ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

11-Desmethoxyreserpine. J. W. E. Harrisson, E. W. Packman, E. Smith, N. Hosansky and R. Salkin. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 688.) The 3:4:5-trimethoxybenzoic acid ester of methyl 11-desmethoxyreserpate known as raunormine, canescine, deserpidine or recanescine was isolated from the mother liquors after the crystallisation of reserpine from extracts of *Rauwolfia canescens*. The substance appeared to be 11-desmethoxyreserpine. On hydrolysis it yielded raunormic acid which differed from reserpic acid in that the infrared spectrum of the hydrochloride lacked the bands at 7·94 and 9·52μ, characteristic of the methoxyl group attached to the aromatic ring, and also the bands at 6·25, 6·35, 11·5 and 12·2 μ, which are characteristic of a 1:2:4-substituted benzene ring. Preliminary studies using mice, rats and dogs showed that the toxicity of raunormine is similar to that of reserpine. G. B.

Rescinnamine, Isolation from *Rauwolfia vomitoria* Afz. D. A. A. Kidd. (*Chem. Ind.*, 1955, 1481.) During the isolation of reserpine from roots of *R. vomitoria*, a further alkaloid was encountered in close association with it, and this was identified as rescinnamine, first isolated from *R. serpentina* and not hitherto found in any other species. Rescinnamine is only a relative minor constituent of *R. vomitoria* (0·6 g. isolated from 4 kg. of dried roots).

A. H. B.

ANALYTICAL

Cholic, Desoxycholic and Dehydrocholic Acids, Determination of. R. Crisafio and L. G. Chatten (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 529.) The following methods were used. (1). Place 5 ml. of methanol and 50 ml. of benzene in a 150-ml. beaker, add 2 drops of thymol blue solution (0·5 per cent. in methanol) and neutralise, using 0·1N potassium methoxide or methanolic potassium hydroxide. Add the sample of bile acid, cover the beaker with a rubber dam and titrate to a blue end-point as rapidly as possible, while stirring with a magnetic stirrer. (2). Place the sample of bile acid in a beaker, add 2 ml. of *NN*-dimethylformamide and stir until the sample has dissolved or is thoroughly wetted. Add 50 ml. of chloroform and 2 drops of thymol blue solution and titrate to a purple-blue end-point. A reagent blank determination is necessary. For the determination of dehydrocholic acid in tablets by method (1) a quantity of powdered tablets equivalent to about 0·2 g. of dehydrocholic acid was dissolved in methanol and benzene and titrated. Using method (2) the sample was dissolved in dimethylformamide and filtered to remove excipients. The filter was washed with chloroform and the washings added to the titration liquid. As an alternative to the use of thymol blue, the end-point was determined potentiometrically using glass and sleeve-type calomel electrodes. Lithium chloride was added to the benzene-methanol solution to decrease its resistance. When titrations were carried out using an open beaker, stirring by hand, the quantity of carbon dioxide absorbed from the air was insufficient to affect the results. Methanolic potassium hydroxide appeared

to give the same results as potassium methoxide solution and had the advantage of being easier to prepare. The results were at least as satisfactory as those obtained by the U.S. National Formulary IX method, which is more difficult to apply. G. B.

Morphine in Opium, Improvement in U.S.P. Method for Determining. H. W. Brickley and F. A. Whipple (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 538.) In carrying out the U.S.P.XIV assay for morphine in opium, the time required for filtration may be reduced considerably by adding calcium phosphate before beginning the assay process and by applying suction during the filtrations. The total time of the assay is reduced by one third while the accuracy of the procedure is unaffected. The addition of magnesium carbonate as a filtering aid was investigated, but found to be of little value, besides giving rise to low results. G. B.

Reserpine and Related Compounds, Paper Chromatographic and Biological Properties of. R. J. Boscott and A. B. Kar. (*Nature, Lond.*, 1955, **176**, 1077.) Trimethoxybenzoic acid, a known hydrolysis product of reserpine, does not affect the fertility of male and female rats. Thus the reported anti-fertility effect of reserpine in rats is due to the molecule as a whole or some fragment other than trimethoxybenzoic acid. A number of paper chromatographic procedures for checking the purity of commercial samples of *Rauwolfia* alkaloids are recommended including the following, (a) Single phase systems, such as the aqueous phase obtained by shaking 10 per cent. v/v acetic acid in 5 per cent. sodium acetate with *n*-butyl ether, *tert.*-amyl alcohol, *sec.*-butanol or methyl*iso*butylketone. A mixture of xylene (200), methanol (75) and methyl*iso*butylketone (25) may also be used. (b) Partition chromatography is also recommended using the solvent system *n*-amyl alcohol (200), water (180), acetic acid (20), or alternatively *n*-hexyl ether (200), methyl*iso*butylketone (50), acetic acid (20), water (180). Tests for locating the spots are described, and typical R_F values of a number of alkaloids are given for the various solvent systems. J. B. S.

Reserpine in Pharmaceutical Preparations, Determination of. R. E. Booth (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 568.) For the determination of reserpine, material is mixed with bromophenol blue and buffer solution, pH 4·0. Bromophenol blue is insoluble in chloroform, but each molecule reacts with 2 molecules of reserpine to produce a complex which can be removed by extraction with chloroform and determined colorimetrically. Absorption measurements are made at 402 mμ, the result being calculated from a standard curve prepared with the aid of pure samples of reserpine. The method is sufficiently sensitive for use in the assay of commercial tablets and elixirs of reserpine. Since reserpic acid reacts in the same way as reserpine, an additional test to determine the extent to which it has been formed by hydrolysis of reserpine is required in the case of aqueous preparations. G. B.

Sodium Carboxymethylcellulose, Determination of. C. R. Szalkowski and W. J. Mader (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 533.) The method depends upon the formation of glycollic acid by reaction of sulphuric acid with carboxymethylcellulose. Carboxymethylcellulose is precipitated from pharmaceutical preparations in the form of its copper salt, which is dissolved in dilute sulphuric acid and heated in a water bath with a 0·05 per cent. solution of 2:7-naphthalenediol in concentrated sulphuric acid. The colour of the solution is measured spectrophotometrically at 530 mμ against a reagent blank, and the

quantity of sodium carboxymethylcellulose is calculated from a calibration curve, prepared by using a standard preparation of sodium carboxymethylcellulose. The standard material must have the same degree of substitution as the sample under test, as this affects the intensity of colour produced. The method is applicable to preparations containing antibiotics and good recoveries have been obtained from samples containing penicillin, procaine penicillin, streptomycin, dihydrostreptomycin and sodium citrate. G. B.

B Vitamins, Quantitative Separation of, by Electrophoresis on Agar Plates. G. Marten. (*Nature, Lond.*, 1955, **176**, 1064.) Mixtures of vitamin B have been quantitatively separated by electrophoresis directly on thin layers of agar jelly as used in macro-electrophoresis. After separation in the electric field is complete a second layer of agar jelly is placed on the first, and contains all the substances required by the test organisms seeded into the agar, except that to be determined. Zones of growth appear in those spots corresponding to the presence of the vitamins in the lower layer, the logarithm of the dose being directly proportional to the diameter of the zone. Chemical determination of the vitamin is also possible after cutting out the appropriate portion of the agar jelly. The method, which will detect as little as 0·002 μg. of vitamin B_1 is described in detail. J. B. S.

ORGANIC CHEMISTRY

Sterculic Acid, Structure of. J. P. Verma, B. Nath and J. S. Aggarwal. (*Nature, Lond.*, 1955, **176**, 1082.) The infra-red spectrum of sterculic acid shows a band at 1008 cm.$^{-1}$. This is considered to support the structure $CH_3.(CH_2)_5.CH{-}CH.CH = CH.(CH_2)_7.COOH$ in which the *cyclo*propane ring is

$$\diagdown\!\diagup \\ CH_2$$

in conjugation with the double bond. More recent work has shown, however, that this could not readily be distinguished from the alternative *cyclo*propene structure. The Halphen reaction is not specific to cottonseed oil, positive results being obtained with oils which do not contain *cyclo*propene fatty acids. The authors do not accept the view that this reaction can be attributed to the presence of a *cyclo*propene group alone. It is also emphasised that there are many instances in which *cyclo*propane groups are no less reactive than ethylene groups, while *cyclo*propene groups have been shown to possess appreciable stability. J. B. S.

Veratrum Alkaloid Group, Application of Paper Chromatography to Structural Problems in. K. Macek and Z. J. Vejdělek. (*Nature, Lond.*, 1955, **176**, 1173). A paper chromatographic method has been devised to assist in the determination of the number of free hydroxyl groups, which form a series of diol systems in the various veratrum alkamines, by exploiting the reaction of such diol groups with boric acid. The compounds were run in two parallel chromatograms with chloroform as the mobile phase on paper impregnated with formamide containing 5 per cent. boric acid in one case and with formamide alone in the other. Since the glycol-borate complexes are highly polar the ratio of movement of compounds containing suitable glycol systems is decreased on the boric acid-impregnated paper. The difference in R_M values for the same compound in the two systems (ΔR_M) gives an indication of the number of free glycol systems. Two glycol systems give ΔR_M values between 0·8 and 1·2 units, whilst a single glycol system gives ΔR_M between 0·3 and 0·45. Changes in

R_M on acylation, and upon partial methanolysis of polyester derivatives give an indication of the number of glycol systems blocked by esterification. The results obtained provide interesting facts concerning the configuration of the glycol system at position 17:20. Contrary to the conclusions of Barton *et al.*, who suggest that it is *trans*, the authors find that the glycol system is capable of complex formation with boric acid, indicating that a *cis* configuration is more probable. Calculation of group constants makes it possible to predict R_M and hence also R_F values for isomeric mono- and poly-esters of the veratrum alkamines, so that for the chromatographic detection and identification of esters of any further acid it is sufficient to determine experimentally the R_F value of a single alkamine ester of the acid. J. B. S.

Vitamin B_{12}, Reduction of. G. H. Beaven and E. A. Johnson. (*Nature, Lond.*, 1955, **176**, 1264.) The two known reduction products of Vitamin B_{12}, now designated as (I) and (II) respectively, have been further examined. Compounds with spectra resembling that of (I) may be obtained by use of a number of different reducing agents. Hydrogenation gives not only (I) but other products of ill-defined structure; chromous acetate is more satisfactory giving a solution of (I) which is stable indefinitely in an atmosphere of hydrogen, and which can be re-oxidised quantitatively to vitamin B_{12a}. Contrary to the reports of Boos *et al.*, the authors find that reduction of vitamin B_{12} with cobalt acetate in a solution buffered at pH 3 with ethylenediamine tetra-acetate gives exclusively (I), and not (II). The latter is obtained by reduction at pH 9·5, with (I) appearing as an intermediate. Both reduction products may be obtained just as readily from vitamin B_{12a} as from vitamin B_{12}, and (II) is also obtained from vitamin B_{12} in dilute potassium cyanide solution. The ready re-oxidation of (I) and (II) to vitamin B_{12a} suggests that the changes merely concern the state of oxidation of the cobalt. In the light of this observation the differences between the spectra of (I) and (II) suggest that there is increased conjugation in the latter, possibly accompanied by rearrangement and release of the benziminazole group from co-ordination. It is thought the spectrum of the vitamin itself could be better explained by the inclusion of an additional double bond in the structures already assigned to it, so that the two alternative structures would then be capable of resonance. Activated groups capable of being chlorinated would still be present in such structures with six conjugated double bonds, and whilst a further increase of conjugation by dehydrobromination would then be unlikely, the spectrum of (II) suggests that the long-wave shift of vitamin B_{12} could be explained without requiring the introduction of still further unsaturation in the molecule. J. B. S.

TOXICOLOGY

Arsenical Poisoning; Studies on some Cases of. H. Griffon. (*Ann. pharm. franç.*, 1955, **13**, 600.) Samples were taken from the bodies of victims of arsenical poisoning, at periods up to 14 years 3 months after death. As the bodies were considerably decayed, relatively small specimens were available and results were inconclusive, except when specimens of hair were examined. These were comparatively well preserved, and arsenic could be estimated at positions along the hair, and a graphic representation showing bands of arsenical impregnation along the hair obtained. In all cases this was in agreement with toxicological and other evidence obtained at about the time of death. G. B.

3:3-Diethyl-2:4-dioxotetrahydropyridine (Persedon), Isolation and Detection of, for Forensic Purposes. F. Dybing. (*Acta pharm. tox., Kbh.*, 1955, **11**, 393.) Persedon is a weaker hypnotic drug than the barbituric acids. It may be extracted from biological tissues by the standard Stas-Otto process, shaking out with 3 successive portions of ether. After evaporation of the solvent, the crude residue is dissolved in methanol and subjected to paper chromatography. After elution with sulphuric acid, the ultra-violet absorption of the solution is measured. M. M.

α-Naphthylthiourea (ANTU), Detection of, for Forensic Purposes. F. Dybing. (*Acta pharm. tox., Kbh.*, 1955, **11**, 388.) A method is described for the isolation of this pesticide. Purification by paper chromatography replaces the saponification of the fat. The material is extracted by adding equal amounts of water and methanol and shaking with *n*-heptane. Most of the fat is extracted by the heptane, while the ANTU remains in the methanol-water mixture. The methanol is removed by distilling under reduced pressure and the ANTU extracted with ether or chloroform. Purification by paper chromatography with a chloroform-formamide mixture for development is followed by identification of the ANTU by its ultra-violet absorption or by a colour reaction with bromine water in the presence of excess sodium hydroxide. M. M.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Cholinesterase, Inhibition of, by 1:2:4-Triazoles. J. B. Polya. (*Nature, Lond.*, 1955, **176**, 1175.) Following an observation that laboratory workers manipulating 1:2:4-triazoles develop symptoms of light nicotine or physostigmine poisoning, it has been shown that simple water-soluble 1:2:4-triazoles may act as inhibitors of cholinesterase activity. The effects of 3:5-dimethyl-(I), 1:3:5-trimethyl-(II), 3:5-dimethyl-1-phenyl-(III), 3-ethyl-5-methyl-1-phenyl-(IV) and 5-ethyl-3-methyl-1-phenyl-1:2:4-triazole (V) as inhibitors of cholinesterase from freshly homogenised sheep brain were examined. Cholinesterase activity was determined manometrically using acetylcholine, acetyl-β-methylcholine, and benzoylcholine chlorides as substrates. A time-lag was observed in all experiments, with pressure dropping, after an intial rise, to a minimum in about 20 minutes. A second 'wave', not adequately explained, was observed with (I) and (II) as inhibitors after about 60 minutes or more. Using rabbit brain tissue anomalous results of this kind were observed with all the triazoles. Similar inhibitory action was also observed with (III), (IV) and (V), and typical experiments with (III) showed that specfic cholinesterase is completely inhibited by (III) for substrate concentrations of 1–30 mg. acetyl-β-methylcholine/100 ml. and triazole/substrate ratios between 0·01 and 0·05. Lower triazole concentrations are ineffective and higher concentrations show slight activation of the enzymes. Similar benzoylcholine and triazole/substrate values activate the unspecific cholinesterase, whilst inactivation occurs at higher triazole concentrations. Inconsistent results were obtained with acetylcholine as substrate. J. B. S.

Oximes and Hydroxamic Acids, Reactivation by, of Cholinesterase Inhibited by Organo-phosphorus Compounds. A. F. Childs, D. R. Davies, A. L. Green and J. P. Rutland. (*Brit. J. Pharmacol.*, 1955, **10**, 462.) Human red-cell and rat brain cholinesterases inhibited by the "irreversible" anticholinesterases,

ethyl pyrophosphate, sarin or dyflos, were reactivated by members of the oxime series and related compounds. There was considerable variation in reactivating activity in compounds of a given type against the different anticholinesterases. Di-*iso*nitrosoacetone and related *iso*nitroso compounds were most effective against sarin, slightly less effective against ethyl pyrophosphate and least effective against dyflos. Picolinhydroxamic acid, on the other hand, was nearly as active as the oximes against ethyl pyrophosphate and dyflos whilst nicotinhydroxamic acid methiodide had equal activity to picolinhydroxamic acid against sarin, but was much less effective against either ethyl pyrophosphate or dyflos. The oximes in general were superior to the hydroxamic acids, especially against sarin-inhibited cholinesterase. The only common factor among the most potent reactivators was a pKa value of about 8. The reactivation, being a nucleophilic reaction, probably occurs through the anion of the oxime, so that, under the conditions of the tests (pH 7·4) with high pKa's the fraction of oxime ionised would be too small and with low pKa's the anion would be too weakly nucleophilic to be an effective reactivator. G. P.

BIOCHEMICAL ANALYSIS

Aureomycin (Chlortetracycline) in Biological Materials, Colorimetric Determination of. T. Sakaguchi and K. Taguchi (*Pharm. Bull. Japan*, 1955, **3**, 303.) Aureomycin appears to exist in the body in the form of chelate compounds with metals, from which it is freed by the addition of a 10 per cent. solution of sodium ethylenediaminetetra-acetate, the reaction of the solution being adjusted to pH 1–2. The liberated aureomycin is extracted by saturating the solution with sodium chloride and shaking with several successive quantities of butanol. The butanol solutions are washed with a 25 per cent. solution of sodium chloride and extracted with a 1 per cent. solution of thorium nitrate, so as to obtain an aqueous solution of the thorium chelate compound of aureomycin. The light absorption of this substance in acetate buffer solution is determined at 400 mμ, and the quantity of aureomycin calculated from a standard curve prepared with the aid of a standard preparation of aureomycin. This method is applicable to the determination of aureomycin in biological fluids containing 2–20 μg./ml. A correction for interfering substances should be applied when examining specimens of urine containing small quantities of aureomycin. The same method may be used for the determination of tetracycline and oxytetracycline. G. B.

Chemotherapeutic Agents, Determination of Bacterial Sensitivity to. G. Czerkinsky, N. Diding, and O. Ouchterlony. (*Scand. J. clin. Lab. Invest.*, 1955, **7**, 259.) A rapid and simple method is described for the determination of bacterial resistance to chemotherapeutic substances using paper strips. Strips of filter paper are impregnated in rectangular zones with sulphathiazole 0·1 mg., penicillin 1 I.U., chlortetracycline 10 μg., oxytetracycline 10 μg., chloramphenicol 20 μg. and streptomycin 50 μg., the zones being separated with linseed oil. Each strip is placed across an agar plate, and one side of the plate is smeared with a suspension of the test bacterium and the other half with a suspension of the standard (usually *Staph. aureus*). After incubation for 18 hours the zones of inhibition are measured for both the test and the standard bacteria from which the degree of resistance is calculated. It is also possible to determine when two adjacent antibiotics exert a synergistic or antagonistic effect. A special machine is described for impregnating the strips. G. F. S.

Heparin in Plasma, Determination of. H. Engelberg, A. Dudley and L. Freeman. (*J. Lab. clin. Med.*, 1955, **46**, 653.) Circulating heparin is strongly protein bound and this new method is based upon the tryptic digestion of the total serum or plasma proteins previously precipitated by methanol-acetone and subsequent dialysis and lyophilization. Add 10 ml. of methanol and 10 ml. of acetone to 5 ml. of citrated plasma, shake vigorously and stand for 30 minutes. Centrifuge and decant the supernatant. Wash the precipitate twice with acetone, centrifuging and decanting each time. Dry the coagulum under a vacuum aspirator. Add 5 ml. of phosphate buffer (0·2M, pH 8·5) and 0·5 ml. of a purified trypsin concentrate solution containing 80 mg. per ml. Digest overnight at 37°C. Heat coagulate for 30 minutes in a boiling water bath cooling the tops of the tubes with a fan. Cool, and decant and drain the clear superantant into a dialysis tube. Dialyze under running cold tap water overnight. Transfer the dialysed solution into a test tube and lyophilize until dry. Remove the dry sample and store at 4°C. The anticoagulant activity of the lyophilized material dissolved in 1·0 ml. of isotonic saline is determined by a semi-micro modification of United States Pharmacopeial method for heparin assay (previously described *Amer. J. clin. Path.*, 1954, **24**, 599). The results of duplicate assays of 25 individual plasma samples showed a ± 13 per cent. deviation from the mean. G. F. S.

Plasma Glycine, Determination of. T. B. Schwartz, M. C. Robertson and L. B. Holmes (*J. Lab. clin. Med.*, 1955, **46**, 657.) A simple, accurate and specific method is described for the determination of glycine in plasma, serum or rat diaphragm using a Conway microdiffusion unit. One ml. of heparinized plasma is diluted with 4 ml. of picric acid solution in a centrifuge tube, stirred, centrifuged and the glycine concentration of the supernatant is determined. 0·5 or 1 ml. of the supernatant is pipetted into the outer well of the microdiffusion unit, evaporated to dryness under an exhaust fan in a hood. The rims of the Conway vessels are greased with soft paraffin, 0·5 ml. of ethyl phosphate is added to the outer chamber near the outside rim of each vessel and the unit gently rotated so that the ethyl phosphate is evenly distributed. 2 ml. of sodium chromotropate solution is added to the centre well and 0·5 ml. of a ninhydrin reagent (2 g. ninhydrin diluted to 100 ml. with citrate buffer) is placed in the outer well. A glass cover is quickly placed over the greased rim to produce an airtight seal, and the unit is allowed to stand overnight in the dark while the formaldehyde distils over into the centre well. The covers of the vessels are then removed, the contents of the inner well are stirred and transferred to test tubes which are heated in a boiling water bath for 30 minutes and protected from the light. After cooling the solutions are transferred to calibrated test tube cuvettes and read at 570 mμ in a spectrophotometer. A glycine standard containing 0·1 μM glycine per ml. and a reagent blank are both run in duplicate through the entire procedure. As little as 0·02 μM of glycine can be determined and recoveries of $98·7 \pm 1·4$ per cent. are obtained from human plasma. As many as seventy-two determinations can be carried out simultaneously using multiple units. G. F. S.

Serum Calcium, Determination of. H. E. Harrison and H. C. Harrison. (*J. Lab. clin. Med.*, 1955, **46**, 662.) The method is based on the precipitation of calcium as oxalate, dissolving the calcium oxalate in a measured excess of disodium ethylenediaminetetra-acetic acid and measuring the excess chelating agent by back titration with standard calcium chloride solution. Pipette 0·2 ml. of serum into a centrifuge tube and add 0·2 ml. of oxalate reagent (consisting

of 0·5 ml. of 0·1 N oxalic acid added to 10 ml. of 0·1 N sodium oxalate). Shake for 30 minutes in a shaker, stand until precipitation is complete, centrifuge for 5 minutes and remove the supernatant. Dissolve the precipitate in 0·5 ml. of a solution of dihydrogen ethylenediamine-tetracetic acid (this solution is prepared daily from a stock solution of 4·5 g. of the salt in 1 litre of distilled water, by taking 2 ml. and diluting to 10 ml. with 0·25 M ethanolamine). Add 0·05 ml. of working indicator solution (0·5 ml. of a 0·5 per cent. solution of eriochrome black in ethanolamine added to 2 ml. of distilled water), and titrate with a standard solution of calcium chloride (0·02 M calcium chloride = 0·8 mg. calcium) from a microburette until the colour changes from a blue green to a purple red. Deduct a blank determination for the reagents. The results agree with the Clark Collip method and show an error of ± 5 per cent. G. F. S.

PHARMACY

NOTES AND FORMULÆ

Diphtheria Toxin: Production in Submerged Culture. F. V. Linggood A. C. Matthews, S. Pinfield, C. G. Pope and T. R. Sharland, (*Nature, Lond.*, 1955, **176**, 1128.) This paper gives details of improvements in the authors' original method of the submerged culture of *C. diphtheriæ*. 80 litre aluminium tanks, vortex stirring and sterile air at a rate of 0·05–0·15 litre per minute are used. A growth period of about 48 hours and an initial inoculum of 200 ml. of a 48-hour culture of *C. diphtheriæ* for a volume of 50–60 litres of medium is satisfactory. By such means the culture filtrate has an Lf value of the order of 180–250 units per ml. Such a method is superior to the surface culture technique. It is preferable to harvest single batches of toxin rather than to run a semi-continuous process. M. M.

Preservatives in Pharmaceutical Products, Efficacy of. E. J. Rdzok, W. E. Grundy, F. J. Kirchmeyer and J. C. Sylvester. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 613.) Vials containing 10 ml. or more of various preparations for injection were inoculated with cell or spore suspensions of the test organisms to give a cell count of about 10,000 to 50,000 per ml. The vials were incubated at 30° C. and after 1, 3 and 7 days and weekly up to 1 month, samples were checked by making plate counts. The organisms used were *Bacillus cereus, Aspergillus niger, Candida albicans, Streptococcus fæcalis, Micrococcus pyogenes* var. *aureus, Escherichia coli* and *Pseudomonas æruginosa*. For use in an aqueous suspension of œstrone for injection 0·9 per cent. of benzyl alcohol was a satisfactory preservative whereas 0·18 per cent. of methyl *p*-hydroxybenzoate with 0·02 per cent. of propyl *p*-hydroxybenzoate was not sufficiently active against *Ps. æruginosa* and *Str. fæcalis*. For preserving a solution of suxamethonium chloride, 20 mg./ml., the mixture of *p*-hydroxybenzoates was satisfactory. Benzethonium chloride, 1 in 5000, was suitable for preserving an ophthalmic preparation, but a lower concentration (1 in 50,000) was not effective against certain strains of *Ps. æruginosa*. Tests were made to assess the value of preservatives in preventing spoilage due to yeast and soil organisms. For this purpose, phenylethyl alcohol was satisfactory for selenium sulphide jelly and 0·2 per cent. benzoic acid with 0·06 per cent. methyl *p*-hydroxybenzoate was satisfactory for a vitamin syrup. G. B.

PHARMACOLOGY AND THERAPEUTICS

Actinomycin C in Hodgkin's Disease. J. R. Trounce, A. B. Wayte and J. M. Robson. (*Brit. med. J.*, 1955, **2**, 1418.) Actinomycin C was given to 6 patients with advanced Hodgkin's disease and one with a reticulum-celled sarcoma. All the patients had previously been treated with radiotherapy, and all but one had received one or more courses of nitrogen mustard. The usual scheme of dosage employed was to start with 100 μg. of actinomycin C intravenously. If no toxic effects were observed the dose was increased so that at the end of a week or 10 days the patient was receiving 400 μg. daily. With doses of 400 μg. or more the drug was dissolved in half a pint of saline and given by intravenous drip over 2 to 3 hours. A total course of 7000 and 10,000 μg. was used whenever possible. Only 2 of the patients showed a marked response to treatment, with disappearance of fever and considerable reduction in the size of lymph nodes and spleen. In one of these patients improvement has been maintained to date—a period of 3 months. Two other patients showed slight improvement. Three cases showed no response. The side-effects of the drug included stomatitis and thrombocytopenia. The results suggest that in advanced reticuloses actinomycin C only occasionally has any great effect on the disease, but it would seem worthy of trial when the other more usual treatments have failed and especially in those patients unable to take nitrogen mustard. S. L. W.

Adrenaline and Noradrenaline, Elimination of, from the Circulating Blood. O. Celander and S. Mellander. (*Nature, Lond.*, 1955, **176**, 973.) An *in vivo* study is made of the manner in which various tissues are capable of eliminating adrenaline and noradrenaline from the blood stream. Cats in which the adrenal glands had been removed and the spinal cord cut were used. Adrenaline or noradrenaline was infused at a constant rate, either intra-arterially to the tissue studied or intravenously into the brachial vein. The contractions of the chronically denervated nictitating membrane of the same animal were used to indicate the amount of amine in the systemic circulation. In most of the experiments the adrenaline or noradrenaline was infused into the lower part of the abdominal aorta in the eviscerated animal and therefore into tissues consisting mainly of skin and skeletal muscle. When the infusion was shifted to the intravenous route, without any change in the dosage, there was a marked contraction of the nictitating membrane, indicating a corresponding increase in the catechols in the systemic circulation. It could be estimated that the muscle and skin of this region destroyed about 90 per cent. of either amine, in one passage of the blood, providing that the amount administered was physiological. This considerable loss in a rather non-specific tissue area can only in part be attributed to the action of amine oxidase since very similar results were obtained after the administration of specific amine oxidase inhibitors. Tissues such as the spleen, kidney and intestines gave similar results. It is reasonable to assume that locally released adrenergic transmitters are inactivated in this way. M. M.

Chlordane: Report to the Council on Pharmacy and Chemistry. (*J. Amer. med. ass.*, 1955, **158**, 1364.) Chlordane is a chlorinated hydrocarbon insecticide available in various forms as an impure mixture. It is a heavy, dark brown, oily liquid insoluble in water but soluble in the common organic solvents. It is

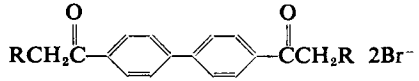
unstable in the presence of weak alkalis. The volatile properties and residual characteristics of chlordane are intermediate between those of dicophane and gamma benzene hexachloride. It is effective in the control of certain agricultural and household pests and is available in the form of oil solutions, emulsion concentrates, wettable powders, and insecticidal dusts, paints and waxes. Lethal action on susceptible organisms results from contact, ingestion or exposure to vapour. It is not approved for over-all treatment of rooms because slow liberation of fumes constitutes a danger. No medicinal use for chlordane in any form has been found acceptable to date. A complete pharmacological evaluation of technical chlordane is difficult because it is not a definite chemical entity. In general, it acts like other chlorinated hydrocarbon insecticides whose sites of action are on the higher motor cortex and the cerebellum. It does not affect vital centres in the medulla. It is absorbed into the body from the gastro-intestinal tract, the respiratory tract and the skin, and it would seem to be capable of a more rapid and greater penetrability of the body barriers than many other synthetic insecticides belonging to the chlorinated class. The minimum lethal dose of chlordane by ingestion for human beings is not known, but death has occurred following the ingestion (with suicidal intent) of 100 mg./kg. body weight of the technical mixture. After dermal application chlordane is more toxic than dicophane as it is readily absorbed through the unbroken skin, and deaths have occurred from such absorption. Little is known about the inhalation toxicity of chlordane, but chemically verified cases of poisoning from combined skin and respiratory exposures have been recorded. The symptoms of acute chlordane intoxication are similar to those observed in poisoning by dicophane. Acute signs of poisoning usually occur within 45 minutes of ingestion, and death may occur within 24 hours though it may be delayed for some days. Chronic poisoning may be manifested by disturbances of the central nervous system; it particularly affects the optic nerve. Treatment of chlordane poisoning depends on the use of symptomatic measures. Details are given of 15 reported cases of systemic chlordane poisoning. S. L. W.

Chlorpromazine, Identification and Pharmacological Properties of a Major Metabolite of. N. P. Salzman, N. C. Moran and B. B. Brodie. (*Nature, Lond.*, 1955, **176**, 1122.) Chlorpromazine, given to man or to dogs, results in the urinary excretion of chlorpromazine sulphoxide. Identification of this derivative was made by extraction from alkaline urine and separation as the picrate. The empirical formula of this metabolite differs from that of chlorpromazine by one additional oxygen atom. This sulphoxide formation is an unusual type of drug bio-transformation. The pharmacological actions of chlorpromazine and its sulphoxide were compared in dogs. The compounds were qualitatively similar, producing sedation, relaxation of the nictitating membrane, adrenergic blockade, orthostatic hypotension, excitement, tremors and finally clonic and tonic convulsions. The dose of sulphoxide which produced minimal sedation was about 8 times that of chlorpromazine but the sulphoxide caused relatively little adrenergic blockade and postural hypotension at sedative dose levels. The sulphoxide was one-eighth as active as chlorpromazine in potentiating hexobarbitone anæsthesia. Since chlorpromazine sulphoxide induces sedation in dogs with relatively little of the orthostatic hypotension observed with chlorpromazine, it is planned to test the drug in man in the treatment of mental illness. M. M.

Ferrous Gluconate, Toxicity of. J. O. Hoppe, G. M. A. Marcelli and M. L. Tainter. (*Amer. J. med. Sci.*, 1955, **230**, 491.) This is a study of a direct comparison of the acute systemic and local toxicity of ferrous sulphate and

ferrous gluconate in experimental animals (mice, rats, dogs and cats). The study clearly establishes that ferrous gluconate is less irritating and less toxic than ferrous sulphate when considered from the standpoint of the total weight of drug administered or in terms of their iron contents. A firm experimental basis for the lack of clinical toxicity and for the therapeutic preference for ferrous gluconate therefore appears to be demonstrable. The magnitude of the acute oral toxicity values when compared with the acute intravenous figures in mice indicates a relatively low order of absorption from the intestinal tract. An additional safey factor is evident from the oral studies in the cat and the dog in which the local irritant effects induce a protective emesis. These data suggest prompt, gentle, gastric lavage along with supportive therapy for shock as an effective emergency measure in cases where vomiting does not occur spontaneously following ingestion of ferrous sulphate, ferrous gluconate or other soluble iron salts. S. L. W.

"Hemicholiniums", a New Group of Respiratory Paralysants. F. W. Schueler, (*J. Pharmacol.*, 1955, **115**, 127.) A series of bis-quaternary ammonium compounds with the general structure,



(where R consisted of a quaternary ammonium nucleus), and some structurally related compounds were investigated for respiratory depressant activity. The compounds fell into three groups according to their main toxic effects:— respiratory depression, neuromuscular blockade or anticholinesterase activity. The presence of an ethanol group in the R radical and hemiacetal formation between this group and the phenacyl grouping were necessary for respiratory depressant activity. To signify the presence of hemiacetal formation and of a choline-like moiety in the R radical the term "hemicholiniums", as a series name, was coined. One of the hemicholiniums, that where R was $-N^{+}(CH_3)_2(CH_2CH_2OH)$, was investigated in detail. In toxic doses this substance induced tonic and/or clonic convulsions, particularly striking in smaller species, i.e. mice and rats, which could be suspended or delayed by artificial respiration. In rabbits under chloralose anæsthesia the knee jerk reflex was depressed with doses causing partial respiratory depression, but only with doses five to ten times the LD50 was there any evidence of parasympathomimetic or neuromuscular blocking actions. When the compound was injected in rabbits and dogs into the cerebrospinal fluid, either intraventricularly or intracisternally, doses of from 10 to 20 times the LD50 caused death only after 2 to 5 times the normal latent period. In toxicity tests on mice, strychnine, picrotoxin, atropine or neostigmine did not antagonize the depressant; physostigmine was effective to a slight degree, but choline afforded by far the greatest protection. The site of action of the depressant appeared to be the spinal respiratory relay centres since in cross-circulation experiments in dogs, where only centres above the level of C-4 received a toxic concentration of the drug from the donor's circulation, there was no respiratory failure. Such a spinal site of action was supported by the depressant action of the drug on the knee jerk reflex. G. P.

5-Hydroxytryptamine, Evidence of Role in Brain Function. B. B. Brodie, A. Pletscher and P. A. Shore, (*Science*, 1955, **122**, 968.) The authors have previously shown that reserpine liberates 5-hydroxytryptamine (HT) from body depots, including the intestines and platelets. With the development

of a sensitive fluorimetric assay method the HT content of the brain has now been shown also to be depleted by this sedative. After the intravenous injection of 5 mg./kg. of reserpine the brain HT content decreased by about 75 per cent. within 30 minutes and by 90 per cent within 4 hours, remained at this low level for about 24 hours and returned slowly to a normal value in 7 days. Doses of reserpine as low as 0·1 mg./kg. lowered the HT content significantly. Reserpine was no longer detectable in brain tissue 12 hours after administration, whereas changes in brain HT levels and sedative effects persisted for longer than 48 hours. This suggests that the sedative effects of reserpine were related to brain HT content rather than to reserpine concentration. During the period of low brain HT levels the urinary output of 5-hydroxyindoleacetic acid (the metabolic product of HT) was appreciable, so that HT apparently was still being formed in the body. The concept was advanced that HT is present in the brain normally in a bound form and after reserpine the binding power is lost so that the HT is metabolized by mono-amine oxidase. HT is still formed during this period and is presumably present in a free form which is considered the mediator of the prolonged reserpine action. G. P.

Isoprenaline, Dilator Reponses to, in Cutaneous and Skeletal Muscle Vascular Beds; Effects of Adrenergic Blocking Drugs. P. A. Walters, T. W. Cooper, A. B. Denison and H. D. Green. (*J. Pharmacol.*, 1955, **115**, 323.) The dilator potency of isoprenaline was compared with that of adrenaline and noradrenaline in cutaneous and muscle vascular beds, and the resistance of these dilator responses to adrenergic blockade by graded doses of azapetine and phenoxybenzamine was determined. Drugs were injected intra-arterially; vascular resistance was determined by an electromagnetic flowmeter. In both vascular beds the initial response to adrenaline and noradrenaline was vasoconstriction, whilst isoprenaline produced vasodilatation. The adrenaline response in muscle was converted to dilatation by a dose of blocking agent approximately 1/30 of that required to block the purely constrictor response to noradrenaline. A dose some twenty times larger than the noradrenaline-blocking dose, abolished the dilator response to both adrenaline and isoprenaline. Approximately the same doses of adrenergic blocking agents which blocked noradrenaline vasoconstriction in muscle, also abolished adrenaline and noradrenaline vasoconstriction in skin without unmasking dilator responses in either case. The failure to unmask vasodilatation in skin in response to adrenaline, may have been due to the fact that constrictor blocking doses of phenoxybenzamine and azapetine also markedly reduced the isoprenaline dilator responses and finally abolished them at a dose only slightly larger. Adrenaline behaved as if it were a composite of noradrenaline and isoprenaline, acting like the former normally, and like the latter after adrenergic blocking agents. G. P.

Morphine and Amiphenazole in Intractable Pain. J. McKeogh and F. H. Shaw. (*Brit. med. J.*, 1956, **1**, 142.) Amiphenazole is one of a series of compounds which have been shown to arouse dogs deeply narcotised with morphine and hyoscine. In human beings it counteracts the morphine-induced respiratory depression, vomiting, narcosis, and depression of the cough reflex without affecting the analgesia. The treatment of 127 cases of intractable pain in terminal carcinoma by the use of morphine and amiphenazole is described. The patients will usually be receiving 1/3 or ½ gr. of morphine before treatment is started. The morphine is increased by increments of ¼ gr. with 20 to 30 mg. of amiphenazole; the solutions may be mixed in one syringe, and the injections given intramuscularly or hypodermically. The injections are repeated when

pain returns. The morphine increments are continued until the analgesia is complete for 6 to 8 hours (it is not necessary to increase the dose of amiphenazole). The patient is then considered stabilised; severe pain may require up to 3 gr. of morphine at a single injection. When the patient is stabilised (in about 2 days) the intramuscular injection of amiphenazole is replaced by oral administration. As amiphenazole counteracts the sedative effect of morphine and itself has a slight stimulant effect, it is possible in most patients to control the degree of alertness by varying the dose of amiphenazole given during the day; the range of oral dosage is 20 to 60 mg. There is only one indication for caution or cessation of treatment, and that is respiratory depression. Acute respiratory depression due to morphine can be countered by intramuscular (or intravenous) injection of 20 mg. of amiphenazole at intervals of 10 minutes for a period of 2 hours. Amiphenazole itself is completely harmless. In over 150 cases there has been no evidence of tolerance or addiction to morphine in spite of the increased dosage. Before treatment all the subjects were suffering moderate to severe pain and exhibited various degrees of depression. After about 2 weeks' treatment a remarkable change was noticeable in the outlook of about 75 per cent.; they took a renewed interest in the life of the ward, in occupational therapy, or in hobbies. Several patients on 60 mg. of amiphenazole had to have their dosage reduced to 20 mg. because they had too much insight into their condition. S. L. W.

Myleran in Chronic Myelocytic Leukæmia. A. Haut, S. J. Altman, G. E. Cartwright and M. M. Wintrobe. (*Arch. intern. Med.*, 1955, **96**, 451.) This is a report on the use of myleran in 16 consecutive cases of chronic myelocytic leukæmia since November, 1952. The interval from the apparent onset of leukæmia to the start of myleran therapy ranged from 1 month to 56 months; in 7 cases it was one year or longer. Myleran was administered to the 16 patients for a total of 40 courses of treatment. To date, 16 first courses, as well as 10 second, 6 third, 5 fourth, 1 fifth, and 1 sixth course have been completed. Usually oral doses of 4 to 6 mg. daily were prescribed, to be taken before the morning meal. The average daily dose for the completed courses of treatment in the whole series was 4·5 mg.; the duration of therapy ranged from 21 to 269 days. For the most part patients received treatment for from 42 to 71 days. Patients were ambulant while receiving the drug and were examined at 1 to 3-week intervals; after therapy had been stopped follow-up examinations were made at 1 to 3 month intervals. Maintenance therapy was not attempted; subsequent courses of therapy were begun when signs of relapse were thought to warrant it. A decrease in the leucocyte count and other favourable changes were observed in all cases and in all courses of treatment. In patients with only partial improvement after the initial course, a subsequent course gave better results. Often within the first 2 weeks of treatment the patients noticed a sense of well-being, a return of appetite and an improvement in endurance. Thereafter, except for 4 patients who died, all remained symptom-free despite evidence of relapse which warranted retreatment in 10 cases. A rising leucocyte count was the first evidence of impending relapse; in most cases therapy was not reinstituted until the count had increased to the range 50,000 to 100,000/c.mm., although even at this point many patients were symptom-free. There were no evidences of gastro-intestinal upset, anorexia, or other undesirable effects, apart from thrombocytopenia, which occurred in 4 cases. The authors conclude that the response to myleran therapy has been at least equal to that expected from X-ray therapy or radio-active phosphorus and superior to that obtained with other chemotherapeutic agents. S. L. W.

Naphthionin, a New Hæmostatic Drug. L. Poller. (*J. clin. Path.*, 1955, **8**, 331.) A study has been made of this new hæmostatic drug, sodium-α-naphthylamine-4-sulphonate. *In vitro* tests have shown the compound to have little effect on the clotting time of blood, but the injection of 1 g. into healthy male volunteers caused a significant reduction in the bleeding time. There was no change in the clotting times or prothrombin times and it is concluded that the hæmostatic action of the compound may be due to the tendency to gel formation resulting from a lowering of the isoelectric point of fibrinogen. G. F. S.

New Bis-Quaternary Series, Including Chlorisondamine Dimethylchloride, Ganglionic Blockade by. A. J. Plummer, J. H. Trapold, J. A. Schneider, R. A. Maxwell and A. E. Earl, (*J. Pharmacol.*, 1955, **115**, 172.) A series of polymethylene bis-quaternary ammonium compounds, where one of the quaternary groups consisted of a tetrachloro*iso*indoline nucleus and the other of an ethyl- or methyl-substituted ammonium grouping, was investigated for ganglion-blocking activity on the cat superior cervical ganglion - nictitating membrane preparation. On this preparation the most active of the series, 4:5:6:7-tetrachloro-2-(dimethylaminoethyl) *iso*indoline dimethylchloride, (chlorisondamine dimethylchloride), was about six times as active intravenously as hexamethonium and twice as active as pentapyrrolidinium. Chlorisondamine had a rapid onset and prolonged duration of action on the nictitating membrane of the unanæsthetized dog after oral administration of 2 mg./kg.; chlorination of the aromatic ring appeared to be an important factor in promoting rapid absorption and prolonging duration of action. Maximal relaxation of the nictitating membrane was consistently obtained with an oral dose of 20mg./kg. given daily for four months. Any tolerance developed was minimal and no toxic effects were observed over this period. In the anæsthetized dog a sustained hypotensive action lasting over five hours was obtained with intravenous doses of 100 to 200 μg./kg. of chlorisondamine. Associated with this there was a lasting diminution of the pressor action of the ganglion stimulant dimethyl phenyl piperazinium. G. P.

Oxamides, Bis-Quaternary Salts of Basically Substituted. A. M. Lands, A. G. Karczmar, J. W. Howard and A. Arnold. (*J. Pharmacol.*, 1955, **115**, 185.) *NN'*-Bis-(2-diethylaminoethyl) oxamide bis-2-chlorobenzyl chloride (WIN 8077) and its bis-2-methoxybenzyl analogue (WIN 8078) were effective antagonists of neuromuscular blockade by tubocurarine in the dog, cat and mouse. They also protected against poisoning with ethyl pyrophosphate in mice. WIN 8077 facilitated transmission at the cat neuromuscular junction, but WIN 8078 was ineffective in this respect, even in large doses. In the anæsthetized dog both compounds were more effective than neostigmine in potentiating the vasodepressor action of acetylcholine, stimulation of the cardiac vagus and the vasopressor responses to acetylcholine after atropine. The effects described were obtained with doses which had little or no detectable inhibitory effect on blood, muscle or brain acetylcholinesterase *in vivo*. *In vitro*, WIN 8077 had high anticholinesterase activity, being about six times as active as neostigmine on erythrocyte acetylcholinesterase. This activity was highly specific for acetylcholinesterase. The enzyme-inhibiting action of WIN 8078 was slight. Acute lethal effects of the oxamides were associated with respiratory depression caused either by a central or by a neuromuscular blocking action. The suggestion was made that the oxamides facilitate transmission in the above cases by an action directly on the receptor mechanism or through an effect on an enzyme system other than acetylcholinesterase. G. P.

Oximes, Reversal by, of Neuromuscular Block Produced by Anticholinesterases. R. Holmes and E. L. Robins. (*Brit. J. Pharmacol.*, 1955, **10**, 490.) Wedensky inhibition of neuromuscular transmission (where the muscle responds only to the first of a train of tetanic stimuli applied to the motor nerve), induced by ethyl pyrophosphate, sarin or dyflos in the isolated rat diaphragm—phrenic nerve preparation, was rapidly reversed by di*iso*nitrosoacetone (I) and mono*iso*-nitrosoacetone (II). Pyridine-2-aldoxime had similar activity, but this could only be demonstrated after washing the preparation, since the oxime had itself a neuromuscular blocking action. With an *in vivo* preparation of the gracilis muscle of the rat, dyflos caused repetitive firing and an apparent increase in conduction velocity, which were abolished by injection of II. Wedensky inhibition in the cat tibialis anterior preparation due to intravenous or close-arterial injection of ethyl pyrophosphate or intravenous sarin, was slowly reversed by intravenous II. The reversals by the oximes of block with anticholinesterases appears to be entirely due to the reactivation of inhibited cholinesterase. Neuromuscular blockade by (+)-tubocurarine, suxamethonium or decamethonium was unaffected. The oximes had a direct action on muscle, decreasing contraction height and slowing conduction velocity. G. P.

Phenylpropylhydroxycoumarin: Anticoagulant Action. M. Toohey. (*Brit. med. J.*, 1956, **1**, 9.) This is a report on the use of phenylpropylhydroxycoumarin (Marcoumar) as an anticoagulant in 104 patients suffering from coronary thrombosis or phlebothrombosis. It is a very potent anticoagulant and in safe therapeutic doses will raise the prothrombin time to within a therapeutic range in 48 hours in 84 per cent. of cases. It has a prolonged cumulative effect, and it may take as long as 7 to 14 days for the prothrombin time to return to normal. Usually, the initial dose should be 24 mg., further therapy being delayed until the prothrombin time 24 to 36 hours after starting treatment is known. According to the prothrombin time at 24 to 36 hours the second dose will be as follows: (*a*) prothrombin time 15 seconds or less, 12 to 15 mg., (*b*) between 16 and 20 seconds, 9 mg., (*c*) between 21 and 24 seconds, 3 to 6 mg., (*d*) 25 seconds and over, nil. The maintenance dose varies between 0·75 and 6 mg. In 80 out of 99 cases it lay within the range of 3 to 4·5 mg. Owing to the prolonged cumulative effect great care is necessary in assessing the maintenance dose, and alterations in the dose should not be made more than every few days. The young, robust and less acutely ill patients require the largest doses, whilst the elderly, frail and seriously ill patients need the smallest; any lowering of renal function will materially reduce the amount of the maintenance dose needed. Frequent prothrombin estimations are necessary in controlling therapy. Phenylpropyl-hydroxycoumarin is particularly free from toxic effects, the only adverse reactions in these 104 patients being 4 cases of microscopic hæmaturia and 1 case of frank hæmaturia. There was an unforeseen and unpredictable rise in the prothrombin time to over 60 seconds in 10 per cent. of cases. Vitamin K_1 is a rapid and effective antidote, but repeated doses may be necessary. Phenyl-propyl-hydroxycoumarin appears to be slightly quicker in action than dicoumarol and less toxic. It is slower in action than phenindione and the variation of response is greater, so that it is more difficult to control. Also, as it is a more potent and cumulative drug any inaccuracy in assessing the dose will have a much more serious effect. However, in those cases where anticoagulant therapy with phenindione is difficult to control phenylpropylhydroxycoumarin appears a useful alternative. S. L. W.

Rauwolfia Preparations and Reserpine in the Treatment of Hypertension. R. W. P. Achor, N. O. Hanson and R. W. Gifford. (*J. Amer. med. Ass.*,

1955, **159**, 841.) In a controlled study of 58 patients with essential hypertension the effects of treatment with a whole-root preparation of *Rauwolfia serpentina* compared with the effects of treatment with the alkaloid reserpine did not appear significantly different. Treatment with these preparations produced a satisfactory reduction of blood pressure in 40 per cent. of the patients. The patients were given initially and alternatively either the whole-root preparation of rauwolfia in a dosage of 400 mg. per day or reserpine in a dosage of 0·4 mg. per day (this dosage ratio of 1 : 1000 has been tentatively accepted as equivalent pharmacologically). These medicines were administered for 2 months, a placebo was given for the next 2 months, and finally the whole-root preparation or reserpine (whichever had not been administered originally) for a further 2 months. Emotional upsets developed in 10 of the 58 patients during the course of study. The mildest form of upset consisted of increased tenseness, restlessness and insomnia; this could progress to an outright period of depression. Three patients experienced a major depression, one of whom required electroshock therapy. Whilst these drugs are quite useful in treating hypertension, and other side-effects attending their use are not serious, the occurrence of severe depressive reactions constitutes a serious objection to their indiscriminate and unattended use. S. L. W.

Reserpine, Psychosis Caused by. H. A. Schroeder and H. M. Perry. (*J. Amer. med. Ass.*, 1955, **159**, 839.) Psychotic behaviour with agitated depression occurred in 5 patients with hypertension treated with reserpine; the usual dose was 1 mg. daily. In 3 of the 5 patients prodromal symptoms consisted of increased nervousness, insomnia, agitation and depression. Continuation of the therapy resulted in sporadic but increasingly recurring bouts of paranoia, with suicidal tendencies, followed by lucid intervals with clear insight. Recovery was slow and was achieved in from one to two months. It is now recognised that administration of reserpine, usually a sedative agent, may cause excitement in some individuals; when a patient taking reserpine for a month or more complains of increased nervousness the dose should be reduced or administration stopped. Other reactions to this drug have been observed, including recurrences of peptic ulcer with bleeding in 3 patients and severe mucous colitis with small ulcerations in one. While it is not certain that gastro-intestinal conditions are influenced by administration of reserpine, the drug could conceivably act as an initiating factor in view of the relative parasympathetic stimulation that is produced. S. L. W.

Uracils as Anticonvulsants. J. H. Burckhalter and H. C. Scarborough (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 545.) A series of 5-substituted uracils was prepared by condensation of an α-formyl ester, $R.CH(CHO).COOC_2H_5$, with urea or thiourea. Substituted thiouracils were converted to uracils by hydrolysis with chloroacetic acid. 1 : 3-Dimethyl-5-substituted uracils were obtained by methylation with dimethyl sulphate. 5 : 5-Diphenylhydrouracil (a homologue of diphenylhydantoin) was prepared by the reaction of ethyl αα-diphenyl-β-aminopropionate hydrochloride with potassium cyanate. A number of α-cyanoureides, $R.CH(CN).CO.NH.CO.NH_2$, were prepared for use as intermediates in the syntheses, but attempts at ring closure were not successful. These compounds were shown to have anticonvulsant activity and submitted for biological testing. Significant protection against leptazol was demonstrated in mice with 1 : 3-dimethyl-5-ethyl- and 1 : 3-dimethyl-6-propyl-6-phenyluracils. G. B.

Uracils and Related Compounds, Anticonvulsant Activity of. D. G. Wenzel (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 550.) A series of 17 uracil derivatives and 5 related α-cyanoureides were administered orally, by stomach needle, to mice. The doses which protected 50 per cent. of the mice against the convulsive effects of leptazol given subcutaneously one hour later were determined. Electric shocks were given at intervals after the drugs had been administered and protective doses were determined. Most of the compounds were active in protecting the animals against convulsions due to electric shocks, the activity increasing with the size of the alkyl group at position 5. The introduction of 1:3-dimethyl groups also seemed to result in improved activity. Several uracil derivatives were effective in preventing convulsions due to leptazol, but in this case no obvious relationship between structure and activity was observed. G. B.

BACTERIOLOGY AND CLINICAL TESTS

Acid-fast Bacilli, Rapid Method for Cultivation of. L. S. Chu. (*Science*, 1955, **122**, 1189.) A 24-hour sputum specimen is shaken with an equal volume of sodium hydroxide solution (4 per cent.) and incubated at 37·5° C. for 30 minutes. An equal volume of medium (containing lecithin, various salts, asparagine, glucose, Tween 80, plasma, blood and penicillin) is added and the mixture incubated at 37·5° C. for 24 hours. The material is centrifuged and slides prepared from the sediment are examined after staining with Ziehl-Neelsen stain. If acid-fast bacilli are not found, 14 glass slide preparations are made from the sediment and air-dried. These are incubated in medium (as above but without Tween 80 and plasma), one slide being removed each day and examined after drying and staining. If at the end of 14 days no acid-fast bacilli have been found the test is considered to be negative. G. B.

Carboxymethylcellulose, Inhibition of Microbial Growth by J. V. Swintosky and A. M. Kaufman (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 540.) The test organisms were suspended in culture media buffered with carboxymethylcellulose. Oxygen uptake of the cultures was measured manometrically at intervals over a period of 4 hours. In the case of *Micrococcus pyogenes* var. *aureus*, *Escherichia coli* and *Streptococcus fæcalis*, the rate of oxygen uptake varied with the pH of the solution in the region pH 3–5. The relationship was in accordance with the equation $\log \frac{dO_2}{dt} = K.pH + C$, K having the value of 0·7 to 1 when $\frac{dO_2}{dt}$ was expressed in μl./minute. K had a much lower value for *Candida albicans*, cultures of which were much less sensitive to changes in pH. On account of its high buffering capacity at about pH 4, and physical properties which are of advantage in compounding pharmaceutical preparations, carboxymethylcellulose may be of use in preparations for the treatment of skin and epithelial tissues. G. B.

Purine and Pyrimidine Analogues: Effect of, on Enzyme Induction in *Mycobacterium tuberculosis*. L. Ottey. (*J. Pharmacol.*, 1955, **115**, 339.) The effect of a series of purine and pyrimidine analogues upon the formation of the adaptive enzymes in *Mycobacterium tuberculosis* BCG 8240 which oxidise

(ABSTRACTS *continued on page* 368.)

useful contribution. Literature and patent references are complete to the end of 1954, although some reference to later work is also included. The extent and seeming completeness of the index, covering both authors and subjects, may be gathered from the fact that it extends over a total of 120 pages. Like many of the earlier volumes in this series, the present one is a reference book for the specialist. Clarity in layout and detailed presentation of information make Volume IX a "must" for any organic chemical library.

J. B. STENLAKE.

(ABSTRACTS *continued from page* 362.)

benzoic acid and myo-inositol was investigated. The *Mycobacterium* was grown on Long's medium; myo-inositol-adapted cells were grown on medium with myo-inositol replacing glycerol. Analogues when present were at a concentration of 1·0 mg./ml. The effect of the drugs on substrate oxidation was measured by conventional Warburg techniques. Cells grew normally in the presence of the analogues (cf. antibiotics) and endogenous respiration was the same as control. The addition of the purine analogues, 6-mercaptopurine and 2:6-diaminopurine, and the pyrimidine analogues, 5-aminouracil, 5-methyl-2-thiouracil, 6-methyl-2-thiouracil, 2-thiocytosine, and 2-thiouracil, inhibited the formation of adaptive enzymes for the oxidation of benzoic acid in this mycobacterium. 2-Thioorotic acid had no effect. The addition of the purine analogues, 6-mercaptopurine and 2:6-diaminopurine, and the pyrimidines, 5-aminouracil, 5-methyl-2-thiouracil, 6-methyl-2-thiouracil, and 2-thioorotic acid, inhibited the formation of the adaptive enzymes for the oxidation of myo-inositol. 2-Thiouracil had no effect. Inhibition by 5-methyl-2-thiouracil, the most effective of the analogues in both cases, was reversed by thymine (1·0 mg./ml.), but not by uracil in the same concentration. Analogue-grown cells oxidised trehalose and glycerol normally: the drugs would appear to affect preferentially adaptive enzyme formation. G. P.

Quaternary Ammonium Compounds, Bacteriostatic and Bactericidal Effect of. O. G. Clausen *Medd. Norsk. Farm. Sels.*, 1955, **17**, 124.) Tests were carried out to compare the bactericidal and bacteriostatic effects of benzalkonium chloride and benzethonium chloride with that of phenol. A series of ærobic and anærobic bacteria were used as test organisms, and also a series of natural inocula (suspension of normal fæces, sputum suspension and dust suspension) were employed in the tests. *Pseudomonas æruginosa* and *Clostridium welchii* were the most resistant organisms encountered in the bacteriostatic tests, while *Bacillus subtilis*, *C. welchii* and suspension of dust were the most resistant materials in the bactericidal determinations. Phenol coefficients were determined, using a 2 per cent. oil-soap solution as an inactivating agent for the quaternary compounds. The results for benzalkonium, benzethonium and cetylpyridinium chlorides and cetrimide using this method of inactivation were lower than those previously reported. It is suggested that *Ps. æruginosa* should be adopted instead of *Salmonella typhosa* as the standard organism for the determination of phenol coefficients. G. B.