ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Alkaloids, Infra-red Spectra in the Determination of. G. B. Pleat, J. H. Harley and S. E. Wiberley. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 107.) The spectra of 22 alkaloids were measured over the range 3 to 15 μ. Each alkaloid had a characteristic spectrum and it was found possible to distinguish not only between the alkaloids but also between an alkaloid and its salts. The method was applied quantitatively by selecting a particular wave length at which other alkaloids did not absorb. Using suitable solvents, a linear relationship was obtained by plotting absorbency against concentration over a range of concentration of 1 to 100 mg./ml. The accuracy of the method varied from less than 1 per cent. relative error for large amounts of alkaloid to 20 per cent. for amounts in the region of 1 mg. G. R. K.

Barbiturtes, Colorimetric Determination of. H. Baggesgaard Rasmussen and B. Jerslev. (*Dansk. Tidsskr. Farm.*, 1951, **25**, 29.) In using the cobalt reaction for the determination of barbiturates, it is necessary to choose the conditions carefully. The concentration of cobalt is important, so that deliquescent cobalt salts should not be used for the preparation of the reagent. Anhydrous cobalt acetate (prepared by warming crystalline cobalt nitrate with acetic anhydride) is stable if kept in a desiccator over calcium chloride and caustic potash. It is used as a 0·177 per cent. solution in anhydrous methyl alcohol. This solution changes in colour; this is not important as a blank should always be done, but the solution should not be more than a few weeks old. The amine preferred is isobutylamine, as this is not too volatile. A considerable proportion of chloroform is desirable in the final solution, as the stability in pure methanol is poor. The reaction is carried out by adding, to 0·25 ml. of the cobalt acetate solution, 1·50 ml. of a solution of the barbiturate in chloroform, followed by 0·25 ml. of M *iso*butylamine in chloroform. The extinction is read immediately at 560 mμ. Standardisation must be done at the same time, as the colour intensity varies from day to day, and interpolation should only be employed between two points at the straight part of the curve, the slope of which varies considerably for different barbiturates. The reaction may be used generally with 5:5- disubstituted and 5:5:*N*- trisubstituted barbiturates. Strong colours are given also by 5:5-diphenylhydantoin, phthalimide, succinimide, theophylline, sulphathiazol, phthalylsulphathiazol, succinylsulphathiazol, and isobutylammonium isobutylcarbaminate. Weaker colours are given by other sulphonamides and theobromine. Organic acids produce a faint colour, while the presence of organic salts decreases the colour given by barbiturates. Colours are also given by extracts of blood, so that barbiturates cannot be determined in such media at concentrations below 0·01 per cent. No colour is given by *N*:*N*'-*di*-, 5:*N*:*N*'-tri-, and 5:5:*N*:*N*'-tetra-substituted barbiturates, while with 5-mono-, *N*-mono and 5:*N*-disubstituted barbiturates the method cannot be used as the colour is too unstable. G. M.

Barbituric Acids, Argentimetric Titration of. B. Danielsson. (*Svensk farm. Tidskr.* 1951, **55**, 125.) In the method of Budde, barbituric acids are titrated with silver in presence of sodium carbonate until a permanent turbidity appears. Results are not very satisfactory, and the potential change at the end point is small. A much greater voltage jump is obtained by using metaborate in place of carbonate, owing to the solubility of silver metaborate being greater than that of the carbonate. Details are as follows: about 0·6 g. of the sample and 0·60 g. of potassium metaborate are dissolved in 25 ml. of water, and the solution is titrated with 0·1N silver nitrate, using 2·0 ml. of 0·25M potassium chromate solution as indicator. The end point is reached when the colour of the mixture differs, for at least 30 seconds, from that of a comparison mixture containing 2 g. of calcium carbonate, 2 ml. of potassium chromate solution and 40 ml. of water. For the assay of sodium barbiturates, the metaborate is omitted. The method can be used for diallyl- and diethyl-barbituric acids and, less successfully, for allyisopropylbarbituric acid, but not for other barbituric acids. The compounds formed contain 1 atom of silver, 1 of potassium or sodium and 2 molecules of barbituric acid. G. M.

Chlorides and Hydrochlorides, Acidimetric Titrations of. T. Higuchi and J. Concha. (*Science*, 1951, **113**, 210.) A novel method is described for the titration as bases of amine hydrochlorides and alkali metal halides depending upon the use of glacial acetic acid as the reaction medium. In this solvent, which is considerably less protophilic than water, hydrochloric acid is largely un-ionised and readily volatile, so that direct titration of the various bases with perchloric acid is then possible. Procaine hydrochloride, under these conditions, required the expected two equivalents of perchloric acid for neutralisation, indicating that the hydrochloric acid was too weak an acid to affect the indicator. Under the same conditions, sodium, potassium and ammonium chloride and potassium bromide all titrated as monacid bases. That the apparent basicity was due to the volatility of the halogen acid formed was demonstrated by the fading of the titration end-point on heating, the stoichiometric end-point being attained only on boiling the solution. Independent verification of these results has been obtained by following the titrations potentiometrically J. B. S.

Cineole, Spectrophotometric Determination of. E. W Martin and J. W. E. Harrisson. (*J. Amer. pharm. Ass., Sci. Ed.*, 1950, **39**, 677.) The following rapid method for the determination of cineole in concentrations as low as 50 μg./ml. in pharmaceutical preparations, is based on the development of a red colour with *p*-dimethylaminobenzaldehyde. The cineole is brought into solution in anhydrous methanol in a concentration of 50 to 250 μg./ml., and 2 ml. of the solution is mixed with sufficient of a 0·5 per cent. w/v solution of dimethylaminobenzaldehyde in 75 per cent. sulphuric acid to produce 25 ml. After 6 minutes, the absorbancy is determined at 555 mμ. The percentage of cineole is calculated by dividing by 28·4. Benzyl alcohol, menthol and camphor do not interfere, but in the presence of thymol, 10 minutes is required for maximum colour development. It is necessary to adhere to the quantities of cineole and reagents suggested, in order to obtain a stable colour at 555 mμ. G. B.

Ethanol, Colorimetric Determination of. Max B. Williams and H. Darwin Reese. (*Anal. Chem.*, 1950, **22**, 1556.) A simple, accurate spectrophotometric method is described for the determination of 0·0010 to 5·800 mg. of ethanol per ml. in aqueous solution. The procedure is based on

the quantitative oxidation of ethanol by a potassium dichromate-sulphuric acid reagent, followed by dilution to a chromium concentration of less than 1 mg./l., at which concentration neither the chromic nor the dichromate ions show appreciable absorption for wave-lengths in the visible region. *s*-Diphenylcarbazide, when added to this dilute solution, forms a deep violet-coloured complex with the dichromate ions, but no colour with the chromic ions. The absorption of this violet-coloured complex is measured at 540 mμ where it accurately follows Beer's law, and so enables the excess dichromate to be determined. The method suffers from the general disadvantage experienced by oxidation methods for ethanol in that other alcohols and other easily oxidisable substances must be removed. The sensitivity of the method makes it possible to analyse a wide range of ethanol concentrations.

A. H. B.

Methionine, Microchemical Identification of. F. Amelink. (*Pharm. Weekbl.* 1951, **86**, 133.) Methionine gives characteristic crystals with copper sulphate (sensitivity 0·25 mg.) and with iodo-platinate (sensitivity 0·1 mg.). The latter reaction is carried out preferably by adding 1 drop of platinum chloride solution to 1 drop of saturated solution of sodium iodide so that the mixture is dark red in colour, and bringing the mixture in contact with a drop of methionine solution. G. M.

Pregnenolone and Pregnenolone Acetate, Determination by Infra-red Spectrophotometry. G. Papineau-Couture and R. A. Burley. (*J. Amer. pharm. Ass. Sci. Ed.*, 1950, **39**, 683.) Pregnenolone acetate exhibits absorption maxima due to vibrations of the following 4 bonds in the molecule: 1736 and 1710 cm.$^{-1}$ due to C=O bonds in the acetate and keto-groups respectively, and 1241 and 1032 cm.$^{-1}$ due to C–O bonds in the acetate group and at position 3 respectively. The position and relative intensity of the absorption maxima are characteristic of the molecule. For example, pregnenolone present as impurity increases the intensity of the 1710 cm.$^{-1}$ band relative to the others; with dehydroisoandrosterone acetate as impurity, the relative intensity of the 1710 cm.$^{-1}$ band is decreased. A method of assay is described, in which the pregnenolone acetate content is calculated by comparing the optical density of a solution in carbon disulphide with that of solutions containing known concentrations of the pure substance. The results calculated separately from measurement at each of the 4 absorption peaks should be in agreement, unless interfering substances are present. A similar method, using absorption maxima at 1708, 1047 and 953 cm.$^{-1}$ is applicable to the determination of pregnenolone. G. B.

ORGANIC CHEMISTRY

Arabic Acid and Sodium Arabate Powder, Preparation of. R. H. Schleif, T. Higuchi and L. W. Busse. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 98.) Arabic acid was prepared as a white, fluffy, finely divided powder by passing a 7 per cent. solution of acacia through a column of Amberlite IR-120 previously treated with 10 per cent. hydrochloric acid and washed with water, and spray-drying the resulting solution at 400°F. Sodium arabate was also obtained as a white finely divided powder by adding sufficient 0·5N sodium hydroxide to the solution of arabic acid to bring its reaction to *p*H 8 and spray-drying at 400°F. Arabic acid obtained from three different batches of acacia had ash values of 0·05 per cent. or less. Differences in viscosity observed in solutions of arabic acid prepared from different lots of

acacia were due to differences in viscosity between the various lots of acacia rather than to a difference in the charge of the acacia molecule, since the viscosity differences persisted in the presence of sodium chloride. The specific rotation of the arabic acid was $-27{\cdot}44°$ and *p*H titration curves showed that arabic acid behaved as a strong monobasic acid. G. R. K.

Pregnenolone and Pregnenolone Acetate, Solubility in Various Solvents. L. Freedman, E. Greenspan and N. A. Levin. (*J. Amer. pharm. Ass. Sci. Ed.*, 1951, **40**, 54.) The following solubilities were determined by heating an excess of the solute with solvent under reflux for 30 minutes, cooling to 25°C. and allowing to stand at that temperature for 3 hours.

Solvent	Solubility (g./ml. of solution)	
	Pregnenolone	Pregnenolone acetate
Carbon tetrachloride	0·005	0·050
Light petroleum	0·001	0·010
Ethyl acetate	0·011	0·079
Acetone	0·006	0·027
Chloroform	0·170	0·550
Ethyl alcohol	0·019	0·025
Benzene	0·009	0·260
iso-Propanol	0·015	0·020

The following solubilities were determined by heating an excess of solute with solvent at 95°C. for 30 minutes, cooling to 25°C. and allowing to stand at that temperature for 3 hours.

Solvent	Solubility (g./ml. of solvent)	
	Pregnenolone	Pregnenolone acetate
Propylene glycol	0·001	0·001
Dioxan	0·031	0·202
Benzyl alcohol	0·081	0·111
Benzyl benzoate	insol.	0·091

G. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

γ-Carotene, A Rich Source of. B. L. Smits and H. L. Mitchell. (*Science*, 1951, **113**, 296.) The heterœcious rust fungus *Gymnosporangium juniperi-virginianæ* Lk. produces telial galls on the common juniper, and the telial spores they produce in spring infect the apple, producing two types of leaf injury, epiphyllous pycnidial lesions and hypophyllous æcia. The authors have investigated the carotenoid pigments produced in the leaves of the crab apple by the pycnidial lesions which, in the leaves used for the investigation, covered 80 per cent. of the leaf surface. The pigments were extracted by

blending 2 g. in a Waring blendor for 5 minutes with 60 ml. of ethanol and 150 ml. of a proprietary mixture of petroleum hydrocarbons consisting principally of hexane. As extraction was incomplete the leaf residue was refluxed for 30 minutes with 40 ml. of 10 per cent. solution of potassium hydroxide in methanol. The carotenoids were removed from the mixed extracts with the petroleum mixture and xanthophylls removed from the petroleum extract by means of methanol. The resulting extract was chromatographed on a mixture of Hyflo Super Cel and magnesia, when two prominent zones were obtained. The lower zone, removable by a 4 per cent. v/v solution of acetone in petroleum naphtha, consisted of β-carotene as shown by spectrum analysis. The upper zone, removed by 8 per cent. v/v acetone in petroleum naphtha, consisted of γ-carotene. The total carotene content of the leaves was 83·2 mg./100 g. of dry material, of which 28·7 mg., or 34·5 per cent., was β-carotene. Normal leaves contained 32·6 mg./100 g. of β-carotene but none of the γ-isomer. γ-Carotene is relatively rare in plants and the concentration found in the infected leaves examined is the highest that has come to the authors' notice. H. T. B.

Cerebrospinal Fluid Globulin, Precipitation of, by Zinc Sulphate. A. M. Donovan, J. M. Foley and W. C. Moloney. (*J. Lab. clin. Med.*, 1951, **37**, 374.) It has previously been reported that zinc salts, which can be buffered more effectively than salts of other metals, for example with barbitone, can be used to precipitate various protein fractions from serum. The authors have applied the same principle to the proteins of the cerebrospinal fluid and find that the results show a high degree of correlation with the incidence of neurosyphilis and multiple sclerosis. The solution used contains 1·44 g. of zinc sulphate ($ZnSO_4,7H_2O$), 3·62 g. of barbitone and 1·20 g. of soluble barbitone in 1,000 ml. of water; its *p*H is 6·9. To 1 ml. of the solution in a 3 ml. 10 mm. bore test tube is added 1 ml. of the cerebrospinal fluid and the result is read after 4 hours as a precipitation reaction on a 0 to 4 plus basis. Ignoring a 1 plus reaction, good correlation was found with the colloidal gold reaction. In 32 out of 37 patients with neurosyphilis, as indicated by a positive Hinton and/or Wassermann reaction in the spinal fluid, a significantly positive response was obtained with the zinc sulphate test. In the remaining 5 cases, the colloidal gold and the zinc reactions were negative. In 21 patients with multiple sclerosis, there was satisfactory correlation between the zinc and the colloidal gold reactions in 20. In the remaining patient the colloidal gold response was negative although the zinc reaction was 3 plus. Both tests gave a small proportion of positive responses in a miscellaneous group of patients with cerebral vascular disease and other conditions. H. T. B.

Gelatin, Cold Water Fraction of. J. Pouradier and M. Abribat. (*Bull. Soc. Chim. biol.* 1950, **32**, 947.) When gelatine is soaked in cold water a small proportion dissolves. This fraction has been examined previously, but, on account of the low concentration, only after concentration of the solution. The results are therefore unreliable because degradation of the soluble fraction is not excluded. The authors have now examined the solution obtained without concentration, using two different samples of gelatin, and comparing the molecular weight and viscosity of the cold water solution with solutions of similar concentration obtained in the usual way. The results show no significant difference, i.e., the dissolution of gelatin in cold water is not due to a modification of the molecule. It appears that the

velocity of dissolution depends on the possibility of molecules detaching themselves from the gel, and therefore on the intermolecular forces in the swollen gel. G. M.

Vitamin A, Concentration of Antimony Trichloride Reagent in Identification Test. J. A. Brown. (*J. Amer. pharm. Ass. Sci. Ed.*, 1950, **39**, 699.) In the Carr-Price test for vitamin A, the difficulty in working due to the fleeting nature of the blue colour may be obviated by the use of a weaker reagent. When a reagent containing 6 per cent. w/v of antimony trichloride is used, the maximum blue colour develops in 7 minutes, compared with 1 minute with the 20 per cent. w/v reagent. The weaker reagent is also less corrosive, and cheaper. The sensitivity of the test is too much reduced when reagents weaker than 6 per cent. w/v are used. G. B.

BIOCHEMICAL ANALYSIS

Dicoumarin in Blood, Estimation of. M. Lubran. (*J. clin. Path.*, 1951, **4**, 63.) The following method, based on extraction with ethylene dichloride and coupling with diazotised anisidine, followed by measurement of the red colour, will detect dicoumarin in a plasma concentration of 0·13 mg./100 ml. Mix 5 ml. of plasma or serum with 1 ml. of 5N hydrochloric acid and 10 ml. of ethylene dichloride, shake for 30 minutes, centrifuge, discard the upper layer and remove 6 ml. of the ethylene dichloride extract by pipette. Add 4 ml. of M/15 disodium hydrogen phosphate, shake for 5 minutes, centrifuge and remove the upper layer, mixing 3 ml. of it with 1 ml. of 2/15 M potassium dihydrogen phosphate in 0·1 per cent. gum ghatti and 0·1 ml. of diazo salt solution. Determine the colour photoelectrically after 10 minutes, using an Ilford filter No. 624. A blank and a standard solution are also required for calculation of the plasma concentration. Phenol, tyrosine and coumarin do not interfere, but salicylates in concentrations greater than 20 mg./100 ml. give a similar colour. G. B.

Histaminase Activity of Biological Fluids, Volumetric Determination of. R. Kapeller-Adler. (*Biochem. J.*, 1951, **48**, 99.) The method is a modification of Zeller's qualitative indigo test and is based on the theory that during the action of histaminase one molecule of hydrogen peroxide is formed for each molecule of histamine oxidised. To increasing amounts of the enzyme solution in test-tubes, 1 ml. of a 0·66 per cent. aqueous solution of indigo carmine and 0·1 ml. of a 1 per cent. solution of histamine dihydrochloride in M/15 phosphate buffer are added and the volume is adjusted to 4 ml. with the phosphate buffer. One drop of chloroform is added as a preservative and oxygen passed through for 1 minute. The tube is stoppered, shaken, incubated at 37°C. for 24 hours, and titrated with 0·002 N potassium permanganate. Control tubes from which the histamine has been omitted are run simultaneously and the differences between the titrations indicate the amount of hydrogen peroxide formed. The results are expressed in permanganate units (P.U.), each unit representing the amount of enzyme which, after incubation at 37°C. and *p*H 7·2 for 24 hours, in an atmosphere of oxygen with 1 mg. of histamine dihydrochloride as substrate and with an aqueous solution of indigo disulphonate takes up 0·1 ml. of 0·002 N potassium permanganate. The method gave good agreement with Anrep's biological method. When applied to the sera from about 230 women in various stages of pregnancy, it was found that histaminase activity became apparent at the end of the second month of pregnancy, reached its maximum between the

twenty-second and twenty-sixth week and somewhat decreased in the seventh month, to remain more or less stationary until delivery. During labour and still more rapidly in the puerperium, the activity tended to decrease. Low results were found in patients with pre-eclamptic toxæmia, high results in most cases of twin pregnancy and a very low result in a case of triplet pregnancy; the activity was also low in several cases of threatened abortion. Purified preparations of histaminase from hog kidneys were found to be four times more active on histamine than cadaverine, whereas serum histaminase from pregnant women was more active on cadaverine than histamine; moreover, on cadaverine, sera from patients with pre-eclamptic toxæmia gave normal results. The discrepancy in the behaviour of histaminase, depending on the origin of the enzyme, suggests that the effect of histaminase on cadaverine, unlike that on histamine, is non-specific and that the name diamine oxidase should be dropped in favour of histaminase for the enzyme which acts specifically on histamine. G. R. K.

Methylamine in Urine, Determination of. A. A. Ormsby and S. Johnson. (*J. biol. Chem.*, 1950, **187,** 711.) The reaction between methylamine and lactose in alkaline solution giving a red colour (maximum at 405 and 545 mμ) has been used for the determination of methylamine in urine. The presence of ammonia gave no colour in the reaction but affected the colour given by the methylamine and increased the sensitivity; on the addition of increasing amounts of ammonia there was at first an intensification of the colour, and then a rapid decrease in intensity. As ammonia was added, the position of the 405 mμ maximum was shifted to longer wavelengths and its height decreased; the maximum at 545 mμ became more pronounced with a slight shift towards shorter wave-lengths. For the routine determination of methylamine in urine, ammonia was added to produce an optimum concentration; details of procedure are given. Dimethylamine and trimethylamine did not interfere unless present in relatively large amounts (20 and 40 times respectively). Using the method devised, analyses of four 24-hour specimens of dog urine gave values of 0·69 to 1·09 mg. of methylamine N per 24 hours. Analyses of six 24-hour specimens of human urine gave a range of 1·11 to 4·40 mg. of methylamine N per 24 hours. Expressed as methylamine, the average daily excretion of the dogs was 1·84 mg. and of the human subjects 5·71 mg. R. E. S.

Penicillin, Quantitative Microbiological Determination of. R. W. Sager and L. Arrigoni. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40,** 104.) A series of tubes containing varying amounts of a standard solution of penicillin in 0·02M phosphate buffer, *p*H 7·0, and 10 ml. of nutrient broth, were inoculated with a suitable lactic acid producing micro-organism and incubated at 37°C. The contents of each tube were then diluted with water and titrated with 0·05 N sodium hydroxide to *p*H 7·0, using a *p*H meter. The volume of sodium hydroxide was plotted against concentration of the penicillin and the curve so obtained used to estimate the potency of unknown solutions of penicillin. Suitable micro-organisms were *Streptococcus lactis, Str. faecalis, Lactobacillus arabinosus, L. casei* and *L. fermentum*. Before use they were activated by a succession of incubations in the nutrient broth used in the test, since maximum susceptibility to penicillin is reached when rapid multiplication is taking place. In addition, ranges of penicillin concentration were established for each micro-organism through which the inhibition of growth, as measured by decreased acid production, remained linear. The

method compared favourably with the Oxford cup and turbidimetric microbiological procedures which are currently used; comparative results on 18 penicillin solutions showed an average error of 7·8 per cent. with the proposed method, 11·1 per cent. with the Oxford cup and 11·6 per cent. with the turbidimetric method. G. R. K.

Protein in Urine and Cerebrospinal Fluid, Estimation of, with Permanent Turbidimetric Standards of Perspex. E. J. King. (*Biochem. J.*, 1951, **48**, 50.) The sulphosalicylic acid procedure for the tubidimetric assay of protein has been successfully used with permanent turbidimetric standards made from cloudy Perspex (Haslam and Squirrel, *Biochem. J.*, 1951, **48**, 48.) Each standard must be calibrated in terms of the sulphosalicylic acid-protein procedure. This may be accomplished by using several dilutions of blood plasma, the protein content of which has been determined by the Kjeldahl method. The accuracy achieved by the use of the Perspex standards is about ± 10 per cent., which is usually sufficient for the purpose. The standards are convenient to use and appear to be permanent. G. R. K.

CHEMOTHERAPY

bis-Choline Derivatives, Curare-like Activity of. H. Vanderhaeghe. (*Nature, Lond.*, 1951, **167**, 527.) The muscarinic, nicotinic and curare-like properties of choline derivatives are known to vary independently of one another, and in an attempt to find substances having only the curare-like activity the succinyl esters of methyl-, phenyl-, and benzyl-choline were investigated. The compounds were obtained by the action of succinyl chloride on the dialkylaminoalkanols in xylene solution. The resulting bis-dialkylaminoalkylsuccinates were converted into quaternary ammonium salts by reaction with alkyl iodide in acetone solution. The pharmacological activities of the compounds were compared by determining the head drop dose in mg./kg. on intravenous injection in the rabbit's ear, the lethal dose in mg./kg. when given in the same way, and the dilution in mg./100 ml. required to obtain curarisation in the rat phrenic nerve-diaphragm preparation. The curare activity of the triethylammonium compounds, $ROOC.CH_2.CH_2.COOR$ where $R = CH(CH_2C_6H_5)CH_2N(CH_3)_3I$ or $CH(CH_2C_6H_5)CH_2N(C_2H_5)_3I$, is the same as, or greater than, that of the corresponding trimethylammonium salts, in contrast with choline derivatives, where opposite results have previously been reported. The lowest head drop dose was 0·2 mg./kg. obtained when $R = CH_2CH_2N(CH_3)_3I$, and this compound also showed the smallest lethal dose, 1 mg./kg., and the lowest dilution, 1 mg./100 ml., necessary to obtain curarisation in the rat phrenic nerve-diaphragm preparation. The corresponding figures for D-tubocurarine are 0·18, 0·2 and 0·075 respectively.

H. T. B.

Curarising Agents; Synthetic. C. A. Winter and J. T. Lehman (*J. Pharmacol.*, 1950, **100**, 489.) 16 compounds were tested for curariform action in mice. Most of the compounds produced marked hypotension when injected intraveneously in cats and dogs, but two in particular were relatively free, namely, *p*-di-β-dimethylaminopropoxybenzene diethiodide (dipropamine) and tris-(triethyl-β-ethoxyammonium)-1,2,3-benzene triiodide (flaxedil). Dipropamine resembles *d*-tubocurarine in some respects (paralysis of muscle to indirect but not to direct stimulation, and raising the threshold to intra-arterial acetylcholine), but differs in others (slowness of onset of

action, irreversibility to neostigmine, failure to antagonise acetylcholine on isolated frog muscle preparation); in many respects it therefore resembles C10. Flaxedil possesses curarising properties closely resembling those of *d*-tubocurarine and is readily reversed by neostigmine. Neostigmine will no longer reverse the action of flaxedil or *d*-tubocurarine in an animal previously given a dose of dipropamine. This effect may be temporarily prevented if flaxedil or *d*-tubocurarine is given immediately prior to dipropamine, but irreversibility to neostigmine eventually sets in and is of long duration. Neuromuscular paralysis produced by flaxedil or *d*-tubocurarine may be relieved by a dose of dipropamine which by itself would cause paralysis; the converse is also true. There would therefore appear to be a mutual competitive inhibition between dipropamine and *d*-tubocurarine or flaxedil, whereas the two last-mentioned are additive in their effects. S. L. W.

PHARMACY

NOTES AND FORMULÆ

Gamma Benzene Hexachloride (Gexane). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **144,** 548.) Gamma benzene hexachloride is γ 1:2:3:4:5:6-hexachloro*cyclo*hexane, $C_6H_6Cl_6$. It occurs as a white, crystalline powder with a musty odour, insoluble in water, slightly soluble in glycerin and ethylene glycol and soluble in alcohol, glacial acetic acid, benzene, chloroform, ether and hot nitric acid. When dissolved in acetone containing 1 per cent. of resorcinol and treated with ammonia, slender needle-like crystals gradually form in 3 to 24 hours. The crystals are colourless at first and become orange in 24 to 48 hours; m.pt. 111·5° to 113·5°C. When dried at 105° for 4 hours, γ-benzene hexachloride loses not more than 2 per cent. of its weight. It contains 99 to 102 per cent. of benzene hexachloride, calculated on a dry basis. It is assayed by heating in alcoholic solution with sufficient sodium hydroxide to decompose it to sodium chloride and trichlorobenzene, and estimating the amount of sodium chloride formed by adding a known volume of silver nitrate, filtering and titrating the excess of silver nitrate with potassium thiocyanate, using ferric ammonium sulphate as indicator. Identity tests and assay processes for lotion and ointment are also described. G. R. K.

Iodine and Sulphathiazole, Diffusion from Ointments. L. D. Lockie and J. B. Sprowls. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40,** 72.) The rate of diffusion was studied by superimposing a layer of ointment on to the surface of agar gel contained in a test-tube, and measuring the depth of penetration of the drug at intervals. The gel contained 1 per cent. of Ehrlich's solution for the studies in sulphathiazole and 0·1 per cent. of soluble starch for the studies on iodine, to indicate the degree of penetration. No measurable diffusion of sulphathiazole was obtained from fatty bases even in a concentration of 20 per cent. and diffusion rates from oil-in-water bases were not significantly increased by changing the concentration of sulphathiazole from 5 to 20 per cent. By using the same base and varying the proportions of drug, it was shown that within the concentrations used for therapeutic purposes there was no significant increase in the rate of diffusion when the concentration was increased. Sulphathiazole seemed to reach a maximum of efficiency at a concentration of 10 to 20 per cent. and iodine at about 5 per

cent. The effect of varying the emulsifying agents in an ointment base on the rate of diffusion of iodine was investigated, using a standard base consisting of white wax 5 g., cetyl alcohol 5 g., heavy liquid paraffin 15 g., emulsifying agent 3 g., and water 72 g. The emulsifying agents used were sodium lauryl sulphate (anionic), Aerosol OT (anionic), diglycol stearate (nonionic), triethanolamine stearate (cationic) and sulphated hydrogenated castor oil. The results showed no significant differences in the rates of diffusion. G. R. K.

PHARMACOGNOSY

***Digitalis* Species, Microscopical Characterisation of.** E. Soos, I. Kaber and A. Mandl. (*Sci. Pharm.*, 1950, **18**, 135.) The following species were compared, *D. ferruginea*, L., *D. lævigata*, W.K., *D. lanata*, Ehrh., *D. orientalis*, Lam., *D. sibirica*, Lindl., *D. purpurea*, L., *D. ambigua*, Murr., *D. lutea*, L., and *D. parviflora*, Jacq. Surface views of the leaves of these species are given. *D. purpurea* is characterised by its net-like nervature, and with its characteristic hairs can hardly be confused with other species. *D. ambigua* also has numerous hairs with a mean length of 700 to 800μ as against 300 to 400μ for all other species. The leaves of *D. lutea*, macroscopically very similar to *D. ambigua*, show on the upper side a cuticular streaking of almost all cells, whereas with *D. ambigua* this is only found round the stomata. With *D. ferruginea* and *D. lævigata*, stomata can be found on the lower surface only. The quantitative microscopic characters are given in the Table.

Species	Stomatal Index		Palisade ratio	Vein islet number
	Under side	Upper side		
D. ferruginea	21·9	0·23	4·56	1·33
D. lævigata	22·3	0·25	5·93	1·21
D. lanata	19·8	17·1	5·58	1·42
D. orientalis	17·3	16·4	5·48	1·17
D. sibirica	22·6	5·40	5·26	0·95
D. purpurea	18·7	3·77	4·30	3·28
D. ambigua	21·1	3·25	5·35	1·22
D. lutea	22·5	1·11	4·91	1·72
D. parviflora	18·4	5·08	5·00	1·53

G. M.

PHARMACOLOGY AND THERAPEUTICS

Antithyroid Substances in the Treatment of Hyperthyroidism. D. M. Dunlop and C. F. Rolland. (*Proc. R. Soc. Med.*, 1950, **43**, 937.) Patients treated with thiouracil or methylthiouracil for a period of 6 months or less showed a very high relapse rate, which was less marked in those treated for 6 or 12 months and much less in those treated for over a year. Treatment should not be stopped even after a year unless all thyrotoxic signs and symptoms have been entirely controlled by as small a dose as 0·05 g. daily for

some months. The longer the remission once treatment has been stopped, the greater the chance of apparent cure. If relapse is going to take place, it will probably occur within a few months of stopping treatment. Toxic effects, which occurred rather more frequently when the patient was taking a large dose, during the initial course of treatment, or when a second course of treatment was started after an interval, were encountered in 11 per cent. of patients and in 7 per cent. were sufficiently severe to necessitate abandonment of treatment. Slight enlargement of the goitre and mild symptoms of myxœdema due to overdosage promptly receded when the dose was reduced or treatment abandoned. Thiouracil is preferable to surgical treatment for the rare cases of thyrotoxicosis in children; for very old thyrotoxic patients with complicating degenerative diseases; for patients who develop a recurrent thyrotoxicosis following thyroidectomy; for patients who have a horror of operation, and for young women with moderate thyrotoxicosis and unobtrusive goitres. It has a place in the preparation of patients for thyroidectomy.

G. R. B.

Aureomycin by Oral, Intravenous and Intramuscular Routes, Evaluation of. M. Klein, S. E. Schorr, S. Tashman and A. D. Hunt, Jr. (*J. Bact.*, 1950, **60**, 159.) Oral administration is at present the method of choice, intravenous injection being used only when excessive nausea, vomiting and diarrhœa make the oral route impracticable. Intramuscular injection was originally stated to be relatively ineffective and to cause considerable pain but preliminary studies by the present authors suggested, on the contrary, that intramuscular administration was unusually effective. The three routes of administration were accordingly investigated in type 1 pneumococcus infections of mice, evaluation of the effects being made by making plate counts after incubation of dilutions in a suitable medium of one drop of tail blood; for comparison parallel tests were carried out with penicillin. For the intramuscular route, 1 mg. of the antibiotic was given immediately after injecting the mice with the bacterial culture. All of 10 mice receiving penicillin died in 48 to 72 hours but all treated with aureomycin survived the 12-day observation period. Blood counts at intervals showed that the difference was due to the rapid absorption and excretion of the penicillin; intramuscular aureomycin is slowly absorbed from the site of injection but rapidly excreted. When given intravenously, the high initial blood level immediately obtained fell rapidly, reaching zero in 24 hours. Orally a moderate initial level was obtained which also fell to zero in 24 hours although mice were protected to some extent even after that time. The antibiotic is only about one-tenth as effective on a weight basis when given orally as when given intramuscularly. The results show that with aureomycin the *in vitro* sensitivity of an organism is not a measure of the concentration in the blood required to inhibit growth *in vivo;* a much lower concentration is effective *in vivo* than is indicated by *in vitro* tests. While there is as yet no generally accepted figure for the minimum effective blood level, 0·03 μg./ml. or less is significantly effective against a heavy dose of pneumococci.

H. T. B.

Curare, Antidotes to, in Man. D. W. Macfarlane, E. W. Pelikan and K. R. Unna. (*J. Pharmacol*, 1950, **100**, 382.) The effects of neostigmine, *m*-hydroxyphenyltrimethylammonium bromide (Ro 2-2561), and *m*-hydroxyphenylethyldimethylammonium bromide (Ro 2-3198) as antagonists to the effects of *d*-tubocurarine in man were studied in

unanæsthetised human volunteers. All drugs were given intravenously over constant periods of time; the injection of the antagonist was commenced 3 minutes after the start of the injection of *d*-tubocurarine in order to coincide with the period of maximum curare effect. Percentage decrease in grip strength, percentage decrease in vital capacity and the time for 75 per cent. recovery in grip strength were determined for each subject. Recovery curves were calculated from control experiments with *d*-tubocurarine and were compared with time-grip strength recovery curves of experiments in which the antidotes were used. Both *m*-hydroxyphenyltrimethylammonium bromide and *m*-hydroxyphenylethyldimethylammonium bromide were more effective than neostigmine in relieving the effects of *d*-tubocurarine but were of shorter duration. The effect of the trimethyl compound was more rapid in onset but of shorter duration than that of the ethyldimethyl compound. Neostigmine had the greatest muscarine-like action and *m*-hydroxyphenylethyldimethylammonium bromide the least. Anticurare action is not dependant upon anticholinesterase activity. G. R. B.

2:5-bis-(3-Dimethylaminopropylamino)benzoquinone-bis-benzylchloride—A new Curarimimetic Drug. J. O. Hoppe. (*J. Pharmacol*, 1950, **100**, 333.) Complete arrest of nerve-impulse transmission was produced by 2:5-bis-(3-dimethylaminopropylamino)benzoquinone-bis-benzylchloride (WIN 2747) in a dose approximately half that of *d*-tubocurarine chloride pentahydrate required to produce a similar effect. By subcutaneous injection in mice, this compound had half the activity and a quarter the toxicity of *d*-tubocurarine; by intravenous injection in rabbits it was 5 times as active, and approximately 4 times as toxic, as *d*-tubocurarine. If rabbits were previously treated with neostigmine the HD50 value of WIN 2747 was raised by a factor of 1·3, and the LD50 value was raised by a factor of 1·6; the factors for *d*-tubocurarine were 1·7 and 2·1 respectively. Doses of WIN 2747 sufficient to produce a neuromuscular block in dogs anæsthetised with thiopentone and soluble barbitone did not produce a fall in blood-pressure; similarly effective doses of *d*-tubocurarine invariably produced a transient fall in blood pressure. The reflex fall in blood-pressure produced by stimulation of the vagus in the dog was abolished for approximately half an hour after a curarising dose of *d*-tubocurarine, whereas corresponding doses of WIN 2747 had no inhibitory effect on this reflex mechanism. Approximately 1,500 times the dose of WIN 2747 required to produce neuromuscular block, infused into the anæsthetised dog under artificial respiration did not produce cardiac arrest, whereas approximately 300 times the curarising dose of *d*-tubocurarine stopped the heart in diastole. G. R. B.

Glyceryl Trinitrate Ointment in Raynaud's Disease. M. S. Kleckner, E. V. Allen and K. G. Wakim. (*Proc. Mayo Clin.*, 1950, **25**, 657.) 25 patients with Raynaud's disease or Raynaud's phenomenon were treated with 2 per cent. glyceryl trinitrate ointment. About 1 g. of the ointment was applied to the fingers of one hand 4 times daily, the other hand being covered with a rubber glove during the period of inunction. Of the 15 patients with Raynaud's disease 3 reported great benefit, 5 moderate benefit, 2 slight benefit, and 5 no improvement, even after a period of treatment varying from 2 to 25 weeks. 7 patients with acrosclerosis were also treated. One with early acrosclerosis reported moderate benefit, 2 slight improvement, and 4 no improvement. Of 2 patients with occupational occlusive arterial disease associated with Raynaud's phenomenon one reported moderate improvement

and the other great benefit. One patient with thromboangiitis obliterans with Raynaud's phenomenon did not obtain improvement. In many of the cases the characteristic episodes of discoloration were benefited by the inunction. In most cases the appearance of a throbbing headache indicated definite systemic absorption of the glyceryl trinitrate ointment, but nitrite syncope, gastro-intestinal symptoms and clinical methæmoglobinæmia were not observed. In previous controlled experiments with lanolin ointment alone no significant changes were noted in the blood flow to the hand, in the amplitude of the digital pulse or in the skin temperature, whereas such changes were observed with the glyceryl trinitrate ointment. While the results of this method of treatment are not wholly satisfactory the benefit occasionally noted justifies a therapeutic trial. The extreme volatility of the glyceryl trinitrate is a disadvantage as the ointment may lose its potency. S. L. W.

Lead Poisoning: Sodium Citrate as Prophylactic. D. O. Shiels, W. C. Thomas and G. R. Palmer. (*Med. J. Austral.*, 1950, **2**, 922.) The prophylactic use of sodium citrate by 48 employees of an accumulator factory, the majority of whom had hitherto not been exposed to any kind of lead hazard is described. The dose used was 4 g. in 1 fl. oz. of water once daily. It was found that this prophylactic measure caused a significant fall in stippled cell counts and in the ratio of monocytes plus large lymphocytes to small lymphocytes in persons exposed to a lead hazard but not showing symptoms of lead poisoning. The authors conclude that the use of this treatment is justified in certain circumstances, though it should not be regarded as an alternative to improving the conditions respecting exposure to lead.

S. L. W.

Radioactive Iodine, Diagnostic and Therapeutic Use of. D. E. Clark, O. H. Trippel and G. E. Sheline. (*Arch int. Med.* 1951, **37**, 17.) By determining the total amount of radioactive iodide in the plasma and the amount in the plasma protein 24 hours after a dose of 1·0 to 1·5 millicuries (Oak Ridge Standard) of I^{131} given orally in the form of sodium iodide in alkaline solution, a "conversion ratio" was calculated as:—Protein-bound plasma $I^{131} \times 100$/total plasma I^{131}. Values ranged from 13 to 42 per cent. in 22 patients with normal thyroids, and from 45 to 96 per cent. in 28 patients with hyperthyroidism. Values of 10 per cent. or less were considered to indicate hypothyroid activity. In the treatment of hyperthyroidism a dose of approximately 0·1 millicurie of I^{131} per estimated gramme of thyroid tissue was given. If this was inadequate other doses were given as indicated. Other anti-thyroid drugs should not be given for 4 weeks before I^{131} therapy is commenced. Of 100 patients treated 88 had a satisfactory remission. Persons with diffusely enlarged thyroid glands required an average of 5·6 millicuries, and persons with toxic nodular goitres needed an average of 11·7 millicuries before a satisfactory state was obtained. In the treatment of carcinoma of the thyroid a dose of 35 millicuries was given orally every 2 weeks. Patients in whom the tumour had an avidity for iodine were treated until there was no longer localisation of I^{131} as determined by external survey with a Geiger-Müller tube. Patients whose tumours showed little or no avidity were treated for approximately 6 months. Of 50 persons treated, 8, whose tumours were of the alveolar and follicular type with varying amounts of colloid, have shown a good response. It appears that radioactive iodide holds some promise for a desirable therapeutic response in the patient whose tumour has a tendency to be differentiated. G. R. B.