect

of impurities likely to be found in commercial concentrated nitric acid, the means of removing interfering substances from concentrated nitric acid and the effect of titrating thiosulphate solution in highly acidified nitric acid solutions. In the presence of concentrated nitric acid, added to standard permanganate prior to the addition of sodium nitrite solution, accurate results are obtained only if the nitric acid has been pretreated with crystals of sulphamic acid to remove nitrous acid, or freshly boiled. Successful titrations were carried out with less than 0·1 g. of sodium nitrite in 48 g. of concentrated nitric acid, and thus the method is suitable for the determination of nitrites in the presence of nitrates. It is shown that iodine may be titrated with thiosulphate in solutions containing approximately 8M concentration of nitric acid, and that the oxidation of iodide to iodine is insignificant within the time required for a thiosulphate titration. Solid sodium nitrite was found to lose less than 0·1 per cent. of its original weight on heating at 115° C. for 28 hours, and solutions of sodium nitrite were stable over 90 days' standing. A. H. B.

Pepsin Assay, Comparison of Methods. H. J. Anderson, D. M. Findlay, M. Targy, H. H. Wiesman and F. W. Wheeler. (*Drug Standards*, 1951, **19**, 135.) A comparison of the official National Formulary procedure and a "milk curdling method" for the determination of pepsin is made. The "milk curdling method" is applied as follows. The milk for the assay is prepared by siphoning the lower layer from 1 quart of pasteurised whole milk, adding 20 ml. of 0·5N hydrochloric acid, mixing quickly and rapidly filtering. Portions (25 ml.) of this milk are pipetted into 1 inch diameter tubes and the tubes placed in a 30° C. bath for 10 minutes. To each tube is added 1·0 ml. of the pepsin standard, or sample, and the contents quickly mixed by inversion of the tubes. The time of addition is noted to the nearest second. The setting time for each tube is noted and the strength of the sample obtained by comparison with the standard solution. The results by this method check well with those obtained by the National Formulary method, and the former is a quick assay involving no expensive or complicated apparatus. Although other enzymes such as rennin and papain curdle milk, tests are available for distinguishing pepsin from these enzymes. A. H. B.

Sulphonamides, Identification of, by Paper Partition Chromatography. R. Robinson. (*Nature, Lond.* 1951, **168,** 512.) The method of ascending paper chromatography described by Williams and Kirby (*Science*, 1948, **107,** 481) has been used for the identification of sulphonamides in small volumes of biological fluids. Although no single developing solvent is suitable for the separation of all sulphonamides, it is shown that these substances fall into two classes, separable with basic and acidic solvents respectively. R_F values for sulphaguanidine, sulphathiazole, sulphanilamide, sulphadiazine, sulphamethazine, sulphamerazine, sulphacetamide and sulphapyridine have been determined using *n*-butanol-ammonia and *n*-butanol-acetic acid respectively. Solutions (0·01 ml.) containing 5 to 15 μg. of sulphonamide in N/1 hydrochloric acid are spotted on the base line, sprayed with Ehrlich's reagent and developed for 6 to 12 hrs. according to the standard technique. Irrigation with the basic developing solvent caused the spots to fade and a second spraying with Ehrlich's reagent may be necessary. J. B. S.

Vanillin, Assay of. L. K. Sharp. (*Analyst*, 1951, **76**, 215.) An examination has been made of various methods available for the assay of vanillin. It was found that the usual volumetric methods including that of the B.P. 1948 and

the hydroxylamine hydrochloride method gave inconsistent results, mainly because of indefinite end-points in coloured solutions. A modified gravimetric procedure was worked out which gave satisfactory results; the procedure involved precipitation with dinitrophenylhydrazine hydrochloride under carefully specified conditions, the resulting precipitate being filtered off, washed, dried at 110° C., and weighed; other gravimetric procedures gave results that were too high. The ultra-violet absorption curve of pure vanillin in 0·01N aqueous hydrochloric acid was determined; it gave the following points of inflexion, $\lambda_{max.}$ 279 mμ, $E_{max.}{}^{1\ per\ cent.}_{1\ cm.}$ 680·0; $\lambda_{max.}$ 307·5 mμ, $E_{max.}{}^{1\ per\ cent.}_{1\ cm.}$ 596·2; λ_{min} 296 mμ; $E_{min.}{}^{1\ per\ cent.}_{1\ cm.}$ 549·5; the figures so obtained were found to be satisfactory criteria of purity. A satisfactory volumetric oxidation procedure was worked out in which 0·4 g. was dissolved in 20 ml. of N sodium hydroxide, 40 to 60 ml. of solution of hydrogen peroxide (20 vol.) were added, and the mixture was then heated on a water-bath until effervescence ceased and then cooled to room temperature; the excess of sodium hydroxide was titrated with N hydrochloric acid, using phenolphthalein as indicator. A blank determination was necessary and an arbitrary factor of 1 ml. of N hydrochloric acid equivalent to 0·2954 g. of $C_8H_8O_3$ was used. R. E. S.

ORGANIC CHEMISTRY

Alginic Acid, Organic Derivatives of. A. B. Steiner and W. H. McNeely. (*Industr. Engng. Chem.*, 1951, **9**, 2073.) Alginic acid reacts relatively rapidly under mild conditions with alkylene oxides, to give water-soluble esters by esterification of the carboxy group of each anhydro-β-D-mannuronic acid residue in alginic acid, the reaction of greatest importance being with propylene oxide. The propylene oxide penetrates the water-soluble fibres of the acid, reaction taking place slowly at room temperature but considerably faster at 75° C. The reaction rate of alginic acid and propylene oxide being high, 50 to 75 per cent. esterification was readily obtained, resulting mainly in 2-hydroxypropylene alginate. Derivatives of reactions with ethylene oxide and short and long chain oxides were also prepared. The decrease in solubility and mole per cent. esterification which was found to occur with increasing chain length of the oxide was to some extent overcome by the addition of glycerol and by partially neutralising the alginic acid when a minimum mole per cent. esterification of 20 mole per cent. could be obtained. Under these conditions the main course of the reaction takes place through the alginate ion rather than the alginic acid when esterification is very slow. When 10 to 20 mole per cent. of the carboxyl groups are neutralised with a base the reaction becomes strongly exothermic, pH rises rapidly and a practicable esterification is obtained in a few hours. The stability and emulsifying and thickening properties of these derivatives, and their uses in pharmacy, are reviewed. J. R. F.

Nicotinic Acid Derivatives, Paper Chromatography of. E. Kodicek and K. K. Reddi. (*Nature, Lond.* 1951, **168**, 475.) Rapid colour and fluorescent tests are described for the detection of tertiary and quaternary pyridinium compounds. The colour test is based upon the formation of coloured compounds when tertiary pyridine derivatives are treated with cyanogen bromide and a primary aromatic amine. The fluorescence test, which allows of the detection of quaternary nicotinamide derivatives, depends on the formation of a bluish-white fluorescence, when quaternary pyridine compounds with a side chain CO–NHR in the β position are treated with methyl ethyl ketone and ammonia. These tests were applied to various nicotinic acid derivatives after descending chromatograms had been run on Whatman paper with different solvent systems. R_F

values of nicotinamide and coramine are more or less constant irrespective of the solvents used, whereas the more acidic compounds, such as nicotinic acid moved slower in neutral or alkaline solutions than in acid solutions.

J. B. S.

PLANT ANALYSIS

***Ammi Visnaga*, Pyrone Content of.** E. Steinegger. (*Phar. Acta Helvet.*, 1951, **26**, 291.) Ammi visnaga is known to contain a number of γ-pyrones related to furochrome. The fruits of the related *Ammi majus* L. contain α-pyrones, and it appeared possible that such compounds might occur also in *Ammi Visnaga.* In order to decide this point, 125 g. of the fruit from a botanically identified culture (of Swiss growth) was exhausted with ethanol (50 per cent.), and the extract was treated with 20 g. of lead acetate in 60 ml. of water. The precipitate was removed by centrifuging, and a solution of 4 g. of monosodium phosphate was added. After centrifuging and concentrating to half bulk, the solution was shaken out with chloroform. The residual solution was dried in vacuo and boiled out with ether, the ethereal solution being washed with 10N sulphuric acid, then with water, and evaporated: 1·173 g. of semi-fluid greenish residue was obtained (α-pyrone fraction). The sulphuric acid extract was nearly neutralised, and the precipitate obtained taken up in chloroform, giving 2·556 g. of semi-solid greenish residue (γ-pyrone fraction). From the latter a total of 1 g. of crystalline products was obtained. The α-pyrone fraction was treated by chromatography on alumina. Two crystalline products were isolated, melting respectively at 97° C. to 100° C., and 187° C. to 188° C. The latter compound was a coumarin, but not identical with any of the three coumarins which have been isolated from *Ammi majus.* G. M.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Adrenotrophic Hormone, Inactivation by Plasma. M. Reiss, F. E. Badrick, I. D. K. Halkerston and C. Plaice. (*Nature*, 1951, **168**, 206.) The rapid inactivation of an adrenotrophic hormone preparation during incubation with heparinised plasma from rats, rabbits and humans was observed. Parallel incubation of the same preparation with the solvent containing an equivalent amount of heparin showed no measurable inactivation when assayed by the Sayers adrenal ascorbic acid depletion assay. R. E. S.

***p*-Aminobenzoic Acid, Metabolism of.** C. W. Tabor, M. V. Freeman, J. Bailey and P. K. Smith. (*J. Pharmacol.*, 1951, **102**, 98.) *p*-Aminobenzoic acid is rapidly absorbed and excreted. In the rat the concentrations in the plasma are appreciably higher than in brain, muscle and erythrocytes. In normal man very little of the drug is excreted as free *p*-aminobenzoic acid and only a small amount is excreted in the acetylated form. Most of it is excreted either as a conjugation product with glycine or as the glucuronate. A small amount of the glucuronate derivative is also acetylated. S. L. W.

Dextran and its Derivatives, Chemistry of. C. R. Ricketts. (*Proc. Roy. Soc. Med.*, 1951, **44**, 558.) Dextran is a polysaccharide formed during the growth of *Leuconostoc mesenterioides* on a medium containing sucrose. The organism produces an enzyme which polymerises the glucose portion of the sucrose to form dextran; the fructose portion is liberated. The glucose units are joined through 1 : 6-glucoside links, and the main chains so formed have

short branches at frequent intervals, probably joined through 1 : 4-glucoside links. The dextrans produced by the various strains of *Leuconostoc* differ in the extent of the branching. In their native state, the chains are composed of about 200,000 glucose units, with a molecular weight of about 40 million and comparable in size to viruses. These large molecules are unsuitable for use as a plasma substitute and are broken down by partial hydrolysis. The hydrolysate contains molecules varying in molecular weight from 1 million to 10,000. On adding alcohol or acetone and cooling, fractional precipitation occurs and makes possible the selection of a sample containing the desired distribution of molecular size. Viscosity and osmotic pressure measurements indicate that dextran molecules are longer and thinner than proteins of comparable molecular weight. Dextran sulphate is prepared by treating dextran with chlorosulphonic acid and pyridine. The free ester is only stable in solution, but solid sodium, calcium and other salts can readily be prepared. These compounds have an anticoagulant action similar to that of heparin, which is itself the sulphuric acid ester of a polysaccharide. G. R. K.

Folic Acid, Stability in Solutions of the B Group Vitamins. A. R. Biamonte and G. H. Schneller. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 313.) The solubility of folic acid in aqueous media increases with increase in *p*H. At *p*H 5·6 and above it is completely dissolved in a concentration of 1 mg./ml.; in the range *p*H 3 to 4 it is substantially undissolved. The dissolved folic acid at *p*H 6 and above is stable for one year at room temperature; at *p*H 3 to 4 the dissolved folic acid is unstable, although mixtures containing any undissolved folic acid at this *p*H range exhibit good stability and are pharmaceutically practicable and useful. In the presence of riboflavine and, to a lesser extent, aneurine, dissolved folic acid is rapidly decomposed, particularly at *p*H 6 to 7; at lower *p*H levels, where folic acid is considerably undissolved, good stability is exhibited. Pyridoxine and pantothenyl alcohol cause some decomposition of dissolved folic acid but none of undissolved folic acid. Nicotinamide has no effect at any *p*H level. The stability of folic acid in syrup is essentially the same as that in aqueous media, but in mixtures of propylene glycol and syrup, the decomposition is considerably increased. The decomposition of folic acid at low *p*H levels and also in the presence of other vitamins of the B group involves the formation of a pteridine and *p*-aminobenzoylglutamic acid. G. R. K.

Malt Extract, Fermentation of. M. P. English. (*Nature, Lond.* 1951, **168**, 391.) An investigation of a sample of fermented malt extract yielded certain yeasts capable of fermenting the yeast-free fresh malt when pure cultures were inoculated back into it. With one exception, these yeasts were found to be species of *Zygosaccharomyces*, a genus which contains many osmophilic species. 5 of the isolates were identified by the Centraal-bureau voor Schimmelcultures Baarn, Holland, as *Z. japonicus* Saito, though they differed slightly in minor cultural characteristics. As the extract originally contained about 50 per cent. of sugars, mostly maltose, any organism capable of fermenting must be highly osmophilic; the organism can grow on substrates of considerably higher osmotic pressure than malt extract as shown by its power of fermenting almost saturated solutions of maltose, and glucose solutions of as high a concentration as 90 per cent. w/v. R. E. S.

Organic Acids, Partition Paper Chromatography of. J. Opienska-Blauth, O. Saklawska-Szymonowa and M. Kanski. (*Nature, Lond.* 1951, **168**, 511.) Acids known to be metabolites in living bacterial cells have been examined. The standard Consden, Gordon and Martin technique (*Biochem. J.*, 1944, **38**,

224) is employed using phenol saturated with water as the developing solvent. The atmosphere in the chromatographic cabinet is saturated with formic acid vapour to prevent the ionisation of the organic acids. The position of the spots is revealed by treatment with an alcoholic solution of bromophenol blue. No apparent influence on R_F values was observed when the concentration varied between 0·5 and 5·0 per cent. and, in general, the size and intensity of the spots was proportional to the concentration of acid used, though some exceptions were noted. Volatile organic acids were not detected and uric acid could not be recovered, probably owing to its insolubility in water. Halogenated derivatives of acetic acid did not give consistent R_F values. In an attempt to find a numerical relationship between chemical constitution and R_F values of organic acids, the theoretical ΔR_F values for a number of different groups have been calculated by the direct comparison of the R_F values for various pairs of related acids. J. B. S.

Vitamins D, Separation by Condensation and Irradiation. J. Green. (*Biochem. J.*, 1951, **49,** 54.) An investigation has been made into the analytical purification of the vitamins D by the methods of maleic and citraconic anhydride condensation and by selective ultraviolet irradiation. The use of selective condensation with maleic or citraconic anhydrides as an analytical step in the assay of irradiation products and fish-liver oils for vitamins D is ineffective in quantitatively removing vitamin A or, alternatively, in removing enough vitamin A to eliminate interference in the vitamin D determination. The condensation procedure of Milas *et al.* (*Industr. Engng. Chem., Anal. Ed.*, **13,** 227) completely destroyed large amounts of vitamin D_3 and over 60 per cent. of calciferol, even when large quantities of the latter are used. The use of ether for the condensation gave good recoveries of calciferol and vitamin D_3 but complete elimination of vitamin A is not possible with a reasonable reaction time. Condensation in benzene gave up to 20 per cent. loss of calciferol and about 40 per cent. loss of vitamin D_3. Selective ultraviolet irradiation to remove vitamin A from vitamin D was not suitable for routine use. Although vitamin A could be smoothly destroyed by filtered radiation of wavelength 300 to 400 mμ, the irradiation, under similar conditions, of small amounts of vitamin D resulted in inevitable losses. R. E. S.

BIOCHEMICAL ANALYSIS

Adrenaline in Blood and Adrenal Gland, Colorimetric Determination of. N. C. Ghosh, C. Deb and S. Banerjee. (*J. biol. Chem.*, 1951, **192,** 867.) In view of the fact that established methods for the colorimetric estimation of adrenaline are unsuitable for its estimation in blood, a new procedure has been worked out. The blue colour formed by the action of Folin's reagent on adrenaline is measured in such a way that the contributions to the total colour of the solution due to the reaction of such substances as ascorbic acid, cysteine, glutathione, ergothionine and uric acid, with the reagent, are eliminated. It has been observed that a 10 per cent. solution of sodium bicarbonate completely destroys a dilute solution of ascorbic acid and cysteine, and partly destroys glutathione, without at the same time affecting adrenaline. On treatment of the resulting solution with 5 per cent. sodium hydroxide only adrenaline is destroyed, uric acid, ergothionine and the remaining glutathione being stable under these conditions. This observation forms the basis of the method used for the estimation of adrenaline in trichloracetic acid extracts of blood and adrenal glands. Two identical samples are treated simultaneously at 30° C. for 30 minutes with 10 per cent. sodium bicarbonate and a mixture of 10 per cent.

sodium bicarbonate and 5 per cent. sodium hydroxide respectively. The blue colour which developed on the addition of the reagent was measured after 1½ minutes in a Lumetron photoelectric colorimeter. The difference of the two colorimeter readings represents the colour due to adrenaline. The adrenaline content of the blood from rats, rabbits, guinea-pigs, monkeys and man, and of the adrenal gland of rabbits, has been determined by this method. J. B. S.

Aminoacids and Peptides, Spectrophotometric Analysis of. J. R. Spies and Dorris C. Chambers. (*J. biol. Chem.*, 1951, **191**, 787.) A simple and rapid spectrophotometric method is based on the colour obtained when combined copper is converted to the copper salt of alanine. Since the colour intensities of equimolar concentrations of amino acid copper complexes vary, solutions of the copper complexes of the various amino acids are treated with alanine, thus largely converting them to the highly soluble alanine copper complex, which is used as the standard. 17 amino acids, 9 dipeptides and 5 tripeptides were studied. Dipeptides, in general, react with copper as though the component amino acids were free, while only two of the amino acids of tripeptides react. The restriction of copper -binding property of peptides to the "end group" (free NH_2 adjacent to peptide-linked carboxyl or free carboxyl adjacent to peptide-linked nitrogen) amino acids, increases the usefulness of the method in that protein hydrolysis can be followed. Excellent agreement was obtained between the values for chromogenic nitrogen found and calculated for (*a*) complete acid hydrolysates of casein, (*b*) β-lactoglobulin and (*c*) fraction CS-54R (cottonseed allergenic protein) based on their amino acid contents. J. B. S.

Progesterone in Blood, New Method for Determination of. W. R. Butt, P. Morris, C. J. O. R. Morris and D. C. Williams. (*Biochem. J.*, 1951, **49**, 434.) The initial separation of steroid hormones from plasma is effected by a solvent partition method. The first ethanol-ether extracts are concentrated and the steroid fraction partitioned between water and ethyl acetate and between water and light petroleum in turn. The solid residue from the light petroleum extract is partitioned on a column of Hyflo grade Supercel with methanol (70 per cent.) as the stationary phase, and *n*-hexane as the mobile phase. Extensive details of column dimensions and packing are described and under those conditions progesterone appears in the second and third ml. of eluate. The eluate is evaporated, the residue is dried over phosphorus pentoxide and treated with a solution of Girard's reagent T in anhydrous acetic acid for 2 minutes at 100° C. Sodium chloride, sodium hydroxide and water are added, the solution is deoxygenated and examined polarographically. The method has been used to examine the concentration of progesterone in the circulation after injection into normal and partially hepatectomised rats. Examination of human pregnancy blood samples has shown that the progesterone level is less than 0·1 μg./ml. In two cases progesterone has been detected in human placental blood and in two further cases a substance giving a polarographic wave resembling that of a Δ^4-3-ketosteroid, but showing no 20-ketosteroid wave, was detected. J. B. S.

Sugars, Detection of, by Paper Chromatography. R. J. Bayly, E. J. Bourne, M. Stacey. (*Nature, Lond.*, 1951, 168, 510.) Some observations of unusual phenomena encountered during the paper chromatography of sugar mixtures are reported. Paper chromatograms of sugars in diabetic urines showed three spots when developed with a mixture of *n*-butanol, ethanol, water and ammonia, and sprayed with certain primary aromatic amines. The R_F value of one of the spots was identical with a reference glucose spot, while the R_F values of the other two were respectively 37 per cent. and 78 per cent. of that of the reference spot.

The slowest moving substance stained with naphthoresorcinol but not with ammoniacal silver nitrate; the middle spot stained with ammoniacal silver nitrate. In *n*-butanol saturated with water the slowest spot moved only 11 per cent. of the distance moved by the glucose spot, while a mixture of *n*-butanol, acetic acid and water as the developing solvent only the glucose spot was observed. Two dimensional chromatography of the urine samples, developed first with butanol-ammonia followed by butanol-acetic acid, showed the complete reversion, in the second solvent, of all spots to glucose. Acid hydrolysates of dextran and starch, subsequently neutralised and submitted to paper chromatography using butanol-ammonia as the developing solvent, indicated the presence solely of glucose, when 20 µg. quantities of sugar were used. A tenfold increase in quantity, however, indicated the presence of a spot, identical in R_F value and reaction to spraying reagents, with the slowest one observed in the urine samples. The same spot was also observed when 200 to 300 µg. of pure glucose was dissolved in water and run on a chromatogram. Other aldoses, including both pentoses and hexoses, behaved similarly, though no additional spots were observed with the ketoses fructose and sorbose. Similar, but less regular, phenomena have also been noted with some partially methylated sugars. J. B. S.

Uric Acid in Blood and Urine, Determination of. D. S. Bidmead. (*J. clin. Path.*, 1951, **4**, 370.) The Folin method was found to give too variable results when used for the determination of uric acid in the blood of the same patient 3 or 4 times a day and an alternative colorimetric procedure was developed, based on precipitation of proteins and treatment of the filtrate with urea-cyanide and an arsenophosphotungstate reagent. The urea-cyanide reagent is prepared by dissolving 25 g. of pure sodium cyanide in 400 ml. of distilled water and adding 75 g. of urea and water to 500 ml. The arsenophosphotungstate reagent is made by dissolving 50 g. of molybdate-free sodium tungstate in 300 ml. of distilled water, adding 25 g. of arsenic pentoxide and when solution is complete adding 12·5 ml. of syrupy phosphoric acid and 10 ml. of hydrochloric acid; boiling for 20 minutes, cooling and diluting to 500 ml. The stock solution of uric acid is prepared by Folin's method. In applying the test to blood, 1 ml. of plasma is centrifuged with 7 ml. of water, 1 ml. of 0·67N sulphuric acid and 1 ml. of 10 per cent. sodium tungstate solution. 5 ml. of the clear supernatant liquid is mixed with 2 ml. of urea-cyanide solution, 0·2 ml. of arsenophosphotungstate solution and water to 10 ml. After 5 minutes, the colour is read in a photoelectric absorptiometer. For urine, a 1 per cent. dilution of the sample is made and 5 ml. of the dilution is used for the test, which is similar to that adopted for plasma. A standard curve is prepared from the results obtained with known amounts of uric acid. Good recoveries were obtained when the procedure was applied to samples both of blood and urine to which additional uric acid had been added. H. T. B.

Vitamin A, Assessment of Potency by Spectrophotometry. T. Boldingh, H. R. Cama, F. D. Collins, R. A. Morton, N. T. Gridgeman, O. Isler, M. Kofler, R. J. Taylor, A. S. Welland and T. Bradbury. (*Nature, Lond.* 1951, **168**, 598). Because of the variations of absorption spectra of many organic solutes upon varying the solvent, the $\epsilon_{max.}$ and $\lambda_{max.}$ values were determined for pure all-*trans* vitamin A alcohol and acetate in *iso*propanol, ethanol, *cyclo*hexane and light petroleum (40° to 60° C.). The figures recorded are the means of the results from 5 laboratories. Analysis of variance of the 40 individual results indicated that the coefficient of variation between laboratories was 1·3

and that the residual coefficient of variation of the one determination was 1·1. Appropriate factors are given for the conversion of values at $\lambda_{max.}$ for vitamin A alcohol or acetate to I.U./g. A. H. B.

Vitamin A, Spectrophotometric Assay of, Geometric Correction of Absorption Curves. N. T. Gridgeman. (*Analyst*, 1951, **76**, 449.) The theory and the reliability of the Morton-Stubbs geometric correction of absorption curves by separating the compound curve into two parts (the major part being the characteristic curve and the minor part due to "irrelevant" absorption) is discussed in its application to the analysis of vitamin A in fish-liver oils and concentrates. The accuracy of the method depends upon the valid scope of two assumptions, (1) that the absorption curves of natural forms of the vitamin are indistinguishable from that of pure all-*trans* vitamin A; (2) that the ultra-violet absorption curves of materials other than vitamin A in natural oils have three linear points at certain wave-lengths. Evidence is presented to show that these assumptions are not always correct, and that comparatively small departures from these conditions may cause appreciable loss of accuracy, and an attempt is made to correlate degrees of departure with accuracy of the result. The influence of the normal observational errors of spectrophotometry on the precision of the result is considered. A. H. B.

Vitamin A, The Precision of the 3-point Correction Method of Spectrophotometric Assay of. D. C. M. Adamson, W. F. Elvidge, N. T. Gridgeman, E. H. Hopkins, R. E. Stuckey and R. J. Taylor. (*Analyst*, 1951, **76**, 445.) The reproducibility of the Morton-Stubbs 3-point geometric method of correcting for irrelevant absorption in the spectrophotometric assay of vitamin A was investigated. For the whole oil in *cyclo*hexane the formula is:—E at 328 mμ (corr.) = 7 × E at 328 mμ − 2·882 × E at 313 mμ − 4·118 × E at 338·5 mμ. Seven independent laboratories determined by photo-electric spectrophotometry $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ at 313 mμ, 328 mμ and 338·5 mμ, each in duplicate (separate weighings), on 5 oils, the solvent being *cyclo*hexane. The geometrically corrected $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ at 328 mμ results were then statistically analysed and it was concluded that the limits of error of a determination in duplicate from any one laboratory are about ±15 per cent. for P = 0·05. The corresponding figure for uncorrected E values is about ±2 per cent. A. H. B.

Vitamin B_{12}, Assay of. Interference with the *Escherichia coli* Response. W. F. J. Cuthbertson, H. F. Pegler, C. Quadling and V. Herbert. (*Analyst*, 1951, **76**, 540.) The effects of various substances on the *E. coli* plate assay of vitamin B_{12} were investigated. Different concentrations of the reagents studied were placed on the assay plates in the absence or presence of the vitamin; when the reagents were tested in its presence the concentration of the vitamin used was always kept at 0·2 μg. per ml. (the same as that used in the provisional assay technique). The growth zones were compared qualitatively with those caused by the vitamin at concentrations of 0·02 and 0·2 μg. per ml. on the same plate; if the test mixtures produced growth qualitatively similar to that obtained with the vitamin alone then the diameters of the growth zones were measured and the apparent concentrations of vitamin B_{12} in the test mixtures were calculated. The allowable concentration of interfering substances may be expressed in the form C = KN per cent. where C is the permitted concentration in the test solution expressed as a percentage, N is the vitamin B_{12} concentration (in μg. per ml.) and K is a constant depending on the interfering substance. Tables are given showing the values of K at levels leading to a 2 per cent. or a 5 per cent. error and showing whether the interfering agents cause an increase or a decrease in the

apparent vitamin B_{12} activity of the solution. Substances studied were ethanol, acetone, ethylene glycol, propylene glycol, ascorbic acid, formalin 40 per cent. (response reduced); butanol, toluene, sodium formate, manganese sulphate, ferrous sulphate, choline, betaine, ammonium sulphate (response unaltered); methionine, thioglycollic acid, phenol, copper sulphate, sodium chloride (response increased); homocystine, potassium cyanide (effect variable). R. E. S.

Vitamin B_{12}, Cup-Plate Assay of, Using *Lactobacillus lactis* Dorner 10697. F. E. Larkin and R. E. Stuckey. (*Analyst*, 1951, **76**, 150.) The routine use of tube assays of vitamin B_{12} with a variety of organisms was abandoned in favour of a cup-plate assay with *Lactobacillus lactis* Dorner 10697 using a modification of the method of Foster, Lally, and Boyd Woodruff (*Science*, 1949, **110**, 507). The effects of variation of the *p*H of the test liquid and of variation in the concentration of cresol present were studied. The most reliable results were obtained on solutions containing crystalline vitamin B_{12}, although considerable variations were experienced; the microbiological assay was found to be particularly useful in determining the stability of samples of vitamin B_{12} of differing purities. With other vitamin B_{12} preparations the microbiological assay was always interpreted in conjunction with organic cobalt determinations since although the organic cobalt assay gave a maximum figure, the microbiological assay often exceeded this result. Concentrated liver preparations gave, in general, concordant results but on autoclaving there was often an increase in microbiological activity, sometimes as much as 70 per cent. Assays of vitamin B_{12} concentrates obtained from streptomyces fermentation liquors gave results that were difficult to interpret, anomalous growth zones being present; it was considered necessary to use the assay in conjunction with a chrómatographic procedure, although some difficulty was experienced in getting a quantitative elution of the small amounts present on the chromatogram. R. E. S.

Vitamin B_{12}, Microbiological Determination of. B. Noer. (*Dansk Tidsskr. farm.*, 1951, **25**, 222.) Four organisms were compared for suitability for vitamin B_{12} assay: *Lactobacillus lactis* Dorner, *L. Leichmannii*, *Thermobacterium lactis* and *T. Yogurth*. The most reproducible results were obtained with *T. lactis*, which also has the advantage that it requires the simplest substrate. The methods usually adopted for the assay are not altogether satisfactory, for the following reasons. It is not possible to be certain that all the other growth factors required are present, since many of them are of an unknown nature and their stability to heat and other conditions is unknown: this applies especially to hydrolysed casein. Growth-hindering substances may be present, and slight alterations in *p*H or *r*H may affect the growth. In order to compensate for these factors it is proposed to use for the test three solutions: a normal standard solution, a test solution, and a test solution to which has been added the optimal quantity of vitamin B. The amount of growth is determined turbidimetrically, using a photo-electric colorimeter. The corrected values obtained by this "compensation method" allow for the extra growth-promoting substances which may be lacking in the culture solution, and for growth-inhibiting substances in the test solution. However, such tests can not alone give a certain figure for the vitamin B_{12} content. Growth resulting from desoxyribosides can be distinguished from that due to the vitamin, as the latter is unstable to heat or light and comparative tests before and after autoclaving may show the presence of such factors. Paper chromatography is also of value for this purpose. When applied to liver extract, agreement between the different methods was satisfactory. An animal food addition product, prepared by fermentation, was

shown to contain both vitamin B_{12} and desoxyribosides. Extract of rye bread gives a misleading result by direct methods, but by combining the results of the "compensation method" before and after autoclaving it was shown that no vitamin B_{12} was present. With products containing a large proportion of the vitamin, a simple activity determination can be used with good approximation, as other substances which affect growth have no appreciable effect at the dilutions used. By the use of the various control methods determinations can be carried out satisfactorily in solutions containing 0·0001 μg. of vitamin B per ml.

G. M.

CHEMOTHERAPY

Antispasmodics. Esters of β-Alkyltropic Acids. A. W. Weston and R. W. DeNet. (*J. Amer. chem. Soc.*, 1951, **73**, 4221.) The synthesis is reported of a series of basic esters of β-substituted tropic acids of general formula $C_6H_5CH(C(OH)R_1R_2)\cdot COO\cdot(CH_2)_nNR_2HCl$ (I). Intermediate β-alkyl-and β-dialkyltropic acids (II) were synthesised by treating either phenylacetic acid or sodium phenylacetate with *iso*propyl magnesium halide and condensing the resulting Grignard complex with the appropriate aldehyde or ketone. The required basic esters (I) were obtained by reaction of the acids (II) with dialkylaminoalkyl chlorides in boiling *iso*propanol. An alternative preparation of the esters was attempted, in which the sodium β:β-pentamethylenetropate, formed by the reaction of the free acid with sodium hydride, was heated with diethylaminoethyl chloride in benzene. The method was unsuccessful, the only basic product isolated being diethylaminoethyl phenylacetate, a tendency which was particularly marked when the substituted tropic acid contained a β-aryl group. Preliminary results indicate that basic esters of type (I) have a pronounced antispasmodic action.

J. B. S.

Antithyroid Substances, 2-Mercaptoglyoxalines, Mercaptothiazoles and Thiohydantoins. C. E. Searle, A. Lawson and H. V. Morley. (*Biochem. J.*, 1951, **49**, 125.) The antithyroid activity has been determined of a further series of 2-mercaptoglyoxalines, several 2-thiohydantoins and a number of 2-mercapto- and 2-aminothiazole derivatives. For assay the screening test of Searle *et al.* (*Biochem J.*, 1950, **47**, 77) was used; all drugs were administered by stomach tube, usually at a level of 0·05 m. mol/kg. body weight. The dose of radioactive iodide (approx. 1 μc. in 0·2 ml. of 0·9 per cent. sodium chloride without added carrier) was injected intraperitoneally after 1 hour and the rats killed after a further 4 hours. The ^{131}I uptakes of the thyroids of the dosed and control animals were then compared using a liquid counter, the results being expressed as the percentage depression of the mean ^{131}I uptake of the control animals. Results are given for 10 glyoxaline derivatives, for 5 thiohydantoins, and for 15 thiazoles; of the mercaptoglyoxalines the 3 most active were 2-mercaptoglyoxaline, and its 1-methyl and 1-ethyl derivatives these being found to be only half as active as thiouracil. The figures for acute toxicity determinations in mice carried out with three representative mercaptoglyoxalines are given. No 2-mercapto- or 2-aminothiazole tested showed activity comparable with the most active thiouracils and mercaptoglyoxalines, the largest depression of ^{131}I uptake being produced with 5-amino-2-carbethoxythiothiazole. 3 simple thiohydantoins had a fairly high activity.

R. E. S.

Nitroparaffins, Antitubercular and Antirickettsial Properties of Derivatives. T. Urbanski. (*Nature, Lond.* 1951, **168**, 562.) A series of nitrocompounds was tested against saprophytic mycobacteria (six strains) *in vitro* in Youman's medium and *in vivo* against *Mycobacterium tuberculosis* ($H_{37}Rv$ strain) in white mice

inoculated intravenously. They were also tested *in vivo* against *Rickettsia prowazeki*. These nitro compounds included 1:3-tetrahydro-oxazines and 1-oxa-3-aza-*cyclo*octanes formed by the interaction of 1-nitropropane with formaldehyde and ammonia and open chain amines derived from these compounds. Condensation products from 2-nitropropane and nitroethane, formaldehyde and ammonia, and also 1-nitropropane, formaldehyde and methylamine were also tested. Most of the compounds were shown to possess a relatively low toxicity. Details of toxicity and activity are tabulated.

A. H. B.

Open-chain Aminoketones Related to Morphine. J. H. Burckhalter and S. H. Johnson, Jr. (*J. Amer. chem. Soc.*, 1951, **73**, 4832.) A series of α-alkyl-α(2-dialkylaminoalkyl)-phenylacetonitriles having the general formula $C_6H_5C{\cdot}(CH_3){\cdot}(CN){\cdot}CH_2CH(R){\cdot}N(CH_3)_2$ with various substituents in the aromatic ring have been prepared from the appropriate phenylacetonitrile and 2 dialkylaminoalkyl halide by means of a sodamide condensation. α-Methyl-α-(2-dimethylaminoethyl)-phenylacetonitrile with ethylmagnesium bromide gave 6-dimethylamino-4-methyl-4-phenyl-3-hexanone (I) in 76 per cent. yield. 6-Dimethylamino-4-methyl-4-(2:3-dimethoxyphenyl)-3-hexanone (II) was obtained by a similar route in 35 per cent. yield. The ketone II could not be reduced catalytically to the corresponding alcohol; attempted demethylations of II were also unsuccessful. Both I and II failed to exhibit analgesic activity in guinea-pigs. This lack of activity is attributed to the 4-methyl group, which replaces the 4-phenyl group in the analogous methadone, the methyl group being too small to effect the necessary steric hindrance which locks the aliphatic chains of methadone into a morphine-like spatial arrangement. J. B. S.

Thiosemicarbazones in the Chemotherapy of Tuberculosis. E. Hoggarth and A. R. Martin. (*Brit. J. Pharmacol.*, 1951, **6**, 454.) Some 60 compounds derived from thiosemicarbazones by a variety of oxidative and reductive processes, or closely related to such compounds, were examined in tuberculous mice. Two groups of "active" compounds were found, namely, 1-benzylthiosemicarbazides and 1-amino-4-phenyl-2:3-diazabuta-1:3-diene sulphonic acids. The activity was of the same order as that found in the parent series of thiosemicarbazones. S. L. W.

PHARMACY

NOTES AND FORMULÆ

Chlorprophenpyridamine Maleate (Chlor-Trimeton Maleate). (*New and Nonofficial Remedies*; *J. Amer. med. Ass.*, 1951, **147**, 128.) Chlorprophenpyridamine maleate is 1-(*p*-chlorophenyl)-1-(2-pyridyl)-3-dimethylaminopropane maleate and occurs as a white crystalline solid, m.pt. 130° to 135° C., soluble in water (1 in 3·4), ethanol (1 in 10) and chloroform (1 in 10), and slightly soluble in benzene and ether; a 1 per cent. solution has *p*H 4·8. When heated in a Bunsen flame on copper wire, the flame turns green (distinction from prophenpyridamine). The yellow crystalline picrate obtained by adding an ethanolic solution of trinitrophenol to a chloroform solution of the base melts at 197° to 200° C. after drying *in vacuo* for 4 hours and then at 105° C. for 4 hours. On adding chloroform and potassium hydroxide to a solution in ethanol and heating, a red colour develops but no odour of isonitriles (absence of primary amines). Chlorprophenpyridamine maleate loses not more than 0·5 per cent. of its weight after drying at 105° C. for 4 hours; residue on ignition, 0·15 per cent. A 0·003 per cent. solution in water exhibits an ultra-violet absorption maximum at 2620 Å ($E^{1\ \text{per cent.}}_{1\ \text{cm.}}$, 143 ± 3), an inflection at about 2680 Å and a

minimum at about 2430 Å. It contains 7·0 to 7·4 per cent. of N, equivalent to 97·5 to 102·5 per cent. of chlorprophenpyridamine maleate. It is used as an histamine antagonist in a dose of 2 to 4 mg. G. R. K.

Cyclopentamine Hydrochloride (Clopane Hydrochloride). (*New and Non-official Remedies*; *J. Amer. med. Ass.*, 1951, **147,** 128.) Cyclopentamine hydrochloride is 1-*cyclo*pentyl-2-methylaminopropane hydrochloride and occurs as a white, odourless, crystalline powder, with a mild characteristic odour, m.pt. 113° to 116° C., soluble in water (1 in 1), ethanol (1 in 1·8), benzene (1 in 23·8) and chloroform (1 in 1·3), and slightly soluble in ether; a 1 per cent. solution has *p*H 6·2. When boiled with sodium nitrite and treated with hydrochloric acid, a yellowish orange oily material separates; on the addition of more hydrochloric acid the oily layer disappears and a white precipitate which is soluble in water forms. When heated on a steam bath with sulphuric acid and potassium cyanate, it yields a white precipitate which melts at 126° to 129° C. after recrystallisation from hot water and drying at 80° for 4 hours. Cyclopentamine hydrochloride yields no odour of isonitriles when heated with chloroform, ethanol and potassium hydroxide; it loses not more than 0·4 per cent. of its weight when dried at 80° C. for 3 hours and leaves not more than 0·05 per cent. of residue on ignition. It is assayed by Kjeldahl determination and contains 98·0 to 102·0 per cent. of cyclopentamine hydrochloride. It is used as a sympathomimetic agent. G. R. K.

Phenacemide (Phenurone). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1951, **147,** 60.) Phenacemide is phenylacetylurea, $C_6H_5CH_2\cdot CO\cdot NH\cdot CO\cdot NH_2$. It is a white to creamy white, odourless, tasteless, crystalline solid, m.pt. 212° to 216° C., slightly soluble in alcohol, benzene, chloroform, ether, and water. When heated with potassium nitrate and sulphuric acid, treated with water and zinc powder and again heated, cooled, diluted and filtered, the filtrate gives a light purple colour when treated as follows: add an equal volume of a 20 per cent. solution of toluenesulphonic acid, dilute with water, and add a solution of sodium nitrite; after 3 minutes add a solution of sodium sulphamate, allow to stand for 2 minutes, add an ethanolic solution of *N*:*N*-dimethyl-1-naphthylamine and allow to stand for 15 minutes. The phenylacetic acid obtained in the assay melts between 75° and 77° C. Phenacemide loses not more than 1 per cent. of its weight when dried at 105° C. for 4 hours, and leaves not more than 0·05 per cent. of residue on ignition. It is assayed by hydrolysing with sulphuric acid, extracting the phenylacetic acid with chloroform, removing the chloroform, dissolving the residue in ethanol and titrating with sodium hydroxide, using phenolphthalein as indicator. It contains 74·9 to 77·9 per cent. of phenylacetic acid, equivalent to 98·0 to 102·0 per cent. of phenacemide. Phenacemide is used as an anticonvulsant in doses of 0·5 g. 3 times a day. G. R. K.

Phenacetin, Acetchloranilide as Impurity in. J. Hald. (*Acta Pharm. Internat.*, 1951, **2,** 27.) Cyanosis has been observed after the taking of impure phenacetin, which was contaminated with a considerable quantity of acet-4-chloranilide, a compound which produces methæmoglobinæmia. This compound is produced in the synthesis of phenacetin and, if present in quantities of not more than 1·5 per cent., the material may still pass pharmacopœial requirements. The permissible limit of this impurity would appear to be about 0·15 per cent.: i.e., 6 mg. would be present in a daily dose of 4 g. of phenacetin. Doses of 10 mg. of acetchloranilide do not in general convert more than 2 per cent. of the total hæmoglobin to methæmoglobin. G. M.

Phethenylate Sodium (Thiantoin Sodium). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1951, **147**, 129.) Phethenylate sodium is sodium 5-phenyl-5-(2-thienyl) hydantoinate. It is a white, odourless, hygroscopic, microcrystalline powder which absorbs carbon dioxide with the liberation of 5-phenyl-5-thienyl hydantoin; in aqueous solution it gradually dissociates. It is soluble in water (1 in 2), and ethanol (1 in 5), very slightly soluble in ether, and practically insoluble in benzene and chloroform; a 1 per cent. solution has *p*H 10·0. When shaken with thiophene-free benzene and a solution of isatin in sulphuric acid, the acid layer becomes green and turns blue after about an hour (distinction from phenytoin sodium). The precipitate formed when hydrochloric acid is added to a solution in water melts at 251° to 259° C. after drying at 105° C. for 4 hours. Phethenylate sodium contains not more than 30 p.p.m. of lead and loses not more than 1·5 per cent. of its weight when dried at 105° C. for 24 hours. It is assayed by extracting an acidified solution with ether, evaporating the ether and drying the residue at 105° C. for 4 hours, and also by determining the nitrogen content. It contains 97·0 to 103·0 per cent. of phethenylate sodium. It is used in the treatment of epilepsy. G. R. K.

PHARMACOLOGY AND THERAPEUTICS

Absorbable Gelatin Sponge in Experimental Surgery. G. Blaine. (*Lancet*, 1951, **261**, 427.) Gelatin sponge is essentially a dried gelatin foam. By heating or whipping a sterile and highly formalised gelatin solution a foam of uniform porosity is produced, which, when dried under suitable conditions, retains its original porous structure. The dried material is cut into required shapes and sizes and packed and sterilised at 160° C. Although gelatin sponge is insoluble in water it is completely digested by proteolytic enzymes. Dry gelatin sponge is soft, springy and light; it can be cut with ease and has sufficient tensile strength to withstand normal handling. Wet gelatin sponge rapidly absorbs moisture and becomes jelly-like. It can be used safely in conjunction with penicillin. Experimental surgical studies were carried out on rabbits. In some rabbits gelatin sponge measuring 3·0 × 2·0 × 0·1 cm. was introduced into the abdominal cavity; in others, gelatin sponge of the same size was introduced into the muscular tissue in the anterior triangle of the neck; and in the remainder the liver was slit for ¾ in. on the anterior surface and gelatin sponge inserted into the wound. The rabbits were killed on the 5th, 11th, 16th, 23rd, 28th and 42nd post-operative days for necropsy, and histological studies were made. From the findings it would appear that gelatin sponge is absorbed or "organised" in about 4 to 6 weeks. It may therefore be considered safe for implantation as an absorbable hæmostatic in surgery. It produces hæmostasis promptly and adheres readily to bleeding surfaces. If too much is implanted it does not become absorbed normally, since it prevents access of phagocytes to the centre of the implant. S. L. W.

Cortisone and Related Hormones; Effect on Responses to Analgesic Drugs. C. A. Winter and L. Flataker. (*J. Pharmacol.*, 1951, **103**, 93.) Cortisone was found to antagonise all the actions of morphine and amidone that were studied in rats and mice. These include: (1) analgesic effect, measured by the D'Amour Smith technique, (2) toxicity of amidone, (3) hyperactivity in mice, (4) hypnosis and catalepsy in rats. The effects of deoxycortone were the opposite of those produced by cortisone, while adrenocorticotrophic hormone produced effects similar to those of cortisone. All the results obtained in these experiments indicate that cortisone has a stimulant effect on the central nervous system. S. L. W.

Heparin, Sublingual Administration of. J. Litwins, J. J. Vorzimer, L. N. Sussman, M. Applezweig and A. D. Etess. (*Proc. Soc. exp. Biol., N.Y.*, 1951, **77**, 325.) Sublingual wafers containing 125 mg. of sodium heparin were given to 10 subjects. Absorption was usually complete within 10 minutes. A therapeutic level was obtained within ½ hour and maintained for 4 hours. The authors suggest that this may become the method of choice for the early anticoagulant treatment of myocardial infarction, pulmonary embolism, and thrombophlebitis, and in vascular surgery. It would also appear to be of value in the early treatment of frostbite in soldiers before hospitalisation. S. L. W.

Morphine, Intravenous, in Ocular Surgery. E. A. Johnson. (*Canad. med. Ass. J.*, 1951, **64**, 429.) A series of 101 unselected ophthalmic surgical cases was operated on under local anæsthesia, using a morphine-scopolamine-ephedrine solution intravenously. The proportions of the drugs used were morphine sulphate ¼ gr., ephedrine sulphate ⅙ gr., scopolamine hydrobromide 1/200 gr., with chlorbutol 0·5 per cent., dissolved under aseptic conditions in 4 ml. of triple distilled water. Premedication consists of the administration of amylobarbitone sodium 3 gr. before retiring, repeated 2 hours before operation. The intravenous injection of the anæsthetic is made very slowly, at an approximate rate of 1 ml. in 2 minutes. The maximum depressant effect occurs in from 7 to 10 minutes, the maximum psychic sedative effect begins in about 10 minutes, and the maximum analgesic effect in about 20 minutes. Apart from mild dizziness in 2 cases, mild tachycardia in 2 cases, and respiratory depression with mild cyanosis in 3 cases, there was a striking absence of severe side-effects. Post-operative reactions such as headache, nausea and vomiting, were also mild and occurred in less than 10 per cent. of patients. S. L. W.

NPH Insulin. K. A. Swallow and A. L. Chute. (*Canad. med. Ass. J.*, 1951, **65**, 23.) The chief distinction between protamine zinc insulin and NPH insulin is that the former is an amorphous material containing a variable quantity of protamine (1·25 to 1·5 mg. per 100 units) which is sufficient to provide an excess, while NPH insulin is a crystalline product in which the protamine is completely combined with insulin. After injection the action of NPH insulin commences in about 2 hours, reaches a maximum in 10 to 20 hours, and is complete in 28 to 30 hours. In 10 out of 18 diabetic juvenile patients, whose ages ranged from 3 to 14 years, and whose total daily insulin requirements varied from 10 to 80 units, as good or better control was achieved by a single injection of NPH insulin given before breakfast as was possible with the double injection of unmodified insulin and protamine zinc insulin. It must be emphasised, however, that each patient's requirement of insulin must be considered as an individual problem, both as regards the type of insulin and the amount required. Since NPH insulin does not significantly alter the effect of added unmodified insulin the use of mixtures of the two insulins has also proved satisfactory by the single injection technique. S. L. W.

NPH Insulin; Composition and Properties. M. Jameson, A. H. Lacey and A. M. Fisher. (*Canad. med. Ass. J.*, 1951, **65**, 20.) Late in 1950 there was made generally available in Canada and the United States a new preparation of insulin under the name "NPH insulin." This was introduced because of the requirement by clinicians for a protamine-insulin preparation acting more quickly than protamine zinc insulin and for only 22 to 26 hours rather than 36 hours as is sometimes the case with protamine zinc insulin. NPH insulin is a buffered aqueous suspension of crystals containing insulin (40 or 80 units per ml.), protamine and zinc. Each 100 units contains 0·03 mg. of zinc and 0·4 mg.

of protamine plus sodium chloride; it has a *p*H of 7·2. In order to distinguish it from other insulins it is distributed only in vials with a square cross section. NPH insulin has the advantage over protamine zinc insulin that when mixed with unmodified insulin for simultaneous administration much of the quick action of the unmodified insulin is retained, whereas with simultaneous administration of protamine zinc insulin and unmodified insulin the quick-acting effect of the insulin is lost. S. L. W.

Phenobarbitone, Mode of Absorption of. M. R. Fabre, M. T. Regnier and M. E. Grasset. (*Ann. pharm. franc.*, 1951, **9**, 98.) The absorption of phenobarbitone is greatly influenced by the type of preparation used. When administered directly into the stomach of dogs, the concentrations (mg. per 100 ml.) in blood and chyle after 8 hours were as follows:

Type of preparation	Phenobarbitone in	
	blood	chyle
Gummy suspension	5.3	0
Oily emulsion	4.2	2.3
Phenobarbitone-sodium, in .. water ..	11.6	8.9

Differences were also observed in the concentrations in the organs of the body, and in the urine. The blood level rises much more rapidly when the drug is administered to fasting animals, than when the stomach is full, and the period before death supervenes is correspondingly less, but the final blood concentration is approximately the same. G. M.

Phenol, Cutaneous Absorption of. M. V. Freeman, J. H. Draize and E. Alvarez. (*J. Lab. clin. Med.*, 1951, **38**, 262.) Albino rabbits subjected, under anæsthesia, to various procedures involving trauma of the skin (scalds, dry heat burns, ultra-violet burns) were exposed to topical applications of 2·3 per cent. of phenol in corn oil, and phenol-camphor (a mixture of 5 per cent. of phenol and 10 per cent. of camphor in corn oil). Urinary analyses showed that phenol is rapidly absorbed and excreted following topical application. A single application of 2·3 per cent. phenol in corn oil mixture does not appear to affect the ability of the skin to absorb phenol. Animals whose skin was irradiated with ultra-violet rays to a point of erythema and slight œdema formation exhibited a retarded excretion of phenol. Urinary phenol values were shown to be in direct ratio to the phenol content of the preparations employed. After treatment, animals with severely burned skin showed much higher values of urinary phenol than animals with intact skin, indicating a potential danger in the indiscriminate use of phenol compresses in severe burns. S. L. W.

α-Phenyl Succinimides, Anticonvulsant Activity of. G. Chen, R. Portman, C. R. Ensor, A. C. Bratton. (*J. Pharmacol.*, 1951, **103**, 54.) This report deals with the laboratory evaluation of some derivatives of α-phenyl succinimides with respect to their actions on the central nervous system. The material, in solution or in suspension with acacia, was administered perorally to fasting rats, a convulsive dose of metrazol solution being injected subcutaneously half an hour later and the animals observed for the time of onset and severity of convulsions for 36 minutes. The anti-electroshock effect was measured either in cats or in mice. The αβ-methyl and ethyl substituted compounds were more effective against metrazol than against electrically-produced convulsions. Methylation of the heterocyclic nitrogen of most of these succinimides resulted

in an increase of anti-metrazol potency, this enhancing effect becoming less with the increasing length and branching of the alkyl chain. The $\alpha\alpha$-diphenyl and $\alpha\beta$-diphenyl succinimides were, however, more effective in suppressing electrically-induced than metrazol-induced convulsions. Methylation of the heterocyclic nitrogen of these compounds diminished their anti-electroshock activity.

S. L. W.

Podophyllin, Toxicology of. M. Sullivan, R. H. Follis and M. Hilgartner. (*Proc. Soc. exp. Biol., N.Y.*, 1951, **77**, 269.) In mice podophyllin and podophyllotoxin administered parenterally were shown to be respectively 1/15 and 1/6 as toxic as colchicine. Death usually occurred within 15 to 18 hours after administration of fatal doses of podophyllin. Diarrhœa, rapid and laboured respiration and a dragging gait were usually observed during the 8 hours after dosing. After 9 hours there was a period of excitation resulting in spastic convulsions. This was followed by flaccidity, dragging gait and slow, shallow, laboured respiration. The most constant finding on microscopic study was acute enteritis. There were increased numbers of polymorphonuclear leucocytes in intestinal sub-mucosa and a striking breaking up of their nuclei, small masses of deeply staining chromatin being found in profusion. Necrosis of the lymphoid tissue beneath the mucosa was also prominent. Increased mitotic activity was present in the epithelium of the tongue. There were no arrested mitoses in the skin. A comparison of the median lethal doses of podophyllin and podophyllotoxin in young and adult rats showed a greater degree of toxicity for young rats. Cumulative toxic effects were produced in rats by giving podophyllin and podophyllotoxin several times a day in individual doses that were in the range of 1/6 and 1/5 of the LD50. S. L. W.

Polymyxin in the Treatment of Burns. D. M. Jackson, E. J. L. Lowbury and E. Topley. (*Lancet*, 1951, **261**, 137.) Local application of 0.1 per cent. polymyxin-E cream every other day to burns colonised by *Ps. pyocyanea* in a controlled trial significantly reduced the incidence of this organism; by the 4th day the organism persisted in 26 per cent. of 19 polymyxin-treated burns compared with 81 per cent. of 16 control burns. Routine local application of this cream protected burns from colonisation by *Ps. pyocyanea* and some coliform bacilli; 7 per cent. of 162 burns so treated acquired *Ps. pyocyanea* compared with 24 per cent. of 207 control burns. Polymyxin-E locally was used for 6 weeks on all burns with no local or general toxic effects, and there was no evidence of acquired resistance by *Ps. pyocyanea.* The use of polymyxin in a controlled prophylactic trial was associated with a significant reduction in the healing time of full-thickness skin-loss burns; 57 per cent. of 28 polymyxin-treated patients were healed in 4 weeks in contrast to 19 per cent. of 43 control patients. The average healing times were 5·2 and 8·5 weeks respectively. There was also a significant increase in the incidence of complete graft takes, and a slight reduction in the incidence of anæmia, pyrexia and death. From the data presented the authors conclude that *Ps. pyocyanea* and some coliform bacilli act as pathogens on burns, and that the routine application to burns of a cream containing penicillin, to combat Gram-positive cocci, and polymyxin, to combat many Gram-negative bacilli, would be more valuable than the penicillin cream widely used to-day. S. L. W.

Pressor Substance in Urine. J. Dekanski. (*Brit. J. Pharmacol.*, 1951, **6**, 351.) Human urine, from which the gonadotrophins had been removed by absorption on kaolin, was treated for the preparation of antidiuretic concentrates, and these concentrates were then tested for other forms of activity.

They were found to have a pressor action in rats which was not due to adrenaline, noradrenaline, *iso*amylamine, tyramine, piperidine, or nicotine, since the action survived the injection of dibenamine, ergotoxine, and piperoxane. It is suggested that the substance responsible for this action is vasopressin. A test for oxytocic activity of the urine concentrates on the guinea-pig's uterus gave negative results and, in terms of posterior pituitary extracts, was certainly less than 1/20th of their pressor activity. S. L. W.

Salicylates: Effect on the Pituitary and Suprarenal Glands. B. S. Hetzel and D. C. Hine. (*Lancet*, 1951, **261**, 94.) This investigation was stimulated by a report of the development of Cushing's syndrome in a patient with rheumatic fever under treatment with aspirin 5 g. a day. Experiments were designed to show whether salicylates, in therapeutic dosage, had an effect on the pituitary and suprarenal glands that was manifested by removal of ascorbic acid from the suprarenal glands and could be abolished by hypophysectomy or by preliminary treatment with suprarenal cortical hormone. The initial dosage was based on the daily dose of sodium salicylate estimated to maintain in the rat an adequate blood-salicylate level of 30 to 40 mg./100 ml.; the dosage of sodium *p*-aminosalicylate was based on similar considerations. These doses were 0·2 g./kg. of bodyweight and 0·3 g./kg. of bodyweight for sodium salicylate and *p*-aminosalicylic acid respectively. The drugs were given in saline solution intraperitoneally. The suprarenal glands were excised 100 to 130 minutes after the treatment. It was shown that in these doses the salicylates cause a significant depletion of the ascorbic acid content of the suprarenals, the effect being directly proportional to the dose. This response can be abolished by hypophysectomy and tends to be inhibited by preliminary treatment with suprarenal cortical hormone. It is concluded that the therapeutic effects of salicylates are mediated by the pituitary and suprarenal glands. The beneficial results of salicylate therapy in rheumatic fever may be due to the production of cortisone-like steroids due to the activation of these glands and salicylate therapy has a beneficial effect in rheumatic carditis according to the dosage and consequent degree of stimulation. S. L. W.

Salicylic Acid, Fatty Acid Esters of. A. Lespagnol, J. Batteur and C. Lespagnol. (*Therapie*, 1951, **6**, 125.) Certain derivatives of salicylic acid, such as diethylacetylsalicylic acid and diethylacetyldi-*iso*propylsalicylic acid, show a resistance to hydrolysis considerably greater than that of acetylsalicylic acid, and it is possible that salts of such compounds would be free from the objections to salts of acetylsalicylic acid, while retaining their advantages. On the other hand, such a substitution might increase the hypnotic activity. With both the acids mentioned, the solubility in oil is considerably greater than that of acetylsalicylic acid. It may further be noted that diethylacetic acid is degraded to methylpropylacetone and the corresponding secondary alcohol, and this is liable to increase the hypnotic power. Further tests are being carried out on these acids. G. M.

Sparteine as Antagonist of Eserine. R. Hazard, E. Corteggiani and A. Cornec. (*C. R. Acad. Sci. Paris*, 1951, **223**, 211.) In view of the antagonistic action of procaine to eserine, sparteine, which resembles procaine as a ganglionary inhibitor, was also tested. The muscular contractions, induced by eserine in the heart of a chloralosed dog, disappeared completely under the influence of sparteine. As with procaine, the action of acetylcholine on the heart is suppressed without affecting the hypotensive action. The action of the two compounds is similar, with some secondary points of difference. G. M.

Tigloidine as a Substitute for Atropine in Parkinsonism. E. M. Trantner. (*Med. J. Austral.*, 1951, **1**, 751.) Tigloidine, the tiglic ester of pseudo-tropine, was tried as a substitute for atropine in 4 cases of parkinsonism, after it had been found that its effect on the action potential and contraction of the muscle-stimulated isolated sartorius of the toad was identical with that of atropine. The drug was employed in the form of the hydrobromide, commencing with a dose of 0·5 mg. 3 times daily. This dose was gradually increased to 4 mg., then to 16 mg., and finally to 64 mg. 3 times daily. The optimum beneficial effect of the drug appeared to be reached with a dosage of 40 to 48 mg. 3 times daily. The therapeutic effect on the symptoms of the disease was found to be identical with that of atropine, but tigloidine does not produce the undesirable side-effects of the latter, namely, excessive mydriasis, dryness of the throat, gastric disturbances and severe headache. The author discusses the clinical and chemical significance of these findings, and the common chemical groupings of atropine, tigloidine and procaine are compared with those of the antihistamine drugs found to be beneficial in parkinsonism. S. L. W.

Toxiferines, Pharmacology of. W. D. M. Paton and W. L. M. Perry. (*Brit. J. Pharmacol.*, 1951, **6**, 299.) The toxiferines are alkaloids extracted from the bark of *Strychnos toxifera*, which is known to be one of the principal ingredients of calabash curare. The object of these experiments was to discover the main properties of the compounds and to analyse the type of block produced at the neuromuscular junction and at the ganglionic synapse. Toxiferines I, II, IV, V, VI, IX, XI and XII were investigated. In most respects the toxiferines share the pharmacological properties of *d*-tubocurarine. The paralysis is such that the muscle is always capable of a full contraction if excited directly, and a typical endplate potential can be recorded, thus demonstrating that toxiferine causes a true neuromuscular block. Further features common to both drugs are the smooth onset of paralysis, antagonism by anticholinesterases, post-tetanic relief of block, and depression of the respiration. The paralysis must therefore be due to a raising of the threshold of the motor endplate to acetylcholine, and the toxiferines must thus be classed with *d*-tubocurarine rather than with drugs such as decamethonium which cause neuromuscular block by a prolonged depolarisation of the endplate. Three important deviations, however, from *d*-tubocurarine are noted: (1) a muscle blocked with toxiferine often responds to a tetanus with a sustained contraction; (2) post-tetanic potentiation seems greater in muscle exposed to toxiferine than in that exposed to *d*-tubocurarine; (3) neostigmine not only reverses block due to toxiferine but also potentiates the normal twitch tension. In their actions on other organs toxiferines show a qualitative resemblance to *d*-tubocurarine. The salient features of the pharmacology of the toxiferines are their high activity and the uniformity with which that activity is displayed in different species. Weight for weight they are more active than any other neuromuscular blocking agent. S. L. W.

Treburon, a new Heparin-like Anticoagulant. C. N. Mangieri, R. Engelberg and L. O. Randall. (*J. Pharmacol.*, 1951, **102**, 156.) Treburon is a synthetic sulphated polygalacturonic acid methyl ester methyl glycoside which has many properties in common with heparin. It has one-half the toxicity of heparin in mice and one-fourth to one-half the anticoagulant activity. Like heparin, it appears to exert its anticoagulant activity chiefly by virtue of its antithrombin activity. It has very little, if any, antithrombin activity at low

doses but measurable activity at high doses. Treburon produces no agglutination of platelets, no change in sedimentation rates and no precipitation of fibrinogen. Like heparin, it is readily neutralised by protamine sulphate, and the clotting-time of the blood which is prolonged can be returned to normal by the injection of an equal weight of protamine. The duration of action of equi-active doses of treburon and heparin is similar. Large doses produce infinite clotting times and the rates of return of the clotting times towards normal are parallel. A dose of 10 mg./kg. intramuscularly in rabbits has an intensity and duration of action at least as great as 5 mg./kg. of heparin. In preliminary clinical trials these findings have been confirmed in human subjects; 150 mg. of treburon had the same anticoagulant activity as 50 mg. of heparin intravenously in 14 cases. The intravenous injection of 500 mg. produced significant prolongations of clotting times. No toxic manifestations were observed in any of the patients. S. L. W.

Triethanolamine Trinitrate; Cardiovascular Effects of. K. I. Melville and F. C. Lu. (*Canad. med. Ass. J.*, 1951, **65**, 11.) In the isolated perfused rabbit heart triethanolamine trinitrate is shown to be an effective coronary vasodilator, comparing favourably with glyceryl trinitrate and exerting a more prolonged action. On repeated injection both substances give rise to the development of a slight tolerance. Glyceryl trinitrate exerts a more marked vasodepressor action, dose for dose, in anæsthetised animals, than does triethanolamine trinitrate. In small doses, both substances exert little significant action on the electrocardiogram or cardiac output (in heart-lung preparation); larger doses of both, however, depress cardiac output and slow the heart rate. Acute toxicity studies on rats and rabbits show both substances to be relatively non-toxic when injected intravenously. S. L. W.

Triethanolamine Trinitrate in Angina Pectoris. J. H. Palmer and C. G. Ramsey. (*Canad. med. Ass. J.*, 1951, **65**, 17.) Triethanolamine trinitrate (metamine) was administered to 5 patients with angina pectoris over 3-week periods. They alternated with 3-week control periods using placebos, (*a*) of enteric-coated chalk, and (*b*) of lactose. The tablets were taken 4 times daily; 3 times before meals and at bedtime. Each dose of the drug was 2 mg. While taking the drug all patients showed a reduction in the daily number of attacks. They were not informed which of the tablets contained the drug and which the placebos. S. L. W.

Tromexan (bis-3:3′-(4-oxycoumarinyl) ethyl acetate), Pharmacology of. M. Stirling and R. B. Hunter. (*Lancet*, 1951, **261**, 611.) Weight for weight tromexan has one quarter of the activity of dicoumarol in the production of hypoprothrombinæmia, but the action is quicker and excretion more rapid, giving greater control by oral administration and less danger of prolonged hæmorrhage. A wide variation of dosage is necessary to induce and maintain hypoprothrobinæmia, indicating that dosage must be decided individually. Divided doses are more effective than a single daily dose. The drug is of relatively low efficiency when compared with heparin therapeutically; its place is essentially in prophylaxis and not in the treatment of thrombosis. Water-soluble naphthaquinones given by mouth counteract tromexan hypothrombinæmia; proof is lacking that they produce any significant effect on that induced by dicoumarol. Sometimes vitamin K oxide was found to produce a rapid rise in prothrombin concentration. J. R. F.