ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Carvone in Essential Oils, Determination of. C. Schooltens. (*Pharm. Weekbl.*, 1950, **85**, 738.) Considerable variations are found in the results of determinations of carvone by different methods. Using a mixture of 65 per cent. of pure carvone with 35 per cent. of limonene, assays were performed by 7 different methods with, generally, low results. The U.S.P. process gave results ranging from 56·7 to 58·5 per cent.; that of the British Pharmacopœia from 60·3 to 63·9 per cent.; the sulphite method of the National Formulary VII gave 64·5 to 65·9 per cent. The latter method is thus to be preferred. Gravimetric determination with 2:4-dinitrophenylhydrazine is fairly satisfactory; results being from 63·7 to 66·0 per cent.

G. M.

Cocaine and Ethocaine, Chromatographic Separation of. R. Fischer and E. Buchegger. (*Pharm. Zentralh.*, 1950, **89**, 185.) The mixed bases (about 20 mg.), dissolved in carbon tetrachloride, are passed through a column of 5 g. of alumina (9 mm. diameter). Cocaine is then eluted by 20 ml. of carbon tetrachloride containing 4·5 per cent. of acetone, and subsequent treatment with 20 ml. of chloroform removes the ethocaine.

G. M.

Isopropyl Alcohol, Identification of. H. Auterhoff. (*Pharm. Zentralh.*, 1950, **89**, 293.) A number of colour reactions given by isopropyl alcohol with phenols are described. These are in general not very characteristic. More satisfactory is that with *p*-dimethylaminobenzaldehyde, as follows. A solution, containing about 20 per cent. of the alcohol, is treated with a little charcoal to remove higher alcohols and other impurities, and the mixture is filtered. A few ml. of the filtrate is layered on a 1 per cent. solution of *p*-dimethylaminobenzaldehyde in sulphuric acid. Isopropyl alcohol gives a bright red-violet ring in a few minutes, gradually becoming brown. Higher alcohols give an immediate brown colour. Brown or reddish brown rings are given by *n*-propyl alcohol, *n*-butylalcohol, *iso*butyl alcohol and *iso*amyl alcohol.

G. M.

Mineral Oil, in Fatty Oils, Detection of. H. Patzsch. (*Pharm. Zentralh.* 1950, **89**, 302.) The presence of unsaponifiable matter in an oil is not conclusive evidence of the presence of mineral oil. The unsaponifiable matter should be refluxed for 6 hours with its own volume of acetic anhydride. If it dissolves completely, the presence of lower wax alcohols is probable, but separation on cooling suggests sterols or high-melting fatty alcohols (e.g., myricyl alcohol). Undissolved material indicates mineral oil, or solid paraffins, which solidify as an upper layer on cooling. The latter may be distinguished by their melting-point, density and insolubility in a mixture of equal parts of alcohol and ether.

G. M.

Sugars, Reducing, Quantitative Paper Chromatography of. J. Montreuil. (*Bull. Soc. Chim. biol.*, 1949, **31**, 1639.) A series of unidimensional chromatograms are produced on a sheet of paper, the initial spots of 5 to 50 μg. of the sugars being spaced 4 cm. apart. After drying,

a single reference band is cut off and developed in order to give the exact localisation of the different spots. From the other part of the paper, a square, cut out large enough to include a spot, is extracted with water at 40°C. for 12 hours. To 5 ml. of the solution obtained 1 ml. of a 0·2 per cent. solution of potassium ferricyanide and 1 ml. of a reagent containing 0·3 per cent. of potassium cyanide and 1·6 per cent. of sodium carbonate are added. The mixture is placed on the water-bath for exactly 8 minutes, cooled quickly, and treated with 0·5 ml. of 2 per cent. oxalic acid solution and 2 ml. of a solution containing 5 g. of ferric sulphate and 75 ml. of phosphoric acid (d = 1·7) in 500 ml. The volume is then made up to 25 or 50 ml. After standing for 30 minutes in the dark, the colour is determined. A similar blank test is made on a piece of paper of the same dimensions.

G. M.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Digitalis Glycosides, Paper Chromatography of. A. B. Svendsen and K. B. Jensen. (*Pharm. Acta Helvet.*, 1950, **25**, 241.) Mixtures of chloroform, methanol and water were found to be suitable for the paper chromatography of digitalis glucosides. The chloroform was first freed from alcohol by washing and drying, and the solvent mixtures were prepared by prolonged shaking in a thermostat, being then allowed to settle. In order to detect the position of the spots, the paper, after drying, was sprayed with a 25 per cent. solution of trichloracetic acid in chloroform, then heated for 2 minutes at 100°C. A positive result is shown by a fluorescence varying in intensity and colour with different glucosides. R_F values observed for 10 of these substances are given in the table below, the solvent mixtures (I, II and III) being prepared from 10 parts of chloroform, 5 of water, and respectively 2, 4 or 8 of methyl alcohol.

	Solvent Mixture		
	I	II	III
Purpurea glucoside A	0·07	0·10	0·15
Digitoxin	0·88	0·91	0·90
Digitoxigenin	0·92	0·93	0·92
Purpurea glucoside B	0·02	0·03	0·05
Gitoxin	0·76	0·81	0·82
Gitoxigenin	0·82	0·84	0·84
Digilanid A	0·37	0·46	0·49
Desacetyldigilanid A	0·07	0·10	0·15
Digilanid B	0·14	0·18	0·25
Desacetyldigilanid B	0·02	0·03	0·05
Digilanid C	0·08	0·12	0·15
Desacetyldigilanid C	0·01	0·01	0·02
Digoxin	0·68	0·75	0·76
Digoxigenin	0·58	0·64	0·61

The process was applied to *Digitalis purpurea* leaves prepared in different ways. The drug, stabilised by alcohol vapour, contained mainly primary glycoside, with small amounts of secondary glycoside and genin. Leaves dried by moderate heat showed only small amounts of primary glycoside, but secondary glycoside and genin in larger amounts. The amount of genuine glycoside appeared somewhat greater in leaves dried at 80°C. than in those treated at 55° to 60°C.

G. M.

Digitoxoside and Gitoxoside, Colour Reactions of. P. Bellet. (*Ann. pharm. franc.*, 1950, **8**, 471.) Suspend about 0·5 mg. of commercial

digitoxoside (" digitaline ") in 5 ml. of phosphoric acid and allow to stand for 5 minutes; an intense yellow colour is produced, and the presence of gitoxoside is indicated by an intense yellow-green fluorescence in filtered ultra-violet light. Less than 1 per cent. of gitoxoside can be detected by this method, compared with 10 per cent., using the Keller-Kiliani reaction. Although the yellow colour in daylight is due to the sugar moiety, the fluorescence in filtered ultra-violet light is due to the aglucone, the actual fluorescent substance being dianhydrogitoxigenin, having conjugated double bands in positions 14-15, 16-17 and 20-22. It may be postulated that resonance at carbon atoms 14, 16 and 20 leads to the formation of mesomeric states responsible for the fluorescence. In anhydrodigitoxigenin, the double bonds are too far apart for appreciable resonance to occur. Oleandrin and honghelin also give a fluorescence with phosphoric acid in ultra-violet light.

G. B.

Starch, Hydrolysis of, by Hydrochloric Acid. A. Leman and P. Didry. (*C.R. Acad. Sci. Paris*, 1950, **231**, 443.) The first stage of the breakdown of starch into non-reducing intermediate products proceeds the more rapidly the more concentrated the acid, but the optimum acidity for complete and rapid conversion into glucose is approximately normal. The actual volume of acid used is unimportant, provided that it is at least 20 ml. per 0·01 mol. of starch. For a given quantity of hydrochloric acid, hydrolysis proceeds much further when it is more concentrated, provided that the concentration does not exceed normal. Using N hydrochloric acid, hydrolysis is practically complete on the water-bath in 50 minutes; 0·5 N acid requires 2½ hours; and 0·4 N 4 hours. G. M.

TOXICOLOGY

Anthisan Poisoning, Acute. A. A. Miller and E. Pedley. (*Brit. med. J.*, 1950, **1**, 1115.) The authors describe a fatal case of poisoning in a child of 16 months who ate several tablets containing 0·1 g. of anthisan; the actual number taken was unknown, but probably was not more than 6. No effects appeared after about 2 hours, when the child began to moan and became unconscious; muscular twitchings occurred and there was profuse catarrhal discharge and foaming at the mouth and nose. Death occurred about 3 hours after eating the tablets. At necropsy a greenish-coloured watery fluid poured from the mouth and nose on moving the body. The main signs were those of acute passive congestion; the meninges, spleen, kidneys and liver were all congested. The stomach contained about 6 oz. of watery undigested food showing a marked greenish tinge. A solution of the base in dilute hydrochloric acid gives a yellowish-green precipitate with Mayer's reagent and a trace of the dry base gives a bright cherry red colour with concentrated sulphuric acid. These reactions were obtained from the stomach contents submitted to the Stas-Otto extraction process. S. L. W.

Barium, Toxicological Determination of. I. L. Castagnou and S. Larcebau. (*Bull. Trav. Soc. Pharm. Bordeaux*, 1950, **88**, 23.) After the administration of a fatal dose (0·11 g./kg. of body weight) to a cat, 76·8 per cent. was recovered from the organs, by far the greater part being in the intestines. With other cats, which received larger doses causing considerable vomiting, the recovery was less than 1 per cent. Since in all these cases the difference between the total barium recovered from all organs, and that from the stomach and intestines, was of the order of 1

to 2 mg., it may be stated that only a few mg., absorbed and fixed in these organs, is fatal. If similar results had been obtained in a toxicological examination, it would not have been possible to affirm that death was due to barium poisoning. Rabbits are much less affected by barium, and in this case appreciable amounts were recovered from all organs. The method adopted for the determination of barium (ashing and fusion with sodium and potassium carbonates) does not appear to be altogether satisfactory, and this question should be investigated. G. M.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Chorionic Gonadotropin, Effect of Colloids on Action of. K. Pedersen-Bjergaard and M. Tønnesen. (*Dansk Tidsskr. Farm.*, 1950, **24**, 271.) In view of the reported action of polyvinylpyrrolidone in delaying the absorption of extracts of the posterior lobe of the pituitary gland, the effect of a number of colloids on the action of gonadotropin was examined, using immature female rats. With hormone from the serum of pregnant mares, the action was not altered by colloids. With subcutaneous injection of chorionic gonadotropin, the effect was increased 4 times by the addition of colloids. The minimum percentage concentration of the colloids which, when used in solution as solvent for 0·3 I.U. of chorionic gonadotropin, caused a positive Allen-Doisy reaction in 50 per cent. of the immature rats used, was as follows: polyvinylpyrrolidone, 25; gelatin, 15; acacia, 15; soluble starch, 10; agar, 1; carboxymethylcellulose, 0·75; tragacanth, 0·125; Irish moss, 0·0625. A similar increase of action was also observed, with polyvinylpyrrolidone, when the chorionic gonadotropin was administered to female Rhesus monkeys. In man, the excretion of the hormone was decreased by 50 per cent. by the addition of this colloid. G. M.

Ribonucleotides, Chromatography of. J. Montreuil and P. Boulanger. (*C. R. Acad. Sci. Paris*, 1950, **231**, 247.) Chromatography has already been applied to the separation of the bases produced by the hydrolysis of nucleic acid. In order to avoid the difficulties resulting from de-amination during acid hydrolysis, the authors have applied the method to the nucleotides. The solution, obtained by hydrolysis with 2 per cent. sodium hydroxide for 24 hours at ordinary temperature, is applied directly to the paper. Alternatively, separation may be facilitated by passing the solution through a cation exchange column, which holds back the adenylic and cytidylic acids, which may be eluted by dilute ammonia. Solvents used and R_F values obtained are as follows:—

	Phosphoric acid	Xanthylic acid	Uridylic acid	Guanylic acid	Cytidylic acid	Adenylic acid
Phenol 40 ; isopropyl alcohol 5 ; formic acid 5 ; water 50 ...	0·17	0·23	0·33	0·44	0·50	0·64
Phenol 50 ; N sulphuric acid 50	—	0·19	0·24	0·28	0·37	0·50
Phenol 45 ; formic acid 5 ; water 50	0·31	0·36	0·44	0·52	0·60	0·70
Isopropyl alcohol 60 ; glycol monochlorhydrin 30	—	—	0·30	0·14	0·24	0·26
N hydrochloric acid 10 ...	0·70	—	0·62	0·36	0·43	0·43

The papers are dried at 80° C. and sprayed with Hanes and Isherwood reagent (ammonium molybdate in hydrochloric-perchloric acids). The colour appears on exposure to sunlight; it may be increased by treatment with hydrogen sulphide. The nucleotide may be determined by cutting out the spot, digesting with sulphuric and nitric acids, and determining the phosphate colorimetrically. The chromatography may be made two-dimensional by using phenolic and alcoholic solvents. G. M.

Vitamin A, Synthesis of Ether-Oxides of. R. Golse and J. Gavarret. (*Bull. Trav. Soc. Pharm. Bordeaux*, 1950, **88**, 57.) β-Ionone is condensed in presence of zinc with propargyl bromide, giving a β-acetylenic alcohol. This compound is condensed with methoxy-4-butanone-2, giving (trimethyl-2′:6′:6′-cyclohexene-1′-yl)-9-dimethyl-3:7-methoxy-1-nonene-8-yne-4-diol-3:7. This compound is converted into vitamin A methyl ether by partial reduction with colloidal palladium, treatment with phosphorus bromide, and removal of hydrogen bromide by potassium hydroxide. By this synthesis, only two stages of condensation are required, and, unlike the similar method of Milas and others, there is no question of any change in the position of the double bond. G. M.

Vitamin B_{12} and Desoxyribosides as Growth Factors for Lactic Acid Bacteria. E. Kitay, W. S. McNutt and E. E. Snell. (*J. Bact.*, 1950, **59**, 727.) Eighteen strains of lactic acid bacteria, representative of 5 different species of *Lactobacillus* and one species of *Leuconostoc*, were found not to grow in a medium complete with respect to known amino-acids and synthetic vitamins, and supplemented by tomato juice and an enzymatic digest of casein. All grew when thymidine was added to the medium. The thymidine could be replaced in most cases by hypoxanthine desoxyriboside, guanine desoxyriboside, adenine desoxyriboside, cytosine desoxyriboside, or by high levels of desoxyribonucleic acid. Vitamin B_{12} could replace thymidine or other desoxyribosides for many, but not all, of the organisms. Ascorbic acid, thioglycollic acid, cysteine or glutathione could also replace thymidine. Vitamin B_{12} and vitamin B_{12b} added aseptically were equally active growth-promoters, but if autoclaved in the medium, the activity of vitamin B_{12b} was only about one-seventh that of vitamin B_{12}. H. T. B.

BIOCHEMICAL ANALYSIS

Creatine, Creatinine and Related Compounds in Urine; Determination by Paper Chromatography. S. R. Ames and H. A. Risley. (*Proc. Soc. exp. Biol. N.Y.*, 1948, **69**, 267.) A 0·025-ml. aliquot of urine is placed on the paper strip and developed for 15 to 20 hours with water-saturated solutions of butyl alcohol, phenol, or lutidine-collidine (equal parts). The strip is air-dried, heated at 100°C. for one hour to convert creatinine to creatine and glycocyamine to glycocyamidine, cooled to room temperature and sprayed with a mixture of 1 part of 10 per cent. sodium hydroxide and 5 parts of saturated trinitrophenol solution. The presence of creatinine or related compounds is shown by the appearance of orange spots. Water-saturated solutions of butyl alcohol and lutidine-collidine separate creatine and creatinine better than water-saturated phenol, although the latter gives an excellent resolution between glycocyamine and the others. The smallest amount of creatine or creatinine which could be detected was 11μg. It is possible to test for amino-acids and creatinine and related compounds on the same strip by spraying the reagent on to the paper previously treated with ninhydrin. In an alternative

procedure for the determination of creatine the strip is not heated, but is air-dried and sprayed with a solution of diacetyl in alcoholic sodium carbonate, when the presence of creatine is indicated by a pink colour. The smallest amount of creatine detectable by this method is 33μg.; creatinine does not react and glycocyamine and glycocyamidine give no colour in quantities up to 200g., but several amino-acids interfere. G. R. K.

Prothrombin Levels in Blood, A New Improved Method for Determination of. A. Goldfeder, D. Bloom and M. Weiner. (*Science*, 1950, **111**, 365.) A simple method of determination of the prothrombin time, using only 5 cmm. of whole blood, is as follows. Calibrated 5 cmm. capillary tubes are filled with a 2 per cent. solution of potassium oxalate and dried in an oven. A prepared capillary tube is filled with blood and, with the aid of a small rubber bulb, the blood is quickly expelled into 15 cmm. thromboplastin solution on a glass slide, and gently mixed. The mixture is drawn gently in and out of the capillary tube, the tube being raised at each filling until a fibrin strand forms. A stop-watch is used to record the time required after the addition of the blood to the thromboplastin solution, for the formation of a fibrin strand. In preparing the thromboplastin solution, whole blood, instead of plasma, is used, the blood being taken into tubes with dry oxalate. In precise work, it is necessary to take into account viriations due to changes in room temperature and, in subjects with low hæmocrit values, the variable plasma volume per ml. of whole blood. G. B.

Testosterone, Colorimetric Assay of. A. T. Nielsen. (*Acta Endocrinol.*, 1948, **1**, 362.) The procedure is based upon the blue colour formed when testosterone is heated with sulphuric acid and subsequently mixed with alcoholic sulphuric acid. Two modifications are described, as follows :—(a) a test-tube (180 × 20 mm.) containing dry testosterone is placed in an ice-bath and 1ml. of sulphuric acid added with stirring. The tube is heated in boiling water for 5 minutes, replaced in the ice-bath, 4ml. of 25 per cent. alcoholic sulphuric acid (1 vol. sulphuric acid diluted with 3 vol. methyl alcohol) added with stirring, and the mixture placed in a thermostat at 29°C. for 30 minutes. After transferring to a photometer cell (1cm.) the extinction is read at 600mμ. The blank consists of reagents without testosterone. (b) The testosterone is dissolved in 0·2 ml. of methyl alcohol prior to the addition of sulphuric acid. The mixture is heated in boiling water for 30 seconds and the test continued as described under (a). At room temperature maximum colour is obtained within 15 to 25 minutes after admixture of the alcoholic sulphuric acid reagent. Colour intensity is plotted against the time (in minutes) elapsing after the addition of alcoholic sulphuric acid. The colour is stable for at least an hour. The colour intensity obtained with testosterone dissolved in methyl alcohol is definitely higher than that given by the dry substance but a more accurate and reproducible action is obtained with the latter, but method (b) is necessary for the determination of testosterone isolated from oily solutions. The accuracy of the method is of the order of ± 5 per cent. 3 substances are known to give a colour reaction very similar to testosterone; Δ^4-androstene-3β,17-dione, deoxycorticosterone and dehydroandrosterone, but the first of these may be effectively removed by means of nicotinic acid hydrazide and in the case of the last 2 the colour intensity may be decreased by extending the heating time. The authors outline the practical application of the method to the analysis of pharmaceutical preparations. S. L. W.

Tyrothricin, *in vitro* Assay of. R. J. Reedy and S. W. Wolfson. (*J. Amer. pharm. Ass., Sci. Ed.*, 1950, **39**, 1.) A routine procedure is reported

in which the normal serial dilution method, a modification of that of Dubos, is used. The organism used, *Streptococcus fæcalis* (M-19), has been found more sensitive to the action of tyrothricin than streptococcus H-69D-5, and the possibility of interference by other ingredients of emulsions and ointment-type products is thus reduced. Details of the method are given for alcohol and propylene glycol solutions and for extraction of tyrothricin from oily and ointment preparations. It was noted that the organism occasionally dissociated, with the development of gramicidin-resistant variants, when transferred daily in the assay broth. This may be overcome by dilution of the nutrients or sub-culturing until the desired sensitivity is regained. A supplementary method for use as a check is also given since the tyrothricin present in many commercial preparations cannot be completely recovered by the routine procedure. It was found necessary to extract the tyrothricin before assay to avoid interference by the bases. G. R. K.

PHARMACY

DISPENSING

Adrenaline, Preservation of Solutions of. M. P. Girard and G. Kerny. (*Ann. pharm. franc.*, 1950, **8**, 463.) Solutions containing 0·025 per cent. of adrenaline in distilled water with the addition of sodium bisulphite, filled into ampoules under nitrogen or carbon dioxide and sterilised by tyndallisation, retain at least 90 per cent. of their adrenaline content for five years. The solution prepared with hydrochloric acid and sodium bisulphite keeps equally well, but the solutions are not well preserved by hydrochloric acid alone. Solutions stored in ampoules of certain kinds of yellow glass deteriorate rapidly; apparently the decomposition is accelerated by the iron which the solution absorbs from the glass. There is good agreement between the colorimetric assay and the biological assay, when bisulphite alone is the preservative, but in the presence of hydrochloric acid and bisulphite, the chemical assay gives the higher result. Colour of the adrenaline solutions cannot be used as a criterion of their state of preservation because some colourless solutions have been found to have lost 60 per cent. of their titre. G. B.

Morphine Solutions, Decomposition of, on sterilisation. C. G. van Arkel and J. H. van Waert. (*Pharm. Weekbl.*, 1950, **85**, 319.) The absorption spectrum of morphine hydrochloride has a maximum at 282·5 mμ and a minimum at 260 mμ. Pseudomorphine, the oxidation product of morphine, shows a higher absorption, so that an increase in the absorption minimum is the best indication of decomposition of a solution of morphine hydrochloride. It was found that a 1 per cent. solution of morphine hydrochloride heated for 1 hour at 100°C., showed 1 per cent. of decomposition, but that this could be completely prevented by the presence of 0·05 per cent. of sodium bisulphite. The decomposition is somewhat greater in more dilute solutions. Keeping of the sterilised solution in brown ampoules for 3 months resulted in a slight discoloration in the absence of sodium bisulphite, but the actual decomposition was too small to permit of spectrographic determination. G. M.

Sterilisation in Free Steam. K. Steiger. (*Pharm. Acta Helvet.*, 1950, **25**, 107.) Tests were made to determine if reliable sterilisation could be attained in 30 minutes at 100°C. in presence of bactericidal substances. The

test organism was a suspension of highly resistant soil spores, which was added in moderate quantity to clear injection solutions. The results showed that sterilisation was attained, without any addition, at *p*H 3·2 or lower. The addition of 0·01 per cent. of sodium fluoride or of 0·1 per cent. of chlorbutol was of little advantage, but 5 per cent. of alcohol resulted in complete sterilisation up to a *p*H of 6·1. Esters of *p*-hydroxybenzoic acid, also in presence of 5 per cent. of alcohol, produced no gain in efficacy, but the combination of 0·1 per cent. of chlorbutol with 5 per cent. of alcohol increased the range of effective action up to *p*H 9. Equally effective (without alcohol) were 0·01 per cent. of potassium oxyquinoline sulphate or 0·0001 per cent. of phenylmercuric borate. With this last addition, 5 minutes' heating in flowing steam was sufficient to produce complete sterility under the conditions of the experiments. G. M.

NOTES AND FORMULÆ

Aurothioglucose (Solganal). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950,**142,** 816.) Aurothioglucose $C_6H_{11}AuO_5S$, is a yellow to yellowish-green, odourless, tasteless powder, soluble in water and insoluble in acetone, alcohol, chloroform and ether; aqueous solutions decompose on standing. When dried *in vacuo* over phosphorus pentoxide for 24 hours the loss in weight is not greater than 1 per cent.; specific rotation $[\alpha]_D^{25°C.}$, +65° to + 73°. It contains 49·4 to 51·0 of gold and 8·0 to 8·4 per cent. of sulphur; it is assayed for gold by boiling with nitric acid, filtering and igniting the residue; the filtrate is used for the assay of sulphur by boiling with hydrochloric acid, diluting with water and precipitating the sulphate with barium chloride. Aurothioglucose is administered by the intramuscular injection of a suspension in oil in the treatment of active rheumatoid arthritis and non-disseminated lupus erythematosus. G. R. K.

Diglycocoll Hydroiodide-Iodine (Bursoline). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **142,** 990.) Diglycocoll hydroiodide-iodine, $2(HO_2C.CH_2.NH_2—HI—NH_2.CH_2.CO_2H) + I_2$, is a dark lumpy powder with a strong odour of iodine, freely soluble in water, almost insoluble in chloroform and only very slightly soluble in alcohol, although the iodine component is soluble in alcohol; a 0·1 per cent. solution in water has *p*H about 3·0. It contains 6·82 to 7·02 per cent. of nitrogen (determined by the Kjeldahl method), 30·5 to 32·0 per cent. of titratable iodine (determined by adding sulphuric acid and potassium iodide and titrating with sodium thiosulphate) and 30·5 to 32·0 per cent. of iodine present as iodide (determined by boiling with potassium iodate and sulphuric acid to remove free iodine, cooling, adding potassium iodide and titrating with sodium thiosulphate). Diglycocoll hydroiodide-iodine is used for the disinfection of drinking water. G. R. K.

Dimethyltubocurarine Iodide (Metubine Iodide). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **142,** 1142.) Dimethyltubocurarine iodide is the dimethyl ether of *d*-tubocurarine iodide and occurs as a white to pale yellow, odourless, crystalline powder, slightly soluble in water, dilute hydrochloric acid and dilute sodium hydroxide, very slightly soluble in alcohol and almost insoluble in benzene, chloroform and ether; when heated to 257°C. it decomposes with evolution of gas. The extinction coefficent $E_1^{1\ \text{per cent.}}_{1\ \text{cm.}}$ at 2800 Å is 74±1·5. It gives a pink precipitate with ammonium reineckate and a yellow precipitate with trinitrophenol; it is distinguished from tubo-

curarine chloride by treating with Folin-Ciocalteu reagent, diluting with water, adding sodium carbonate and heating in a water-bath; the final solution is colourless or very faintly blue. Dimethyltubocurarine iodide loses not more than 7 per cent. of its weight when dried *in vacuo* at 75°C. for 8 hours. It contains 2·80 to 3·10 per cent. of nitrogen (by the Kjeldahl method) and 98 to 102 per cent. of dimethyltubocurarine iodide, determined by measuring the optical density of a 0·005 per cent. solution at 2800 Å and dividing by 7·4; the specific rotation $[\alpha]_D^{25°C.}$ of a 0·25 per cent. solution in water is +150° to +160°. The potency is determined by observing the head-drop response following intravenous injection in rabbits. Dimethyltubocurarine iodide has an action similar to that of tubocurarine chloride but is more potent, having a shorter onset and more prolonged action. G. R. K.

Hydroxyamphetamine Hydrobromide (Paredrine Hydrobromide). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **142**, 816.) Hydroxyamphetamine hydrobromide is 1-(*p*-hydroxyphenyl)-2-aminopropane hydrobromide, $HO.C_6H_4.CH_2CH(NH_2).CH_3,HBr$. and occurs as a white crystalline powder with a faint odour, m.pt. 189° to 192°C., very soluble in water and alcohol, and almost insoluble in benzene and ether; a 2 per cent. aqueous solution has *p*H 4·5 to 5·5. The free base, obtained by saturating an aqueous solution with potassium carbonate, extracting with ether and evaporating, is a white to faintly yellow crystalline solid, m.pt. 127° to 129°C., soluble in acids and alkalis. Hydroxyamphetamine hydrobromide is distinguished from other amines by the emerald-blue colour it gives with ammonium molybdate and sulphuric acid, and the purple colour with ferric chloride. When diazotised and extracted with chloroform, the chloroform is coloured amber. It loses not more than 0·5 per cent. of its weight when dried at 110°C. for 3 hours; ash, not more than 0·1 per cent. A 0·0015 per cent. solution shows ultraviolet absorption maxima at 2550 Å ($E^{1\ per\ cent.}$ = 370±5) and 2780 Å, with a minimum at 2440 Å; the ratio of the observed optical densities at 2250 Å and 2780 Å is 4·3 to 5·4. The content of hydrogen bromide is 34·0 to 35·2 per cent. and the content of hydroxyamphetamine hydrobromide, 97·5 to 101·0 per cent.; the latter is determined by liberating the base by the method described above, dissolving in sulphuric acid and titrating the excess of acid. Hydroxyamphetamine hydrobromide is used locally as a 1 per cent. solution to reduce swelling of the nasal mucosa. G. R. K.

Mephenesin (Oranixon). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **143**, 655.) Mephenesin is 3-*o*-toloxypropane-1:2-diol, $CH_3.C_6H_4.O.CH_2.CHOH.CH_2OH$, and occurs as an odourless, crystalline white powder, m.pt. 67° to 72°C. It is freely soluble in alcohol, chloroform and ether, and sparingly soluble in benzene and water; a saturated aqueous solution has *p*H 6·0. The extinction coefficient $E_{1\ cm.}^{1\ per\ cent.}$ at 2700 Å is 81±3. When mephenesin is dissolved in sulphuric acid and treated with formaldehyde an intense red colour develops. The loss on drying *in vacuo* over phosphorus pentoxide for 24 hours is not more than 0·1 per cent. The content of mephenesin is 98 to 102 per cent., determined by measuring the light absorption of a 0·006 per cent. solution at 2700 Å. Mephenesin resembles curare in action and is used for the production of muscular relaxation in light surgical anaesthesia. It may also be tried in the treatment of spasticity and tremor in Parkinson's disease, of muscle spasm in hemiplegia, tetanus and certain other spastic conditions, and of athetoid or

choreiform movement. It antagonises the action of strychnine and potentiates that of barbiturates. It has a low toxicity and is given in a dose of 1 g. 3 to 5 times a day, usually as tablets or elixir. G. R. K.

PHARMACOGNOSY

***Colchicum Autumnale*, New Compounds from.** F. Santavy. (*Pharm. Acta Helvet.*, 1950, **25**, 248.) By chromatographic separation of an extract of *Colchicum autumnale*, the following substances were separated: substance I, substance F, substance G, substance J, colchicine, substance D, substance B and a crystalline mixture of substances C and E_1, a phytosterol mixture, saccharose and 2-hydroxy-6-methoxybenzoic acid. The quantities of substances F and G obtained were comparable with that of colchicine. Substance G was partially converted into colchicine by chromatography on alumina. G. M.

Coriander Fruit, Dutch. F. H. L. van Os. (*Pharm. Weekbl.*, 1950, **85**, 732.) A number of strains of coriander, differing in the size of fruit, are generally recognised. An examination was made of coriander grown in Holland with a view to determining whether different varieties, characterised by large and small fruits, were present. This did not appear to be the case, the different sizes of fruit in the harvest being apparently derived from umbels flowering at different periods. In the determination of the essential oil, prolonged distillation results in fatty acids distilling over. A correction should be made for this by determining the acid value of the distilled oil. It is important that the material should not be ground too finely before distillation, as this results in a loss of essential oil. G. M.

Stramonium, Assay and Comparative Method of Drying. M. Rubin and L. E. Harris. (*J. Amer. pharm. Ass., Sci. Ed.*, 1950, **39**, 477.) Freshly collected stramonium leaves were dried (1) in air, (2) at 40°C. and (3) by freeze-drying. The fresh leaves contained 89 per cent. of water, and after drying in air they contained about 9·45 per cent. At 40° C. the water content was 8 per cent. and 5·45 per cent. after freeze-drying. These figures were determined by the U.S.P. toluene method. Assays were then carried out on each sample by (a) U.S.P. XIII method of acid titration of the isolated bases (b) the hydrolytic method of Reimers (*Quart. J. Pharm. Pharmacol.*, 1948, **21**, 470), and (c) a chromatographic method. For the last method 10 g. of the sample was macerated with 10 ml. of ammonia solution (27 per cent. NH_3 w/w) and 30 ml. of ether for 1 hour. This extract was adjusted to 100 ml. It was then run through an alumina column and the adsorbed alkaloids were eluted with alcohol. After elution the alkaloidal solution was evaporated to dryness on a water bath, heated for 15 minutes and the residue was dissolved in 20 ml. of ether and 15 ml. of 0·02N sulphuric acid was added. The ether was then driven off and the excess of acid titrated against 0·02N sodium hydroxide with methyl red as the indicator. Results obtained by the U.S.P. method were slightly lower than those obtained by the other methods, which were almost identical. The hydrolytic method was the least time-consuming. A. D. O.

PHARMACOLOGY AND THERAPEUTICS

Aureomycin in the Treatment of Poliomyelitis. E. Appelbaum and R. Saigh. (*J. Amer. med. Ass.*, 1950, **143**, 538.) 38 patients with non-paralytic poliomyelitis were treated with aureomycin during the early phase

of the disease, while 66 patients served as controls. Aureomycin was given in a dosage of 1 to 1·5 g. daily to children up to 4 years of age, 4 g. to those of 5 to 16 years of age, and 6 g. to patients above that age. The treatment was continued for 7 days. The drug was well tolerated except for transient nausea or vomiting in some cases. The clinical results were about the same in the treated and the control patients and the authors conclude that in this study the early use of aureomycin did not appear to exert any favourable effect on the clinical course of the disease. S. L. W.

Benzodioxan, Action of, in Man. F. T. G. Prunty and H. J. C. Swan. (*Lancet*, 1950, **258**, 759.) The experiments described were planned to contrast the effect of benzodioxan (933F, 2-(1-piperidylmethyl)-1 : 4-benzodioxan) on hypertension produced by adrenaline in normal subjects with that produced by noradrenaline and to observe the resultant circulatory changes. The benzodioxan was given intravenously in a dose of 0·25 mg./kg. of body weight to 4 patients undergoing adrenaline infusion and 3 undergoing noradrenaline infusion. Following the injections there was an initial fall in blood pressure due to peripheral vasodilatation succeeded after a few seconds by a rise to a level above that observed before injection. Both the fall and the rise were accompanied by tachycardia, which may have been responsible for an increase in cardiac output, resulting in the rise in blood pressure. In no patient was there a sustained fall in blood pressure as reported by other workers, and there is therefore some doubt about the reliability of the drug in differentiating phæochromocytoma with hypertension from hypertension due to other causes. G. R. K.

Chloramphenicol in Typhoid Fever. R. A. Good and R. D. MacKenzie. (*Lancet*, 1950, **258**, 611.) A clinical trial in 13 cases of typhoid fever, alternate cases being given chloramphenicol, showed that this antibiotic has a specific clinical effect on the disease. The most striking effect was its control over the patients' temperature which fell to normal in 72 hours. At the same time it relieved the persistent headache and reduced the toxæmia, the patients felt better, appetites returned and abdominal discomfort was lessened. Half the treated cases developed relapse symptoms and in 2 these were sufficiently severe to justify further treatment. All responded satisfactorily to a second course of treatment, which may indicate that longer courses of treatment, larger doses, or a routine second course should be given to all primary cases. The relapses in the treated cases occurred later than in the untreated controls. The condition of the 3 control cases which relapsed became so severe that chloramphenical was given, to which they responded satisfactorily. The course of treatment with chloramphenicol lasted 8 days, 4 g. being given in the first hour, followed by 0·25 g. two-hourly until temperature was normal, and then 4-hourly for the rest of the course. No toxic effects due to the drug were noted, and there was no evidence that *S. typhi* developed an increased resistance to the drug. From the bacteriological point of view the drug did not prove efficient as in 3 of the treated cases fæcal excretion of *S. typhi* which stopped temporarily during treatment started again either before or at the end of the course. S. L. W.

Chloramphenicol in Typhoid Fever. A. L. K. Rankin and A. S. Grimble. (*Lancet*, 1950, **258**, 615.) Chloramphenicol is effective in the treatment of typhoid if given in adequate dosage over a minimum period of 18 to 20 days, an initial dose of 4 g. being followed by 3 g. daily until the patient is apyrexial, then 1·5 g. daily for a week, and 1 g. daily for the final

week. The incidence of relapses is about the same with or without the drug. Thus, in this series of 17 cases there were 8 relapses, 4 among the 9 cases treated with chloramphenicol and 4 among the 8 cases not treated with the drug. In cases treated with chloramphenicol for 8 or 9 days *S. typhi* was isolated from the fæces and/or urine both during and after treatment, but in cases treated for 18 days *S. typhi* was not isolated from the fæces and/or urine after completion of the course. The avera e duration of fever in cases treated with chloramphenicol was 3 days whereas in controls it was 14½ days: there was very noticeable, and in some cases dramatic, improvement in the patients' condition within 48 to 72 hours. No toxic or side-effects were observed in any of the patients treated with chloramphenicol. S. L. W.

Ch'oramphenicol in Typhoid Fever. A. H. El Ramli. (*Lancet*, 1950, **258,** 618.) In a series of 200 cases treated with chloramphenicol the average duration of fever after commencement of treatment was 3·5 days, the relapse rate in patients followed up for at least three weeks was 27·5 per cent., and the mortality was 6·5 per cent. Relapses were fewest on 12-hourly doses. The response to the drug was little affected by the severity of the condition or duration of symptoms before treatment. Toxic effects observed, due to chloramphenicol, were anorexia and gastric upset, stomatitis and glossitis, and mental apathy. The best scheme of dosage appears to be 50 mg./kg. of body weight over the first 2 hours, and then 25 mg./kg. every 12 hours until the temperature becomes normal, and half that dose for 14 days in convalescence. Rest in bed and symptomatic treatment should continue, as before chloramphenicol therapy, for at least another week. Chloramphenicol provides the most efficient treatment for typhoid fever so far devised. S. L. W.

D'gitalis, Differences in Standardisation of. E. Keeser. (*Arch. Pharm., Berl.*, 1950, **283,** 166.) Considerable differences were found between biological assays of digitalis preparations in different laboratories, although the results in both cases were compared with the international standards. In order to obtain uniform results, it is essential to specify details of the method of assay, and also of the preparation of the solution. It is well known that the rate of administration of the preparation is of importance, especially with slow-acting glucosides such as digitoxin. Exact definition of the end-point is essential. In the case of specialities produced by commercial firms, it is desirable that they should publish the essential features of the method of preparation and declare any additions. G. M.

Dinaphthalene Methane Silver Disulphonate, Trichomonicidal Action of. G. R. Sluming. (*Brit. med. J.*, 1950, **1,** 1116.) A 1 per cent. aqueous solution of this substance, known under the proprietary name of viacutan, was successfully employed in the treatment of 20 resistant cases of trichomonal vaginitis. The treatment consisted of 6 weekly paintings of the vagina with the solution without any preliminary cleaning, followed by insufflation of acetarsol compound powder in non-pregnant cases and acetarsol compound pessaries daily during the course of treatment. In none of the cases was a relapse reported within 6 months. Viacutan has *p*H 4·5 to 5 and contains a wetting agent to assist spreading and a water-soluble yellow dye to indicate the areas treated. It has the property of attaching itself to and penetrating animal tissue. It inhibits the growth of both Gram-positive and Gram-negative organisms in 24-hour broth cultures in a dilution of 1 in 16,000, and its activity is enhanced by the presence of blood, pus or serum.

In addition to its use in trichomonal vaginitis it may also be employed for pre-operative sterilisation of the skin in obstetrical and gynæcological operations.

S. L. W.

β-Naphthyldi-2-chloroethylamine (R48) in Leukæmia, Hodgkin's Disease and Allied Diseases. W. B. Matthews. (*Lancet*, 1950, **258**, 896.) The author reports on clinical trials with this substance on 17 patients. Five cases of Hodgkin's disease all showed some improvement, but the results were on the whole disappointing and were certainly no better than those to be expected with nitrogen mustard. Two cases of reticulosarcoma and 2 of acute leukæmia did not respond. Good remissions were obtained in 2 out of 3 cases of chronic myeloid leukæmia, and in 2 out of 4 cases of chronic lymphatic leukæmia. One case of polycythæmia vera was still in remission a year after treatment. Gastric disturbances with R48 were less common and less severe than with nitrogen mustard, and the only other toxic effect was cystitis which occurred in one patient. The drug was given by mouth in tablets each containing 100 mg., which were chewed before being swallowed and taken after meals: the taste was inoffensive. The daily dose never exceeded 600 mg., and usually 300 to 400 mg. was given in divided doses. The duration of each course of treatment was determined by the response of the leucocyte count and varied within wide limits. In patients with a non-leukæmic blood picture treatment proceeded until the leucocyte count fell to about 3,000 per c.mm.; in chronic leukæmia the aim was to reduce the leucocyte count to about 20,000 per c.mm. On roughly comparable doses the average duration of a course in Hodgkin's disease was 48 days; in chronic myeloid leukæmia 45 days; and in chronic lymphatic leukæmia 20 days. The author concludes that the action of R48 is essentially similar to that of nitrogen mustard but slower and more easily controlled.

S. L. W.

Œstrogens, Artificial, Activity of, Determined by Experiments on Rats. G. L. M. Harmer and W. A. Broom. (*Lancet*, 1950, **258**, 850.) Using stilbœstrol as the reference substance, and giving it an arbitrary value of 100 for its potency by mouth and subcutaneously, the following relative values were obtained for other œstrogens in large scale tests with ovariectomised rats (the subcutaneous value is given in parentheses): hexœstrol 10 (66), dienœstrol 68 (28), potassium hexœstrol sulphate 7·3 (0·98), 7-methyl-bis-dehydrodoisynolic acid 500 (46), œstrone 2 (29), premarin 5 (8), ethinyl-œstradiol 18 (233) and divinyl stilbœstrol 103 (45). Premarin consisted of the naturally occurring œstrogens in their water-soluble form, expressed as sodium œstrone sulphate. The results agree with clinical findings for some of the substances but with others such as ethinylœstradiol and 7-methylbisdehydro-doisynolic acid there were large discrepancies. Such discrepancies are serious because they may lead to a clinically useful synthetic œstrogen being discarded because of unfavourable animal tests. It is also clear that clinical assay is still necessary before the value of a new œstrogen can be established and that there is an urgent need for an animal test which will give the same results as clinical tests.

G. R. K.

Procaine Penicillin. Choice of Preparation. R. W. Fairbrother and K. S. Daber. (*Brit. med. J.*, 1950, **1**, 1098.) Two different types of preparation, with a particle size of 20μ or less, were subjected to investigation, the main object of which was to determine the blood levels produced by a standard intramuscular injection of 1 ml. The preparations were:—Type A: procaine penicillin (300,000 units) in arachis oil with 2 per cent. w/v

aluminium monostearate, plus crystalline potassium penicillin G (100,000 units) in 1 ml. Type B: a stable aqueous suspension of procaine penicillin in a finely divided state and containing 400,000 units of procaine penicillin in 1 ml. With Type A preparation effective levels of penicillin were maintained for the 24-hour period in all of 28 individuals (15 out of 21 gave assayable levels after 48 hours) but some difficulty was experienced in administration owing to its viscosity. Type B was injected without difficulty and gave satisfactory levels for a 24-hour period in all of 40 cases. In the authors' view this is the most convenient preparation for general use, though Type A should be very useful when it is desirable to give the injection at longer intervals than 24 hours. S. L. W.

Procaine Penicillin in Oil, Histological Reactions to. P. Story. (*Brit. med. J.*, 1950, **1**, 1467.) This paper gives an account of the histological changes occurring in the deltoid muscles and adjacent lymph nodes of a patient who had received intramuscular injections of procaine penicillin in arachis oil. The preparation used contained 300,000 units of penicillin G in combination with 120 mg. of procaine in 1 ml. of oil. Examinations at necropsy showed that underneath the needle marks seen on the skin of each deltoid region was an area in the muscle about 6 x 3 cm. which was greasy to the touch and showed some free oil in its deepest part. No scarring of muscle was observed macroscopically but some minute cystic areas and one small hæmorrhage were observed. Histological examination of tissues from both deltoid muscles showed that the muscle was œdematous and the perimysium contained a large number of oily globules. This oily material was associated with a marked cellular reaction in the connective tissue and some degeneration of adjoining muscle bundles. The cellular reaction was composed mainly of small round cells and large mononuclear phagocytes with smaller numbers of eosinophils and a few multinucleate giant cells. In some places however eosinophils were particularly numerous and in areas immediately surrounding the oil globules large mononuclear and multinucleate phagocytes were predominant. Fat-bearing phagocytes were plentiful around the oil and in adjacent lymph nodes. S. L. W.

Progesterone and Anhydrohydroxyprogesterone. A Comparative Study of Oral Administration. W. Bickers. (*J. Lab. clin. Med.*, 1950, **35**, 265.) Patients who had had a bilateral oophorectomy or who had amenorrhœa secondary to functional ovarian failure received 4,000 I.U. of mixed natural œstrogens daily for 20 days. A progestational response was produced by subsequent oral treatment with progesterone or anhydrohydroxyprogesterone (120 mg. daily for 5 days). Secretory response in the endometrium was less with anhydrohydroxyprogesterone than with progesterone. The invagination of the glandular epithelium and migration of nuclei was similar for the two drugs but secretion in the gland lumen, œdema in the stroma and tendency towards decidua-like cell formation in the stroma occurred only with progesterone treatment. G. B.

Salicyl Derivatives, Renal Excretion of during Aspirin Therapy, Influence of Urinary *p*H on. W. S. Hoffman and C. Noble. (*J. Lab. clin. Med.*, 1950, **35**, 237.) When aspirin is administered, the renal clearance of free salicylate, calculated on the total plasma salicylate, is generally below 2 per cent. of the creatinine clearance. The true value, calculated on an estimate of the unbound plasma salicylate is 4 to 5 times higher. This represents about 20 per cent. of the total salicylate, the remainder appearing in the urine in conjugated forms. When sufficient sodium bicarbonate is adminis-

tered to render the urine alkaline the free salicylate clearance is increased 3 to 13 times, in proportion to the urinary *p*H. It is suggested that the greater part of the free salicylate in the glomerular filtrate is reabsorbed by the tubules if the urine is acid, but not if it is alkaline. Conjugated forms of salicylate occur only in traces in the plasma, and must be either produced in the renal tubules, or excreted by them. A modification of a method for estimating urinary free salicylate, salicylurate and salicyl glycuronides, based on the differential ease of extraction with ethylene dichloride and carbon tetrachloride, and on the ease of hydrolysis with boiling acid, is described.

G.B.

BACTERIOLOGY AND CLINICAL TESTS

Ethylene Oxide, for the Sterilisation of Hospital Equipment. S. Kaye. (*J. Lab. clin. Med.*, 1950, **35**, 823.) Bedding, books and papers known to be contaminated with *Streptococcus hœmolyticus, Bacillus globigii* and BCG organism were rendered completely sterile by treatment with ethylene oxide. A suitable method for the sterilisation of hospital blankets, etc., is as follows. The materials are placed in an autoclave which is then closed and evacuated. Carboxide (ethylene oxide, 10 per cent., in carbon dioxide) is introduced from a cylinder and allowed to remain in the autoclave overnight, after which it is removed by vacuum pump, and passing air through the material for about one hour. Woollen and other delicate fabrics are not harmed by this treatment. G. B.

Menstruum for Drying Organisms and Viruses. J. W. Hornibrook. (*J. Lab. clin. Med.*, 1950, **35**, 788.) A suitable suspending medium for the freeze-drying of organisms and viruses which are required to retain their viability may be prepared as follows. Dissolve 1·35 g. of potassium citrate (monohydrate), 2·45 g. of sodium citrate (dihydrate), 0·61 g. of potassium phosphate, 0·6 g. of magnesium chloride (hexahydrate), 1·0 g. of potassium carbonate (sesquihydrate) and 57·5 g. of lactose in 500 ml. of water, mix with a solution of 1·33 g. of anhydrous calcium chloride in 500 ml. of water, adjust to *p*H 7·0 by addition of lactic acid, and filter through a Berkfeld filter. This solution approximates in composition to milk dialysate. For cholera organisms, this medium is preferable to Naylor's solution, milk, broth or lactose solution, and the medium may also be used for drying yellow fever vaccine. The inclusion of cysteine does not improve the medium, but it is possible that some of the salts are superfluous. G. B.

Toxin Production in Aerated Cultures of *Corynebacterium Diphtheriæ*. F. F. Howatt and G. B. Reed. (*Canad. J. Res., Sect. E.* 1950, **28**, 23.) When *C. diphtheriæ* is grown in aerated cultures, toxin production occurs earlier than in pellicle cultures. The yield of toxin is increased, provided that a further quantity of fermentable carbohydrate is added to the aerated cultures. The following method of aerated culture was adopted. 200 ml. of Wadsworth and Wheelers' infusion free proteose peptone medium was used in a 1000-ml. flask. 5 hours after inoculation the flask was placed on a mechanical shaker to give aeration at the rate of 2 to 3 volumes of air per minute. A further amount of a solution of sodium lactate, maltose and glucose, equivalent to half the amount in the original medium was added after 24 to 36 hours' incubation. The toxin concentration reached a maximum in 60 hours, compared with 120 hours for non-aerated (pellicle) cultures.

G. B.