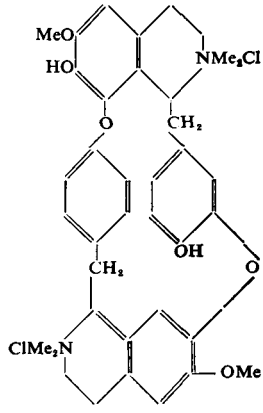
ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS



Curare Alkaloids, Constitution of *dextro*-Tubocurarine Chloride. H. King. (*J. chem. Soc.*, 1948, 267.) *dextro*Tubocurarine chloride, on O-ethylation, gave amorphous O-ethyltubocurarine chloride which when submitted to a two-stage Hofmann degradation gave the nitrogen-free O-ethylbebeerilene identical in properties with the substance obtained from bebeerine. The same distribution of methoxy- and phenolic groups is therefore present in *dextro*tubocurarine chloride and bebeerine; since their particular orientation is known in bebeerine, *dextro*tubocurarine chloride must have the structure shown.

R. E. S.

ANALYTICAL

Antihistaminic Drugs of the Thenyl Series, Identification of. T. J. Haley and G. L. Keenan. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 85.) The optical crystallographic properties and physical constants of antihistaminic drugs of the thenyl series are recorded. Those studied were thenylene or histadyl (N : N-dimethyl-N-(2-pyridyl)-N-(2-thenyl)-ethylenediamine) and its halogenated derivatives, chlorothen (N : N-dimethyl-N-(2-pyridyl-N-(5-chloro-2-thenyl)-ethylene diamine) and bromothen (N : N-dimethyl-N-(2-pyridyl)-N-(5-bromo-2-thenyl)-ethylenediamine). A means for the identification and differentiation using 6 common alkaloidal colorimetric reagents is described. Tests were made by placing a drop of reagent on a microscope slide and adding about 1 mg. of the drug to it. Changes taking place were observed for about 30 minutes. The alkaloidal colorimetric reagents gave better results than three precipitation reagents also investigated but as the tests were almost identical for each of the thenyl compounds the optical crystallographic properties described offered the best means for their identification.

G. R. K.

Barbiturates; Xanthydrol as an Identification Reagent. R. S. McCutcheon and E. M. Plein. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 24.) Xanthydrol may be prepared by treating an alcoholic solution of xanthone with sodium amalgam or by reducing xanthone with zinc dust, Xanthone may be prepared by refluxing phenyl salicylate and distilling the product at high temperature. Xanthyl derivatives of 18 barbiturates were obtained by reaction of the barbiturates with xanthydrol in glacial acetic acid. Pure white crystals with characteristic m.pts. were obtained for most, clearly identifying these barbiturates. Two of the group of 20 studied were N-substituted barbiturates and did not react. The derivatives were dried to constant weight and analysed for nitrogen by the Kjeldahl-Gunning method. Deviation of the percentages found from those calculated did not exceed

9·20 except for barbitone (calculated 5·15, found 4·86). M.pts. were determined by both the block and U.S.P. methods. The former checked consistently and were useful; rate of heating was 0·5°C. per minute. G.R.K.

Paper Chromatography, Streaming Potential in. L. Rutter. (*Nature*, 1949, **163,** 487.) The net resultant flow of solvent through the capillary channels of paper used in chromatography is in a direction away from the point of feed, resulting in the setting up of a streaming potential. Distilled water flowing through a washed and dried strip of No. 3 Whatman paper showed a potential gradient of approximately 10 mV. per cm. With a 1 per cent. thorium nitrate solution in place of water, the potential was reduced almost to zero; 1 per cent. sodium chloride solution showed a potential gradient of approximately 4 mV. per cm. The rate of flow of liquid and the nature of the electrodes used for measurement affect the values. Such potentials effect chromatographic development and it was found that development of 0·01 ml. of 0·1 per cent. aqueous solution of a mixed colour (Edicol Green 37113) with distilled water failed to separate the components, the band moving with the solvent front, whereas development with 1 per cent. sodium chloride solution achieved complete separation into blue and yellow bands. With either the strip or central feed technique, the flow of one liquid over another in partition chromatography may result in streaming potentials of varying sign, of possible significance in considering mechanisms of partition separations. R. E. S.

Paper partition Chromatography, Deposition and Simultaneous Concentration of Dilute Solutions in. K. F. Urbach. (*Science* 1949, **109,** 259.) The concentrations of histamine encountered in ordinary paper partition chromatographic procedures, where 0·01 to 0·1 ml. volume of fluid is used, were too low relative to the sensitivity of the colour reaction used as indicator in the development of the chromatogram; a procedure was devised therefore so that the entire 3 to 5 ml. of butyl alcohol extract could be deposited without allowing excessive spreading of the solvent on the strips. Horizontal paper strips were fixed over a hot plate and maintained at 60° to 70°C. The extract was then dropped on to the strip from a capillary ended tube at a controlled rate of 1 ml. per hour, at which rate the solvent evaporates. The total spread of the spots is not more than 2 to 3 cm. Details of the apparatus and capillaries, together with diagrams are given. Other organic solvents, such as ether, acetone, alcohol, dioxane, etc., can be applied in a similar manner, although aqueous solutions spread excessively on the paper; solutions containing weak hydrochloric or sulphuric acid charred the paper even at moderate temperatures. R. E. S.

Stilbœstrol, Polarographic Studies of. L. E. Bingenheimer Jr. and J. E. Christian. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38,** 117.) Electrolysis of solutions of stilbœstrol in concentrations of 10^{-4} to 3×10^{-4} M failed to give polarographic oxidation or reduction waves when the supporting electrolyte was N/10 potassium chloride in alcohol (30 per cent.) or in N/10 potassium hydroxide. Stilbœstrol suppressed the oxygen maximum in N/1000 potassium chloride solutions in alcohol (10 per cent.) or N/100 potassium hydroxide. The suppression was complete when the concentration of stilbœstrol was as little as 10^{-5} molar but negligible when the concentration was reduced to 10^{-6} molar. Repeated electrolysis decreased the size of the maxima and lowered the *p*H; buffering did not counteract this

effect but interfered, since the buffer exerted a suppressive action. Tablets of stilbœstrol were extracted by the U.S.P. method except that an extra step was necessary to remove gelatin which interfered with the results. The residue after removal of the ether was taken up in sodium hydroxide solution, potassium chloride was added and the solution diluted to correspond to $0{\cdot}4 \times 10^{-5}$ M stilbœstrol in N/100 potassium chloride and N/1000 potassium hydroxide. Standards corresponding to the U.S.P. limits of 90 and 110 per cent. of the labelled strength were similarly prepared. When the solutions were saturated with oxygen and electrolysed, the size of the maximum in the unknown solution was between those in the standard solutions. The main advantage of this method over the U.S.P. method is the saving of about 1 hour. G. R. K.

Tragacanth Flake, Evaluation of. Report No. 2 of the Tragacanth Sub-Committee of the Analytical Methods Committee of the Society of Public Analysts. (*Analyst*, 1949, **74**, 2.) The following recommendations are made: *Viscosity*—The flake is ground rapidly until all passes a No. 30 mesh sieve and a quantity of the powdered gum equivalent to the required weight of dry gum is wetted with 5 ml. of 95 per cent. alcohol. Cold distilled water is then added quickly, the mixture is shaken, allowed to stand for 1 hour and is then heated in a boiling water bath, the determination being completed as described in Report No. 1 (*Analyst*, 1948, **73**, 368; *J. Pharm. Pharmacol.*, 1949, **1**, 44.). *Suspending Power*—Owing to the variations in suspending power in gums of the same viscosity it is recommended that the purchaser should carry out a form of test using the concentration of tragacanth normally employed in his process and using all the materials that he desires to suspend. *Ash*—Direct ashing failed to give concordant results and, after preliminary treatment the sulphated ash (at about 850°C.) was chosen. *Volatile acidity*—The method used was described in " Methods of Analysis of the Association of Official Agricultural Chemists," 6th Edition, 1945, p. 709. The detailed procedure necessary to obtain concordant results is given in each case. R. E. S.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Digitalis Glycosides, Chemistry and Pharmacology of. E. W. McChesney, F. C. Nachod, M. E. Auerbach and F. O. Laquer. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 364.) Analytically pure samples of gitoxin, digitoxin and their aglucones have been prepared. Experiments on rats showed gitoxin to be only slightly less toxic than digitoxin. It is known that gitoxigenin gives a red colour with ferric chloride in the presence of strong sulphuric acid, the intensity of the colour increasing rapidly up to 5 minutes and then fading. Without the ferric chloride the red colour develops slowly, reaching a maximum in about 24 hours and remaining unchanged for several days. Digitoxigenin treated similarly gives a pale lemon-yellow colour which gradually deepens. The glycosides give similar colour reactions but the aglucones are much better adapted to colorimetric work, since with the glycosides there is admixed a brown colour resulting from the reaction of sulphuric acid on the digitoxose. The absorption spectra of the aglucones in sulphuric acid were studied and their different behaviour was found to provide the basis for an analytical method which gives the proportion of digitoxin and gitoxin plus gitalin in a mixture.

S. L. W.

ESSENTIAL OILS

Ascaridol, Assay of; Iodination of Terpenes. A. Halpern. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 465.) The assay of ascaridol in oil of chenopodium depends on the liberation of iodine from potassium iodide. The author shows that the major part of the iodine is released within the first minute; this is followed by a slower steady release of iodine. In order to elucidate whether the olefinic linkage was responsible for this property an attempt was made to iodinate several related unsaturated hydrocarbons under conditions similar to those of the assay process. No iodination of the olefinic linkage occurred, so that the atypical behaviour of ascaridol toward the iodide reagent cannot be explained on this basis.

J. W. F.

GUMS AND RESINS

***Sterculia setigera*, Composition of the Gum of.** E. L. Hirst, L. Hough and J. K. N. Jones. (*Nature*, 1949, **163**, 177.) The analysis of reducing sugars by means of paper partition chromatography yields inconclusive results for mixtures of sugars with very similar R_g values. The use of a column of powdered cellulose was found to afford a method for the separation of sufficiently large amounts of the individual sugars to allow identification by the normal determination of physical constants; results are given from a study of the gum of *Sterculia setigera*. Partial hydrolysis of the gum gave a mixture of sugars and a degraded material containing the uronic acids. The uronic acid portion had properties corresponding to a trisaccharide containing two D-galacturonic acid residues and a sugar residue, probably mainly L-rhamnose. The chief components of the non-acidic portion were D-galactose, L-rhamnose and a ketose, which, from its properties, could be fructose, tagatose or sorbose. A column of powdered cellulose was used to separate the sugar mixture with a solution of *n*-butyl alcohol saturated with water as the mobile phase. Two fractions crystallised spontaneously yielding D-galactose and L-rhamnose hydrate; D-tagatose was obtained from an intermediate fraction after oxidation of the accompanying aldose to the aldonic acid, which was removed as the barium salt. The D-tagatose thus isolated was identical with the sugar prepared synthetically. In all, five fractions were obtained containing respectively: a ketose, probably a methyl pentose; L-rhamnose; an aldose and D-tagatose; an aldose, tagatose and D-galactose; and D-galactose; D-tagatose has not hitherto been reported as a constituent of any natural product.

R. E. S.

ORGANIC CHEMISTRY

***p*-Aminobenzoic Acid and its Sodium Salt.** C. J. Kern, T. Antoshkiw and M. R. Maiese. (*Anal. chem.*, 1948, **20**, 919.) Sodium *p*-aminobenzoate was purified by charcoal treatment and three recrystallisations from aqueous solution. Purified *p*-aminobenzoic acid, prepared from the sodium salt by precipitation with hydrochloric acid, washing and drying at 100°C., melted at 187° to 187·5°C. A curve is given of the *p*H changes during titration of the acid with standard alkali and the equivalence point of 7·85, $pk^{\wedge}=4.65$ is derived. For acidimetric determination an indicator with a colour change between 7·0 and 8·7 is therefore suitable. A characteristic absorption spectrum was obtained in *iso*propyl

alcohol, wave length maximum = 288mμ. $E_{1\ cm.}^{1\ per\ cent.} = 1370$. In water both *p*-aminobenzoic acid and its sodium salt show about the same characteristic wave length, maximum = 266mμ, and $E_{1\ cm.}^{1\ per\ cent.} = 1070$, Beer's law being obeyed in both *iso*propyl and aqueous solutions. A table is given of comparison of results obtained by the spectrophotometer, titration, and diazo methods, all three being suggested for complete characterisation of pure *p*-aminobenzoic acid. R. E. S.

2-Amino-3-hydroxybenzoic Acid, Synthesis of. J. F. Nye and H. K. Mitchell. (*J. Amer. chem. Soc.*, 1948, **70**, 1847.) This substance was synthesised by two routes. In the first, 2-nitro-3-methoxybenzoic acid was reduced by catalytic hydrogenation to 2-amino-3-methoxybenzoic acid, followed by demethylation with hydriodic acid. The second method involved oxidation of 8-methoxyquinoline to give 2-(N-methyl-N-formyl)-amino-3-methoxybenzoic acid; appropriate treatment with hydriodic acid gave 2-amino-3-hydroxybenzoic acid m.pt. 254 to 255° C. (corr.). The compound of Keller (*Arch. Pharm.*, 1908, **246**, 1) reported as 2-amino-3-hydroxybenzoic acid was in reality the 3-methoxy derivative. Graphs of the ultraviolet absorption spectra of the 3-hydroxy and 3-methoxy derivatives are given. R. E. S

Mercurial Derivatives of Sulphanilamide. G. Rodighiero. (*Ann. Chim. appl. Roma.*, 1949, **39**, 27, 34.) In organic mercurial compounds the bond C-Hg usually shows greater resistance to reagents than the bond N-Hg and this influences the toxicity, antisyphilitic action and the uses in therapy and hygiene of these compounds. Mercurial derivatives of sulphanilamide are particularly interesting since they give the possibility of the mercury being linked to a carbon atom of the aromatic nucleus or to amino nitrogen or amido nitrogen. One molecule of sulphanilimide with 1 molecule of sodium hydroxide and 1 molecule of mercuric acetate gives (A) $H_2N.C_6H_4SO_2.NH.HgOH$. This is a white powder, decomposing on heating, insoluble in water, organic solvents and alkalis, but dissolving in hydrochloric acid to give the chloride (B) $HCl.H_2N.C_6H_4.SO_2.NH.HgCl$. This can be crystallised, has m.pt. 155° to 157°C. and is soluble in alcohol. These compounds have a notable antibacterial activity. The substance B dissolved in water and 0·5 to 1 molecule of sodium nitrite added at 0°C. gives (C) $HOHg.HNO_2SC_6H_4.NH.=NC_6H_4SO_2NH.HgOH$. This is a yellow powder insoluble in water, organic solvents and alkalis, but dissolves in hydrochloric acid, the solution liberating nitrogen on warming. The substance A, dissolved in 4 or more molecules of hydrochloric acid gives on the addition of 1 molecule of sodium nitrate at 0°C. (D) $H_2N.SO_2C_6H_4.N=NCl.HgCl_2$. This is a white powder which can be crystallised from water; soluble in acetone and alcohol, insoluble in ether, benzene, and chloroform. It deflagrates on heating. One molecule of the substance D dissolved in acetone and shaken with 2 molecules of powdered copper gives (E) $H_2N.O_2S.C_6H_4.HgCl$. Crystallised from acetone, this occurs in white needles, melting with decomposition at 315° C. It is insoluble in water and dilute acids, but soluble in sodium hydroxide, being reprecipitated unaltered on acidification. It is insoluble in ether and light petroleum, slightly soluble in hot alcohol, readily soluble in pyridine. If the substance E is dissolved in pyridine and poured into water a white amorphous unstable compound is precipitated, which when kept in a vacuum desiccator over sulphuric acid finally loses its

pyridine leaving (F) $C_6H_4.SO_2NH$ (with Hg bridging) This is a white, amorphous powder insoluble in water, acids, bases and organic solvents, except pyridine, slightly soluble in acetic acid. It does not melt on heating and is unchanged at 320° C. H. D.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Aminobenzoic Acids, Production of, by Sulphonamide-resistant Bacteria. R. Lemburg, J. P. Callaghan, D. E. Tandy and N. E. Goldsworthy. (*Austral. J. exp. Biol.*, 1948, **26**, 9.) It is widely held that drug-resistance can be explained by an increased rate of production, or an increased total production, of *p*-aminobenzoic acid by a resistant strain of bacteria as contrasted with the susceptible parent strain. The main difficulty in demonstrating this has been the lack of specific methods for the isolation of *p*-aminobenzoic acid from the bacterial cultures. Attempts have therefore been made to develop a method of extraction and separation of aromatic animo-acids which would be sufficiently sensitive to be used with the low concentrations of these substances found in bacterial cultures. The method adopted by the authors consisted in the transformation of the aromatic amino-acids into azo-dyes by diazotisation and coupling with dimethyl-α-naphthylamine, in the separation of the acidic from the non-acidic dyes, and in separation of the former by chromatography on alumina. A strain of *Bacterium coli*, growing in synthetic Kisch's medium, produced diazotisable amino-acids in the supernatant; these were identified for the most part as anthranilic acid and *p*-aminobenzoic acid. After this strain had become adapted to sulphathiazole it did not produce more *p*-aminobenzoic acid that the parent susceptible strain, and the amount of *p*-aminobenzoic acid was insufficient to account for the resistance to sulphathiazole. The same held for the sum of *o*- and *p*-aminobenzoic acid produced. The production of anthranilic acid was variable and decreased with the number of sub-cultures. The authors therefore concluded that *p*-aminobenzoic acid production does not explain acquired drug-resistance so far as *Bacterium coli* is concerned. It may be true that the method of drug adaptation is different in different organisms. S. L. W.

Antibiotic Activity, as Shown by a Highly Amylolytic Strain of *Bacillus subtilis* B. S. Lulla. (*Nature*, 1949, **163**, 489.) *Bacillus subtilis* when grown on wheat bran medium, showed a pronounced antibiotic activity, the maximum being found in the aqueous extract from a 24-hour old culture; during this period the amylase formation was found to be low, but steadily increased with further incubation. Results are given of a study of the relationship between the antibiotic production and amylase formation by *B. subtilis* (N.C.T.C.: 2027 N) when grown on wheat bran. The antibiotic activity, at a maximum on the first day of growth, gradually disappears with further incubation, while amylase production, although negligible on the first day, steadily increases as the incubation period proceeds, and reaches its peak value on the fourth day. There is therefore a relationship between amylase formation and the production of antibiotic substance. R. E. S.

Insulin, Regeneration from Insulin Fibrils by the Action of Alkali. D. F. Waugh. (*J. Amer. chem. Soc.*, 1948, **70,** 1850.) Reversion by alkali treatment of insulin fibrils produces a crystalline product (termed r-insulin) similar to native insulin. A detailed method is given for the complete conversion of insulin into freely suspended insulin fibrils by heating crystalline zinc insulin at 100°C. with 0·05N hydrochloric acid in sealed glass ampoules. Limiting conditions for regeneration procedure were determined by studying the effect of alkali on native insulin. Using 0·5 ml. of 2 per cent. insulin (10 mg.) and 5·0 ml. of sodium hydroxide, experiments indicated that 0·03N alkali, 0°C. and a 45-minute treatment time gave the best results. Reversion of the fibrils was greatly accelerated by increasing the number of available fibril ends (by a freezing and thawing cycle which breaks up the longer fibrils into short segments), suggesting that disaggregation occurred mainly at these positions. The presence of sodium chloride in N/1 concentration in the alkali caused a 90 per cent. inhibition of reversion; the repulsive forces between similarly charged groups may thus play a part in the mechanism of disaggregation. The crystalline product from reverted fibrils was not found to be significantly different from native insulin in crystallisation properties, in biological activity (20 I.U. per mg.), in ultracentrifuge pattern (sedimentation constant 3·3 to 3·6), and in fibril formation (at 20° and 100°C.). The irreversible loss of one or more of the characteristic properties following demonstrable changes in internal structure was not found with r-insulin; tests for changes in labile groups, such as amino and disulphide, have been negative. The retention by r-insulin of characteristic insulin properties known to be sensitive to structural changes, the absence of changes in labile groups, and the fact that fibril elongation may take place at low temperatures in the *p*H region of maximum stability, are interpreted as showing that only small structural changes take place during fibril formation and that the process is one in which globular or corpuscular units are linked endwise. R. E. S.

Neomycin, a New Antibiotic. S. A. Waksman and H. A. Lechevalier. (*Science*, 1949, **109,** 305.) The organism producing neomycin was isolated from the soil, and is related to *Streptomyces fradiæ*. When the newly isolated culture was grown in various media containing a source of nitrogen, a carbohydrate, and salt, it was found to produce neomycin under both stationary and submerged conditions of culture. The antibiotic can easily be removed from the culture medium and concentrated by the methods of adsorption and elution applicable to streptomycin. Neomycin is a basic compound, most active in an alkaline medium. It is soluble in water and insoluble in organic solvents. It is thermostable. It is active against numerous Gram-positive and Gram-negative bacteria, especially mycobacteria, but not against fungi. The antibiotic spectrum of crude neomycin is quite distinct from that of streptomycin or streptothricin. Neomycin preparations were found to posses the following desirable properties: (1) similar activity against both streptomycin-sensitive and streptomycin-resistant bacteria; (2) considerable activity against various forms of -resistant mycobacteria; (3) limited or no toxicity to animals; (4) activity against various bacteria *in vivo*, including Gram-positive and Gram-negative organisms and against both streptomycin-sensitive and streptomycin-resistant organisms; (5) lack of resistance against neomycin among the organisms sensitive to it, or only limited development of such resistance. Neomycin has not

yet been obtained in crystalline form, but preliminary results point to its being distinctly different chemically from streptothricin and from streptomycin.
S. L. W.

Penicillin, Diffusion of. L. Mosonyi, R. Held and Ch. Kocsàn, (*Acta med. scand.*, 1942, **132**, 487.) The diffusion properties of penicillin are different from those of other crystalline substances. It is evenly absorbed by collidal substances, such as agar, without losing its efficiency and its diffusion is greatly influenced by this adsorption. A thrombin-fibrinogen membrane was shown to exert an adsorptive action on penicillin similar to that of agar, a fibrin layer of 3 mm. preventing the action of 0·5 to 1 unit of penicillin when interposed between the penicillin and an infected agar plate, though a fibrin layer of this thickness is not able to inhibit the action of penicillin when used in larger quantities (5 to 10 I.U.). As the concentration of penicillin in the blood, even when given intravenously, reaches only 0·3 to 0·4 units, and this for a very short time only, this explains why bacteria at the base of vegetations such as occur on the endocardium in endocarditis, and which often exceed a thickness of 4 or 5 mm., are found to retain their full virulence in spite of administration of penicillin. This adsorptive action of fibrin can be lessened, and the penicillin rendered more diffusible, by the addition to the penicillin solution of 20 per cent. of sodium dehydrocholate. S. L. W.

Penicillin. Enhancement of Therapeutic Activity. G. A. Hobby, T. F. Lenert, W. Reed and D. Renne. (*J. Bact.*, 1949, **57**, 247.) As a result of further work to discover the reason for the enhanced activity of impure penicillin as compared with crystalline penicillin G, evidence has been found suggesting that certain degradation products of penicillins G and dihydro-F respectively enhance the activity of highly purified samples of the two penicillins. The products possibly responsible are *p*-hydroxyphenylacetic acid, caprylic acid, penillic and penicilloic acids. H. T. B.

Progesterone, Stability of. R. B. Woolf and W. M. Allen. (*Proc. Soc. exp. Biol., N.Y.*, 1948, **67**, 79.) Samples of α and β-progesterone isolated from pigs' ovaries in 1936 were found on assay to be as active, after storage for 10 years, as the first preparations obtained by Wintersteiner and Allen in 1934. The preparations had been stored in small, unsealed glass-stoppered vials at room temperature. Both α and β-forms were assayed according to a slight modification of the original Corner-Allen method and the results were compared with the data obtained in 1934 by the original method. The comparison showed that the quantities necessary to produce full proliferation, and the quantities which produced little or no proliferation, were virtually the same, establishing beyond doubt that there had been no great change in activity in the interim. The dosage response curves from the data of 1934 were found not to differ significantly from those obtained from assay in 1946. S. L. W.

Steroids, Deuterium-labelled. Infra-red Spectrometry in Metabolic Studies. K. Dobriner, T. H. Kritchevsky, D. K. Fukushima, S. Lieberman, T. F. Gallagher, J. D. Hardy, R. N. Jones and G. Cilento. (*Science*, 1949, **109**, 260.) Infra-red absorption spectra of steroids, with one or more of the hydrogen atoms replaced by deuterium, are of value in the detection, analysis and identification of these compounds. The spectrum of pregnanol-3-(α)-one-20 is compared with the spectrum of the same compound where a hydrogen atom at C-11 and at C-12 has been

replaced by deuterium. Two absorption bands appear at 2,165 and 2,125 cm^{-1} in the deuterium-containing compound and in the neighbourhood of 1,200 $cm.^{-1}$ pronounced differences are apparent in the two spectra. The C-D absorption bands in the neighbourhood of 2,150 $cm.^{-1}$ are useful for the identification of a deuterium-containing molecule as this region, in the concentrations used, is transparent in the absence of deuterium. The method was very sensitive and the presence of deuterium could be established in as little as 25 μg. of pregnan-11:12-d_2-ol-3-(α)-one-20 containing 5 atoms per cent. excess of the isotope. In a metabolic experiment allopregnan-5,6-d_2-ol-3-(β)-one-20-acetate was injected into a normal woman and the urine and fæces were collected over 20 days; a fractionation procedure for treatment of urine and fæces followed by spectrum examination showed that the isotope was present in both the crude α- and β-hydroxy-ketonic fractions as well as in the α- and β-hydroxy non-ketonic fractions from both urine and fæces. Graphs are given of the infra-red spectra of normal and deuterium steroids in carbon disulphide solution for pregnanolone and pregnan-11:12-d_2-ol-3-(α)-one-20.

R. E. S.

Tricothecin, Isolation and Chemical Properties of. G. G. Freeman and R. I. Morrison. (*Biochem. J.*, 1949, **44**, 1.) The isolation of trichothecin from the culture filtrate of *Tricothecium roseum* is described. The nitrate was extracted with chloroform and the residue after evaporation of the chloroform was dissolved in ether and fractionated on a column of activated alumina using ether to develop the column. After fractional precipitation if inactive material from light petroleum and chloroform, the residue obtained was dissolved in carbon tetrachloride and again fractionated on an alumina column until pure tricothecin, m.pt. 118°C., was obtained. The substance dissolved in chloroform, ethyl alcohol, acetone, and benzene, and was slightly soluble in water (400 mg./l. at 25°C.); it had optical activity $[\alpha]^{18°C.}$ + 44° (*c*.1, in chloroform); analytical data were consistent with the molecular formula $C_{15}H_{18}O_4$ or $C_{15}H_{20}O_4$. The molcule contained one ketone group, one ethylenic group and three methyl groups attached to carbon; free carboxyl, hydroxyl, alkoxyl and aldehyde groups were absent. On hydrolysis with alcoholic potassium hydroxide, tricothecin combined with one equivalent of alkali. The ultra-violet absorption spectrum of tricothecin contained two main bands which, together with the shift of the bands occurring with a change in polarity of the solvent, indicated that the molecule contained conjugated ethylenic and carbonyl groups. Tricothecin was found to be relatively stable in acid solution and at *p*H 10, but at *p*H 12 hydrolysis took place with liberation of a carbonyl group and with virtually complete loss of antifungal activity in 6 hr. at 20°C. Acidification of the inactivated alkaline solution led to the formation of an inactive neutral ketone.

R. E. S.

BIOCHEMICAL ANALYSIS

Alcohol in Blood and other Biological Fluids, Colorimetric Determination of Microquantities of. R. J. Henry, Carol F. Kirkwood, S. Berkman, R. D. Housewright and J. Henry. (*J. Lab. clin. Med.*, 1948, **33**, 241.) The method depends upon the oxidation of the alcohol to acetaldehyde and determination of the latter colorimetrically with *p*-hydroxydiphenyl. Up to 20 ml. of sample is diluted with 20 ml. of water and 0·1 ml. of 10 per cent. sodium hydroxide solution and distilled. The

distillate, which should contain all the alcohol, is added to a solution of potassium dichromate and sulphuric acid and again distilled. The distillate is collected in a graduated tube in an ice-bath, the quantity collected depending on the amount of alcohol in the original sample. The loss of alcohol by complete oxidation to acetic acid is controlled by strict adherence to the routine described. The acetaldehyde is estimated by adding to 1 ml. of the distillate cooled in ice, 1 drop of 5 per cent. copper sulphate solution, 6 ml. of arsenic and nitrogen-free sulphuric acid, and, with constant shaking, 0·1 ml. of a 1·5 per cent. solution of *p*-hydroxydiphenyl in 0·5 per cent. sodium hydroxide solution. The mixture is warmed at 30° C. for 30 minutes, placed in a boiling water-bath for 90 seconds to dissolve excess of reagent, and cooled in ice to room temperature. The deep violet colour, which is stable for several hours, is read in a photoelectric colorimeter against a blank prepared by using 1 ml. of water instead of the distillate. Substances which interfere with the results include oxalacetic acid, α-glycerophosphates, glyceraldehyde and other alcohols. Among the non-interfering substances listed are methyl alcohol, glucose, acetone, pyruvic acid, urea and various amino-acids. The method is suitable for the determination of blood in amounts obtainable from finger puncture. The blood should be diluted 1 in 20, and the proteins and erythrocytes precipitated with tungstic acid. The method gives results within ±6 per cent. on a single blood determination.

G. R. K.

Alcohol, Ether and Volatile Reducing Substances in Blood and Gases, Determination of. A. Hemingway, L. A. Bernat and J. Maschmeyer. (*J. Lab. clin. Med.*, 1948, **33**, 126.) Various methods used for the determination of alcohol and ether in blood and air are reviewed and their disadvantages are examined. In the procedure adopted the reducing substance is absorbed in a known excess of standard potassium dichromate solution in the presence of sulphuric acid and the excess of chromic acid is determined by titration with ferrous sulphate solution using the redox indicator barium diphenylamine sulphonate. In the presence of phosphoric acid the indicator gave a sharp end-point from violet-blue to colourless, which is the main advantage of the method. Details are given for the determination of reducing substances in small quantities of blood using the Widmark flask method, and also for the determination in gases. The effect of variation in distillation time is studied and results are given of tests carried out on the reducing power of sulphuric acid, on the oxidation of acetic acid, and on the recovery of ether from prepared solutions of ether in blood.

R. E. S.

***p*-Aminosalicylic Acid in Blood and Urine, Estimation of.** H. G. Dickenson and W. Kelly. (*Lancet*, 1949, **256**, 349.) To a solution of 1 per cent. sulphanilic acid in 10 per cent. hydrochloric acid, cooled in ice, add 10 per cent. sodium nitrate solution until the reaction is just positive to starch iodide; then add the sulphanilic-acid solution until the starch iodide test is negative. This solution is kept cold and made up freshly for each test. Blood serum (2 ml.) containing *p*-aminosalicylic acid equivalent to 10 to 20 mg./100 ml. diluted with water (2 ml. is deproteinated with 10 per cent. trichloracetic acid (2 ml.), and filtered. The filtrate (2 ml.) is made strongly alkaline by adding 30 per cent. sodium hydroxide solution (0·25 ml.) and the diazo solution (0·25 ml.) added. The stable cherry colour produced is compared with that obtained from standard aqueous solutions of *p*-aminosalicylic acid equivalent to 10 to 20

mg./100 ml. Sulphonamides and *p*-aminobenzoic acid give no coloration, and normal blood only a negligible coloration. Salicylic acid gives a coloration only about 5 per cent. of that obtained for *p*-aminosalicylic acid. The recovery of added *p*-aminosalicylic acid from blood and blood serum is from 90 to 100 per cent. For the estimation in urine, 5 to 10 drops of a 10 per cent. calcium chloride solution is added to an aliquot (10 ml.) of the urine sample, the *p*H brought to 8 to 9 with 3N ammonia, the solution filtered, and the filtrate and washings adjusted to *p*H 2 with 10 per cent. hydrochloric acid. The intensity of colour on adding 2 drops of 10 per cent. ferric chloride solution is observed on a sample, and the urine diluted to give a colour approximately equivalent to that obtained from a standard solution of *p*-aminosalicylic acid. A colorimetric estimation can be made with an accuracy of ± 5 per cent. Salicylic acid will, of course, interfere with this estimation.

S. L. W.

Anti-Pernicious Anæmia Factor, Estimation of. W. F. J. Cuthbertson. (*Biochem. J.*, 1949, **44**, v.) The microbiological assay of Shorb for the growth factor present in highly refined liver extracts was found to be unsatisfactory. The organism used, *Lactobacillus lactis* Dorner ATCC 8000, required, in addition to the medium of Shorb, tomato juice and "Tween 80" as well as the anti-pernicious anæmia factor. Thymidine allowed the growth of the organism on vitamin B_{12}-deficient media and it was not possible to obtain a response to the anti-pernicious anæmia factor using the technique of Shorb. The cup-plate assay was adaptable to the determination of the anti-pernicious anæmia fraction and to the detection of other members of the B_{12} group of microbiological growth factors present in purified liver extracts. The medium used was that found suitable in the ordinary microbiological assay with the addition of 2 per cent. of agar. For an assay the sterile medium is melted, held at 45°C., and inoculated with a culture of *Lb. lactis* Dorner ATCC 8000; 12·5 ml. samples are then poured into Petri dishes. Holes are cut in the covered agar plates with a 10 mm. cork borer. Three drops of test or standard solution are placed in each of the appropriate holes and the plates are incubated overnight. After 16 to 24 hr. the colonies developing around holes form sharply defined zones of exhibition; zone diameters are proportional to the logarithms of the anti-pernicious anæmia fraction concentrations over the range 0·02 to 0·5μg./ml. Both factors contribute to microbiological activity and unless the ratio of clinical to microbiological activity is the same for both of these substances this test alone will not exactly predict the clinical potency of liver extracts. The method is rapid and simple, but it is relatively insensitive and somewhat susceptible to interference by other members of the B_{12} group, preservatives and antibiotics.

R. E. S.

Penicillin. Paper Strip Chromatography. R. G. Kluener. (*J. Bact.*, 1949, **57**, 101.) A modified paper strip technique which can be completed in 24 hours is described for determining separately penicillins X, G, F and K. The method is based on the differences in the distribution coefficients of the varieties of penicillin between ether and a phosphate buffer. The ether used for development must be anhydrous and of reagent grade. In analysing 35 known mixtures in buffer solution and 24 known mixtures in broth, the difference between percentages added and those found did not exceed 13 per cent. and averaged ± 5 per cent.

H. T. B.

Penicillin G, Determination of. G. B. Levy, D. Shaw, E. S. Parkinson and D. Fergus. (*Anal. Chem.*, 1948, **20**, 1159.) The light absorption in the ultra-violet region due to the benzyl group in penicillin G, is used to determine this component in mixtures of penicillins. The total light absorption of a penicillin mixture is due to a "background" non-selective absorption upon which is superimposed the benzene band spectrum which can be evaluated by several methods; graphs are given illustrating this procedure. For routine analysis of penicillin G preparations, a simplified technique was used based on the fact that commercial penicillin G preparations usually do not contain penicillin X or inactivated penicillin G, but, besides benzylpenicillin, only small amounts of penicillins F, K, and dihydro F, and some inert pigment. Under these conditions an average value for the angular displacement of the spectrum due to impurities, i.e. the slope of the "background" absorption—together with the measurement of the height of a characteristic band (maximum and minimum) affords a rapid method for determining the benzyl content of penicillin G preparations. Comparisons of assays by this method with the gravimetric procedure, based on the precipitation of penicillin G by N-ethyl piperidine, showed that the average deviation for the gravimetric method is 1·43 per cent., and for the spectrophotometric method 1·40 per cent., while the maximum deviation is 4·5 and 5·2 per cent. respectively. The method evolved is particularly suited to routine use but extraneous benzyl groups will interfere. A photoelectric spectrophotometer is necessary to produce the required accuracy of extinction readings. R. E. S.

Sodium in Biological Fluids, Microcolorimetric Determination of. A. A. Albanese and M. Lein. (*J. Lab. clin. Med.*, 1948, **33**, 246.) The reagent is a solution of uranyl zinc acetate prepared by adding a boiling solution of 10 g. of uranyl acetate in 50 ml. of water and 2 ml. of glacial acetic acid to a boiling solution of 30 g. of zinc acetate in 50 ml. of water containing 1 ml. of glacial acetic acid, allowing to stand overnight, filtering, diluting with an equal volume of alcohol (95 per cent.), cooling at 4°C. for 48 hours and again filtering. A mixture of 0.2 ml. of urine or spinal fluid and 1 ml. of reagent is cooled at 4°C. for one hour, and centrifuged. The supernatant liquid is discarded, the residue drained and washed with 2 ml. of alcohol (95 per cent.), again centrifuged and drained, and dissolved in 5 ml. of water. Any turbidity due to an excess of phosphate is removed by centrifuging and the intensity of the yellow colour of the solution measured in a photoelectric colorimeter. A parallel determination is done for 0.2 ml. of a standard solution of sodium chloride in water containing 2 mg. of sodium per ml. The content of sodium per ml. is calculated from: reading of unknown/reading of standard × 0.4 mg. of Na × 5. Fer sera, plasma and whole blood, 0·2 ml. is treated with 0.6 ml. of a 20 per cent. solution of trichloracetic acid, centrifuged, and 0.4 ml. of the supernatant liquid treated as described. The experimental error is about ±5 per cent. G. R. K.

Steroids in Urine, Determination of. S. L. Tompsett. (*Analyst*, 1949, **74**, 6.) A review is made of the methods available for the isolation and identification of the steroids found in human urine. The origin of the principal steroid hormones, male and female, is given, together with a detailed table of the principal natural steroids found in man. Steroids in urine are separated from other materials by three procedures: (1) extraction of the steroid conjugates with butyl alcohol followed by acid hydrolysis, (2) a short acid

hydrolysis followed by extraction of the free steroids with an organic solvent, (3) simultaneous hydrolysis by acid and extraction with a comparatively high-boiling solvent. Methods for the determination of total 17-ketosteroids, of non-alcoholic 17-ketosteroids, and of α- and non-alcoholic 17-ketosteroid content after precipitation of the β-alcohols with digitonin, are given. The 3:20-ketosteroid fraction is discussed and a method is given for the determination and separation of ketonic and non-ketonic steroids using Girard reagent T. Results are quoted for urine samples from a wide variety of clinical cases. For a complete picture of steroid hormone metabolism the following determinations are necessary: œstrogens; 17-ketosteroids; total ketones indicative of the presence of the 20-ketosteroids; the non-ketones; pregnanediol-3(α):20(α); the corticosteroids. R. E. S.

CHEMOTHERAPY

Diamidines as Antibacterial Compounds. R. Wien, J. Harrison and W. A. Freeman. (*Brit. J. Pharmacol.*, 1948, **3**, 211.) In the diphenoxyalkanes there was a graded increase in bacteriostatic activity, which was maintained in the presence of blood, against staphylococci, rising to a maximum from the propane to the hexane and nonane derivatives. This increase was accompanied by an increase of intravenous toxicity but by only a relatively small increase in local toxicity to phagocytes. Gram-positive bacteria were more susceptible than Gram-negative bacteria. The introduction of halogen into one or both benzene nuclei in the diphenoxyalkanes increased bacteriostatic activity, with little alteration in local toxicity to phagocytes. The mono-halogen derivatives were more active than the di-halogen against staphylococci, whereas the di-halogen derivatives were more active against Gram-negative bacteria. Dibromopropamidine and iodohexamidine were amongst the most active of the compounds examined for their possible use in surface infections. They both showed bacteriostatic and bacterical activity, these effects being decreased in an acid and increased in an alkaline medium. Drug-resistant strains of bacteria could easily be induced by repeated sub-cultivation *in vitro*. Cross-resistance experiments showed that: (1) staphylococci resistant to penicillin or to 5-amino-acridine were susceptible to diamidines, (2) staphylococci and streptococci resistant to one diamidine were resistant also to other diamidines, (3) staphylococci resistant to diamidines were not resistant to penicillin or 5-amino-acridine. Little therapeutic activity can be demonstrated when the compounds are given by injection. S. L. W.

Miracil D. D. M. Blai, and F. G. Loveridge. (*Lancet*, 1949, **256**, 344.) Miracil D was given by mouth twice a day for 3 to 6 days to African school-children. Of 82 children who received a total dosage of at least 60 mg./kg. of body weight 74 ceased to pass living eggs or active miracidia, and none of the cured cases had relapses up to 12 weeks after treatment. There seems to be no advantage in giving more than 15 mg./kg. daily. This dosage caused no symptoms in about half the children. The others complained of abdominal pain, loss of appetite, nausea, and/or headache and dizziness, but none were ill enough to seek medical aid. A few children infected with *S. mansoni* were also treated with the same dosage but the results were unsatisfactory. S. L. W.

PHARMACY

GALENICAL PHARMACY

Cetyltrimethylammonium Bromide, Efficacy of in Ointment Bases. L. P. Prusak and A. M. Mattocks. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 67.) Six ointments comprising 1 per cent. of cetyltrimethylammonium bromide in hydrophilic ointment U.S.P., hydrophilic petrolatum U.S.P., simple ointment U.S.P., tragacanth glycerite N.F., pectin paste N.F., and a carbowax ointment base prepared by mixing carbowax 1500 with 10 per cent. of water, were added in amounts of 0·5 g. to 5 ml. quantities of nutrient broth inoculated with *Staphylococcus aureus*, and the mixtures were incubated at 37°C. for 3- and 24-hour periods. One loopful (0·05 ml.) of each mixture was sub-cultured in a medium containing lecithin, which inhibits the action of the bactericide, incubated for 48 hours and the tubes were read for turbidity. Control tests were carried out to determine the extent to which the ointment bases interfered with the action of the bactericide. Pectin paste and carbowax ointment base proved to be suitable ointment bases. The ointments prepared with hydrophilic ointment and hydrophilic petrolatum had no bactericidal activity, while those prepared with simple ointment and tragacanth glycerite released such small amounts of bactericide as to be practically ineffective. G. R. K.

Saccharated Iron Oxide for Intravenous Administration. J. H. Nissim and J. M. Robson. (*Lancet*, 1949, **256**, 686.) Samples of saccharated iron oxide differ widely in toxicity owing to differing methods of preparation. The following method permits of better control and provides material of lower toxicity. Dissolve 25 g. of anhydrous sodium carbonate in 1 l. of distilled water and add 50 g. of ferric chloride ($FeCl_3,6H_2O$). Carbon dioxide is evolved and the ferric hydroxide formed dissolves in the excess of ferric chloride to give a dark purple solution. Add sodium carbonate solution gradually to reprecipitate all the iron as ferric hydroxide, and adjust to *p*H 7. Wash away the sodium chloride formed by repeated additions of distilled water in quantities of 1000 to 2000 ml., allowing the ferric hydroxide to settle and decanting, continuing until the supernatant fluid acquires a brownish tinge. Add 166 g. of sucrose, followed by 30 ml. of 15 per cent. sodium hydroxide solution, pour the mixture into a flat dish and heat in the oven at 130°C. The saccharated hydroxide gradually dissolves to an almost black solution. If to a sample of this solution (enough to colour 10 ml. of distilled water red-brown) dilute hydrochloric acid is added drop by drop it precipitates when *p*H 8 is reached. With continued heating, the *p*H at which this precipitation takes place falls gradually from 8 to 3. When the required precipitation point is reached (the original specimen used clinically with success had *p*H 5·7) the temperature of the oven is reduced to 90°C., the product is evaporated to dryness, and dissolved in 500 ml. of distilled water to give a solution containing 2 per cent. of elemental iron. After filtering through a Whatman No. 50 filter paper, and autoclaving, it is ready for use. Variations in the method of preparation are discussed and an account given of toxicity tests with these preparations leading to the selection of the best samples for intravenous administration. The macroscopical and microscopical findings in mice receiving lethal doses are described and the manner in which saccharated iron oxide produces

its toxic effects is discussed. Until the method of preparation is sufficiently reliable to ensure the constant production of satisfactory samples the authors consider that biological standardisation is necessary. S. L. W.

PHARMACOGNOSY

Indian Belladonna, Studies on. R. Chatterjee and J. K. Lahiri. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 11.) Indian belladonna, *Atropa acuminata* Royle, was compared morphologically with *A. belladonna* Linn. and found to differ mainly in the structure of the leaves. As a result of a critical study of specimens of *Atropa* from the Calcutta Herbarium, the authors suggested that the Indian species should be reduced to a variety of the type species and henceforward be called *A. belladonna* Linn. var. *acuminata* (Royle) R. Chatterjee and J. K. Lahiri. *A. lutescens* Jacquemont was said to be synonymous with *A. belladonna* Linn. var. *acuminata* (Royle), hence the statement by Chopra that *A. lutescens* (which is of low alkaloidal content) is used as an adulterant to Indian belladonna seemed without foundation. The hyoscyamine content of the roots from Indian sources is fairly high; commercially the roots are used for the preparation of atropine, and can be used for hyoscine since this constitutes about 15 per cent. of the total alkaloids. G. R. K.

***Juniperus occidentalis*, Hooker, Sierra Juniper Wood.** E. F. Kurth and H. B. Lackey. (*J. Amer. chem. Soc.*, 1948, **70**, 2206.) Trees of this species grown in Oregon were collected, the bark removed, and representative specimens of sapwood, heart-wood, whole wood, stumpwood and root-wood obtained. After room-drying to a moisture content of less than 10 per cent. the various samples were extracted with ethyl ether; the extractive ranged from 2·96 per cent. (stumpwood) to 6·50 per cent. (heart-wood). Approximately 1 per cent. of additional material, soluble in acetone, was chiefly a catechol phlobaphene. The ether extract was soluble in light petroleum to an extent of 2·53 per cent. on the weight of the sap-wood and 3·01 per cent. on the weight of the whole wood. The light petroleum extract consisted of resin acids, oleic acid and high molecular weight lactonic acids, a mixture of α- and β-sitosterol, and a hydroxyresene $C_{18}H_{31}O$. The volatile oil from the trunk of the tree ranged from 0·9 to 1·25 per cent. and appeared to consist of cedrol; the oil obtained from the rootwood contained cedrene and cedrol. R. E. S.

***Remijia pedunculata*, Observations on the Bark of.** H. W. Youngken. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 27.) Studies of the physical characteristics and histology of *Remijia pedunculata* bark, a recognised source of quinidine sulphate in the U.S.P. XIII, and of quinine during the war, are reported. The materials and methods used in these studies are outlined. The unground bark is described and shown to differ from cinchona in having brittle cork which readily separates from the bark and is therefore absent or partly absent from most commercial specimens. A detailed description of the histology of the stem bark is given and differences between it and cinchona bark noted, a striking difference being the absence of microcrystals. New additional anatomical data are also reported and the powdered bark is described. Illustrations of pieces of the unground drug, the cross section of the stem bark and the diagnostic tissue elements of the powdered

bark are included. *Remijia pedunculata* bark is shown to satisfy the identification test for cinchona in the N.F. VIII; hence it is suggested that this test be deleted in the N.F. IX or made more specific for cinchona. G. R. K.

***Opuntia vulgaris*, a new Source of Pectin.** H. Diacon and V. Massa. (*Ann. pharm. Franc.*, 1949, **6**, 457.) *Opuntia vulgaris* Mill., which is common in Tunisia, gives a good yield of pectin. From 2 kg. of the fresh twigs, 14 g. of calcium magnesium pectate were prepared. The anti-hæmorrhagic action of this material was determined, and it was concluded that the action was superior to that of the pectin used by previous authors. This superiority, it is considered, is due solely to the high content of calcium and magnesium. G. M.

PHARMACOLOGY AND THERAPEUTICS

Amethocaine Hydrochloride. Severe Toxic Effects when used for Bronchoscopy. C. A. Jackson. (*Brit. med. J.*, 1949, **1**, 99.) The occurrence of toxic reactions in 2 patients treated with amethocaine to secure local analgesia before bronchoscopy is reported. The procedure adopted was to give 2 lozenges of benzocaine 200 mg. to suck 40 minutes pre-operatively followed after 10 minutes by 11 to 16 mg. of morphine. In the anæsthetic room, the fauces, the posterior pharyngeal wall and both pyriform fossæ were painted with less than 2 ml. of a 2 per cent. solution of amethocaine hydrochloride containing adrenaline 1 in 5000, and finally 2 ml. of the same solution was injected between the cords. Soon after injection, both patients became unconscious and had convulsions. In one patient, endotracheal oxygen and carbon dioxide and venepuncture effected recovery, but in the second, who had received 4 ml. by injection, deep cyanosis supervened and the pulse stopped, necessitating cardiac massage. Although the total period of cardiac arrest was about 4 minutes and unconsciousness persisted for 4 days, complete recovery took place. To avoid the occurrence of severe reactions the following precautions are suggested: (a) a barbiturate should be given by mouth pre-operatively; (b) an amethocaine pastille should be sucked 30 minutes before examination; (c) the total dose of amethocaine should not exceed 80 mg., and the solution should contain adrenaline, 1 in 5000; (d) application by spray should not be used; (e) amethocaine should not be applied to inflamed, traumatised or highly vascular surfaces (especially in the urethra), and (f) it should not be used for allergic, severely debilitated or cachectic patients. Treatment of toxic reactions should include artificial respiration with oxygen-carbon-dioxide mixture, using an endotracheal tube if necessary, intravenous administration of a rapidly-acting barbiturate to control the convulsions, and administration of respiratory and cardiac stimulants. G. R. K.

Antabuse, Preliminary Report on Clinical Trials. R. G. Bell and H. W. Smith. (*Canad. med. Ass. J.*, 1949, **60**, 286.) Antabusé is the Danish trade name for tetraethylthiuramdisulphide. Patients receiving this drug have an abnormal reaction to alcohol in the body, though the drug itself has few effects when administered in daily doses of 0·5 g. over a period of several months. The symptoms produced by alcohol after administration of antabuse are probably due to interference with the oxidation of alcohol so that abnormally high levels of acetaldehyde are produced in the body.

This report is mainly concerned with the symptoms produced when alcohol is taken after antabuse. 5 to 10 minutes after ingestion of a moderate amount of alcohol (20 g. of ethyl alcohol) there is a sensation of heat in the face, accompanied by flushing of the face and upper part of the body, with throbbing of the head and neck and accelerated pulse. After larger doses of alcohol (40 to 50 g. of ethyl alcohol) nausea may begin 30 to 60 minutes after the cardiovascular symptoms and may result in copious vomiting. A considerable fall in blood pressure may occur. The duration of the symptoms may last from half an hour to several hours but after a few hours sleep the patient feels completely well again. Antabuse will prove a valuable adjunct in the treatment of the alcoholic patient. S. L. W.

Curare; A Method of Assay Using Rats. M. G. Allmark and W. M. Bachinski. (*J. Amer. pharm., Ass., Sci. Ed.*, 1949, **38**, 43.) A unit consisting of 15 separate compartments floored partly with wire netting and partly with galvanised iron and fitted with a cover to keep the rats in their respective compartments was attached to a frame at a 60° angle. Commercial samples of intocostrin and *d*-tubocurarine chloride were used for the tests, diluted with water to a concentration required to produce responses when injected subcutaneously into each rat. After injection, each rat was placed in a separate compartment and observed for 20 minutes; if it fell off the wire netting within this time it was considered a reactor. It was found that very few rats fell off after 20 minutes. To test the validity of the method several three-dose assays were carried out using 15 rats on each dose. *d*-Tubocurarine chloride was used as a standard for each assay. Results were tabulated and the methods of Bliss were followed in making the calculations. The slopes of the regression lines were found not to differ significantly for the 10 assays reported and in no assay did the slope of the regression line for intocostrin differ from that of *d*-tubocurarine chloride, nor could it be determined that the weighted means of the slopes differed, indicating that the responses in rats are the same for both products. Further tests of accuracy were recorded using solutions of known potency. The mean actual error for these assays was 5·7 per cent. Changing the slope of the frame to 75° did not increase the accuracy of the method. It compared favourably with the rabbit head-drop cross-over test and the mouse method. G. R. K.

Decamethonium Iodide (C10) in Anæsthesia. G. Organe. (*Lancet*, 1949, **256**, 773.) The use of decamethonium iodide in 150 operations of many different types, have established that this drug is a safe and satisfactory substitute for *d*-tubocurarine and that it may be used in unselected cases. It is roughly five times as potent as *d*-tubocurarine chloride but has a shorter effect, and it produces a neuromuscular block which is not affected by anticholinesterases. A single intravenous injection of 3 mg. in light surgical anæsthesia produces in most patients good muscular relaxation without unduly depressing respiration. Its action is relatively evanescent and further injections are made at intervals of 10 to 40 minutes as required. The dose depends on the preceding interval—after 40 minutes a further 3 mg. will probably be necessary. Pentamethonium iodide in a dose 10 times that of decamethonium iodide is an effective antidote. Thoracic and abdominal breathing fail, and recover, together. It seems to act similarly to *d*-tubocurarine in reducing laryngeal muscular irritability. There appears to be no

direct effect on the cardiovascular system, even with relatively large doses. Post-operative vomiting occurs in less than 25 per cent. of patients, post-operative collapse seems considerably less frequent than after *d*-tubocurarine chloride, and there have been no cardiovascular complications. Urinary retention lasting for 24 hours occurred in 9 per cent. of patients. All the common anæsthetic agents have been used with no obvious difference in effect, and a mixture of decamethonium iodide 4 mg. with thiopentone 1 g. has been used successfully. S. L. W.

Dextran as a Plasma Substitute. G. Thorsén. (*Lancet*, 1949, **256**, 132.) A proprietary form of dextran is described as a 6 per cent. solution of a polydispersoid glycose-polymer dextran, in which most of the molecules have been hydrolytically given a molecular weight conforming to that of an albumin, with 0·9 per cent. of sodium chloride added. Its viscosity lies between that of blood and plasma, and its specific gravity somewhat exceeds that of human plasma. It is non-toxic and does not injure the tissues either locally or systematically. After an intravenous injection of 1 or 2 l. of dextran the plasma-dextran level rises to 1 to 2·5 g./100 ml., and after an initial fall due to elimination of a low molecular fraction through the kidneys it falls at an even rate. During the initial renal excretion, when about a quarter of the dextran given is excreted, the urine-dextran level rises to 7 g./100 ml. without sign of renal injury. After that no dextran can be detected in the urine, the remainder of the dextran of a higher molecular weight being presumably metabolised. In Sweden today the hospital transfusion services rely to a large extent on dextran for emergency cases. It has been given to 5000 patients, and as much as 4 l. has been given in a single infusion. Very good results have been obtained both in the treatment and prevention of shock, and it is stated to be as good as plasma in shock from burns. Its use has not been found to affect fertility, foetal development or growth. S. L. W.

Dimethyl Ether of *d*-Tubocurarine Iodide, Pharmacology of. H. O. J. Collier, S. K. Paris and L. I. Woolf. (*Nature*, 1948, **161**, 817.) The dimethyl ether of *d*-tubocurarine iodide was compared with *d*-tubocurarine in the following ways: (1) in the intact mouse, rat and rabbit; (2) in a preparation of the rat under nembutal anæsthesia, similar to that of Raventos, in which the contractions of the rectus femoris muscle, in response to repeated condenser charges applied to its motor nerve, are recorded on smoked paper; (3) in the rat phrenic nerve-diaphragm preparation *in vitro*. In the intact rabbit the intensity of action of the dimethyl ether compound was shown to be many times greater than that of *d*-tubocurarine, while the slopes of the two duration curves were closely similar, indicating that the two substances are removed from their site of action at similar rates. The dimethyl ether compound, therefore, has a greater specific action at the myoneural junction in this species; this is again shown in the rat, though to a somewhat less extent than in the rabbit, by the *in vitro* experiments on the phrenic nerve-diaphragm preparation. In the intact mouse, the myoneural junction is slightly less sensitive to the dimethyl ether compound than to *d*-tubocurarine, but the rate of elimination of the former is slightly less than that of the latter. In the rat, *in vivo*, the duration of action of the dimethyl ether compound is many times greater than that of *d*-tubocurarine, whereas it has only about three times the intensity of action, which indicates that it is removed much more slowly from its site of action in this species. In the

Raventos preparation of the rat, the weight of the dimethyl ether compound required to reduce the tension of the muscular response to a given extent was one-third to one-half that of *d*-tubocurarine, when each drug was administered by jugular cannula, and the reduction lasts considerably longer. Ligation of the renal arteries and veins in the preparation prior to administration of either substance increases duration of action of each but does not prevent recovery of the muscular contraction. The dibenzyl and di-isopropyl ethers of *d*-tubocurarine iodide were prepared. The dibenzyl compound exhibited in the mouse about one-third the activity of *d*-tubocurarine and about one-half the intensity of action in the rat and the rabbit; it did not exhibit any more prolonged action. The dimethyl ether of *l*-bebeerine di-methochloride was also prepared. The ED50 and LD50 of this compound were found to be about twice those of *d*-tubocurarine. It was three times as potent as *d*-tubocurarine in reducing tension of muscular contraction in a Raventos preparation, and the effect lasted four times as long. S. L. W.

Local Anæsthetics, a Comparison of. H. S. Hamilton, B. A. Westfall and J. K. W. Ferguson. (*J. Pharmacol.*, 1948, **94**, 299.) A new series of indices, to be called Relative Ratings (R.R.) is proposed for the comparison of potency and toxicity of local anæsthetics in relation to cocaine or procaine. For each the drug of reference should be named, e.g., the Relative Rating with reference to cocaine should be designated R.R. (cocaine). In addition, the bases of comparison should be specified in tables or in the text. Example for any drug X:

$$\frac{\text{LD50 of cocaine}}{\text{LD50 of X}} = \text{Relative Toxicity (cocaine) or Toxicity relative to cocaine.}$$

$$\frac{\text{EC50 of cocaine}}{\text{EC50 of X}} = \text{Relative Potency (cocaine) or Potency relative to cocaine.}$$

Then:

$$\frac{\text{Relative Potency (cocaine)}}{\text{Relative Toxicity (cocaine)}} = \text{Relative Rating (cocaine).}$$

LD50 values (by intraperitoneal injections in mice), tissue toxicities (by intradermal injections in guinea-pigs), and EC50 values for infiltration anæsthesia (by intradermal injections in guinea-pigs), and surface anæsthesia (of the guinea-pig cornea) were determined for procaine, metycaine, monocaine, naphthocaine, butacaine, octocaine, cocaine, amethocaine (pontocaine) and cinchocaine (nupercaine). The LD50 values decreased in magnitude in the order given. Adrenaline hydrochloride in solution with each anæsthetic increased the systemic toxicity of procaine and metycaine and decreased the toxicity of amethocaine and cinchocaine significantly, but had no effect on the toxicity of the other drugs. From these determinations Relative Rating indices for the 9 drugs are defined and estimated by the authors for infiltration anæsthesia, localised block anæsthesia, and corneal anæsthesia. The two relatively new drugs naphthocaine (*β*-diethylaminoethyl-4-amino-1-naphthoic acid) and octocaine (2-(1-methylheptyl)2:2-dimethylethyl *p*-amino-benzoate) have high Relative Ratings for all four types of anæsthesia. S. L. W.

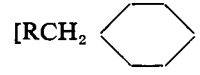
Orthoxine, Pharmacology of. B. E. Graham and M. H. Kuizenga. (*J. Pharmacol.*, 1948, **94**, 150.) In a study of a large series of phenyl propylamines, for the purpose of obtaining compounds more active than ephedrine

as bronchodilators and possessing little or no pressor activity, the methoxyphenyl*iso*propylamines appeared most interesting. The intravenous toxicities, pressor actions and bronchodilator properties of 14 of these amines are recorded. *ortho*-Methoxy-β-phenyl*iso*propyl methylamine hydrochloride (Orthoxine), because of its high activity and low toxicity, was investigated further. Perfusion experiments with constrictor agents on isolated lungs showed it to be a much more effective agent than ephedrine for relieving bronchoconstriction. Using isolated strips of jejunum or ileum, it was 4 to 8 times as effective as ephedrine in relieving intestinal smooth-muscle spasms induced by histamine, acetylcholine and barium chloride, and 5 times as effective in quieting the normal contractions of unstimulated intestinal muscle. On the non-pregnant uterus it was only half as active as ephedrine in stimulating the muscle to contraction. It produces little or no pressor response, and 4 times as much orthoxine as ephedrine must be administered to normal dogs to produce the bradycardia characteristic of the latter. Intestinal smooth-muscle tests indicate that it possesses anti-histaminic properties of the order of 1/20 that of benadryl but much greater than that of ephedrine. Its toxicity, chronic and acute (except for higher intravenous toxicity), is of the same order as that of ephedrine. The authors suggest that the drug may be of value in the treatment of asthma, and clinical trials are being carried out. S. L. W.

BACTERIOLOGY AND CLINICAL TESTS

Dithiocarbamic Acid Derivatives: Action Against Human Pathogens. C. R. Miller and W. O. Elson. (*J. Bact.*, 1949, **57**, 47.) The activity of compounds in this group against plant pathogens prompted the investigation of their *in vitro* activity against human bacterial and fungal infections. The substances tested included series of dithiocarbamates, thiuram monosulphides and thiuram disulphides, and a number of miscellaneous sulphur-containing compounds. The most active antibacterial compounds studied were tetramethylthiuram disulphide and sodium dimethyldithiocarbamate, their activity being greatest against *Streptococcus pyogenes*, much less against *Str. fæcalis*, *S. aureus* and *Eschericha coli*, and weakest against *Pseudomonas aeruginosa*. The same compounds were also the most active against the fungi tested, *Trichophyton gypseum* being most affected, *Epidermophyton floccosum*, *Microsporum canis*, *Sporotrichum schenckii*, *Blastomyces dermatitidis* much less, and *Candida albicans* least. H. T. B.

Quaternary Ammonium Sulphonamides, Antibacterial Actions of. C. A. Lawrence and G. R. Goetchius. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 424.) Six members of a series of compounds of the general structure



have been examined for antibacterial action against a number of organisms. The two most active compounds were those in which R = tetradecyldimethylamino-, R′ = R″ = H, and R = pyridine, R′ = dodecyl, R″ = H. These two compounds were highly active against both gram-positive and gram-negative organisms but not against *Pseudomonas æruginosa*. The antibacterial actions of these compounds are due to the quaternary ammonium component in the molecule and not to the sulphonamide radical.

A. L.