ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

***N*-Allylnormorphine Hydrochloride, Identification and Determination of.** W. J. Seagers, J. D. Neuss and W. J. Mader. (*J. Amer. pharm. Ass. Sci. Ed.*, 1952, **41**, 640.) Infra-red absorption spectroscopy may be used for the identification of *N*-allylnormorphine and for the detection of morphine and normorphine in it. *N*-Allylnormorphine exhibits an absorption maximum at 11·60 μ, which is absent from the spectra of morphine and normorphine, whereas these two substances show an absorption maximum at 11·47 μ which *N*-allylnormorphine does not. Samples of 20 mg. of the hydrochloride may be ground and examined in 0·05 ml. of liquid paraffin. Parenteral solutions should be extracted with absolute methanol, evaporated and dried, and the residue examined in liquid paraffin. Solutions may be assayed by ultra-violet absorption, using the datum, $E^{1\,\text{per cent.}}_{1\,\text{cm.}}$ at the absorption maximum 285 $\pm$ 1 mμ = 44·0. Autoclaving enhances the absorption and gives rise to results about 6 per cent. high, while a measurable absorption appears in the region 310 to 360 mμ. Results can be corrected by subtracting the absorption at 310 mμ from that at 285 mμ. The absorption is due to the hexahydrophenanthrene system, and does not differentiate *N*-allylnormorphine from normorphine and morphine. *N*-allylnormorphine in 2 M lithium chloride exhibits a polarographic wave of half-wave potential $-1{\cdot}6$ v., measured against the saturated calomel electrode, apparently due to reduction of the allyl group. For assay purposes 5 ml. of a 0·02 per cent. solution in water is mixed with 15 ml. of 2·65 M lithium chloride and 10 ml. placed in an H-type polarographic cell at 25° C., deoxygenated by passing nitrogen through the solution, and the polarogram recorded with a dropping mercury electrode. The result is calculated by comparison with a standard *N*-allylnormorphine hydrochloride of known purity. G. B.

Aminoacetic Acid and Histidine Monohydrochloride, Assay of. S. W. Goldstein. (*Drug Standards*, 1952, **20**, 223.) A formol titration is suggested for the N.F. assay of aminoacetic acid and histidine monohydrochloride in place of the present nitrogen determination by the Kjeldahl method. Comparative assays of histidine monohydrochloride showed that Kjeldahl results (100·5, 99·8 per cent.) were higher then microbiological results (99·5, 99·2 per cent.), while the highest results (101·0, 101·0 per cent.) were obtained by the formol titration assay. Since the formol procedure yields slightly higher results than those obtained with the present Kjeldahl nitrogen assay, it is suggested that the assay limits for both amino-acids should be 98·5 to 101·5 per cent. R. E. S.

Caffeine in Tablets, Determination of. C. M. P. Wirth. (*Drug Standards*, 1952, **20**, 226.) A method was studied for the rapid determination of caffeine in tablets in the presence of aspirin and phenacetin. The determination of caffeine was based upon the fact that it forms, in acid medium with iodine-potassium iodide solution, an insoluble precipitate of caffeine periodide, thus permitting, after separation of the precipitate, a titration of the excess of iodine with 0·1N sodium thiosulphate. Recovery experiments with known amounts of caffeine were satisfactory. R. E. S.

Cardiac Glycosides, Colorimetric Determination of. C. H. Hassall and A. E. Lippman. (*Analyst*, 1953, **78**, 126.) The reaction of a cardiac glycoside and *m*-dinitrobenzene in the presence of dilute sodium hydroxide or an alkaline buffer at a *p*H between 11 and 13, results in a red colouration ($\lambda_{max.}$ 560 mμ); a blue colouration is produced when the red is treated with more concentrated alkali. The red colouration can be used for the determination of dilute solutions of cardiac glycosides and aglycones. In the reaction an approximately 0·1 per cent. solution of the glycoside in absolute ethanol is treated with 0·3 ml. of *m*-dinitrobenzene solution, ethanol being added to 1·2 ml.; after the addition of 0·1 ml. of sodium hydroxide solution the mixture is allowed to stand for 10 minutes and the absorption is determined at 560 mμ. The colour is distinct from that given by simple ketones. R. E. S.

Heavy Metal Determinations by Amperometric Titrations. A. D. Cooper and M. W. Green. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 1.) Certain cations react with *n*-dodecylmercaptan forming a precipitate of metallic mercaptide, and the excess of reagent may be titrated with silver nitrate solution. The end-point may be determined amperometrically since there is a rapid rise in the diffusion current with a rotating platinum electrode when an excess of silver nitrate is added. An applied potential of – 0·23 volt measured against a saturated calomel electrode is sufficient for the electrode reaction with the silver ions, but is insufficient to reduce any dissolved oxygen present. The titration should be performed in ammoniacal solution to prevent the precipitation of any silver chloride which would interfere. The method is applicable to the determination of small amounts of silver, lead, cupric and mercuric ions, but not to ferric ions because ferric mercaptide is decomposed on the addition of solution of ammonia. The *n*-dodecylmercaptan solution is standardised amperometrically against a solution containing a known amount of heavy metal ion, and then used to determine the quantity of heavy metal in a solution, or present as a contaminant in a pharmaceutical chemical. A blank test to allow for oxidation of the reagent is performed at the same time. The method gives satisfactory accuracy and reproducibility, and the results are compared with tests by colorimetry of the heavy metal sulphides. Some chemicals interfere by inhibiting complete cathodic depolarisation. G. B.

Isoniazid, Spectrophotometric and Polarographic Determination of. J. D. Neuss, W. J. Seagers and W. J. Mader. (*J. Amer. pharm. Ass. Sci. Ed.*, 1952, **41**, 670.) The infra-red absorption spectrum is a means of identifying isoniazid (*iso*nicotinyl hydrazide) and detecting the presence of the isomers nicotinyl hydrazide and picolinyl hydrazide, and the corresponding acids. Bands occur in the isoniazid spectrum at 8·78, 9·48, 10·05, 11·25, and 11·82 μ, but the isomers do not show the strong absorption bands at 10·05 and 11·82 μ. The latter is shifted to 11·98 μ in the case of nicotinyl hydrazide and 12·19 μ for picolinyl hydrazide. Tablet excipients interfere and should be removed. In the assay by ultra-violet absorption measurements, a 0·0015 per cent. solution in 0·1 N hydrochloric acid should show an absorption maximum at 267 $\pm$ 1 mμ and a minimum at 235 $\pm$ 2 mμ. Results are calculated from the absorption at 267 mμ ($E_{1\,cm.}^{1\,per\,cent.}$ = 374). Ultra-violet absorption data for isoniazid, its isomers and related acids are tabulated. When examined polarographically, solutions of isoniazid of *p*H 1·5 to 7 show two waves suitable for assay purposes. *iso*Nicotinic acid, ethyl *iso*nicotinate, nicotinic acid, nicotinyl hydrazide and picolinic acid and its hydrazide do not interfere in the *p*H range

1·5 to 5. The half-wave potentials at *p*H 1·5, measured against a saturated calomel electrode are − 0·52 and − 0·70 volt. The cell solution, prepared by mixing 10 ml. of a 0·02 per cent. solution with 10 ml. of buffer solution, is deoxygenated with nitrogen and polarographed in an H-type cell with a conventional dropping mercury electrode. The result is calculated by comparison with isoniazid of known purity. Corn starch, lactose and magnesium stearate, which may be present in tablets do not interfere in the assay. G. B.

Pentose and Hexose in Mixtures of Sugars, Determination of. W. R. Fernell and H. K. King. (*Analyst*, 1953, **78**, 80.) It was found that pentose and hexose could be estimated when present together in mixtures by heating them with orcinol and acid either alone or with ferric chloride. When orcinol is heated with sugars in the presence of sulphuric or hydrochloric acid the product has an absorption band in the blue end of the spectrum. Pentoses and hexoses both give a peak at 425 mμ, but the absorption with the hexoses is the lesser. If the colour is read on a colorimeter incorporating the appropriate filter, the colour ratio of pentose to glucose is about 1·3 to 1, or 1·1 to 1 if molar concentrations are considered. If, however, the reaction is carried out in the presence of ferric chloride, pentoses give, in addition, a strong absorption band in the red, which is not given by hexoses. In the method recommended the mixture of sugars is heated with orcinol and acid, alone or with ferric chloride, and the absorption is read at the most suitable wavelengths, viz., 425 mμ and 660 mμ, respectively. The concentrations of both pentose and hexose can then be read from a nomogram. A specimen nomogram is given for the calculation of pentose and hexose from colorimetric readings. R. E. S.

Phenols, Titration of, in Nonaqueous Solvents. J. S. Fritz and R. T. Keen. (*Analyt. Chem.*, 1953, **25**, 179.) Three procedures are given for the nonaqueous titration of phenols. Phenols which have a negative group in the *o*- or *p*-position are stronger acids than unsubstituted phenols and naphthols; thus the -CHO, -COR, -COOR, $-CONH_2$, or $-NO_2$ groups in the *o*- or *p*- position increase the acidity of phenols sufficiently to permit accurate titration in dimethylformamide using azo-violet indicator. *Ortho*-halogen-substituted phenols can also be titrated by this procedure. Unsubstituted and alkyl- or aryl-substituted phenols and naphthols are too weakly acidic to be titrated using azo-violet but can be titrated in ethylenediamine using *o*-nitro-aniline indicator. Carboxylic acids can often be determined in the presence of most phenols by differential titration using *p*-hydroxyazobenzene as indicator. The accuracy of the methods varied from ±0·3 to ±0·6 per cent. R. E. S.

Phosphorus in Biological Material, Microdetermination of. H. W. Harvey. (*Analyst*, 1953, **78**, 110.) The method proposed is suitable for 1 to 70-μg. amounts of phosphorus and consists in decomposing the organic material with sulphuric acid and hydrogen peroxide, heating with 5N acid to convert the pyrophosphate to orthophosphate, decomposing residual hydrogen peroxide with sulphite and converting the orthophosphate to phosphomolybdic acid. The phosphorus is then determined by the molybdenum blue method after controlled reduction by stannous chloride; details of the experimental procedure are given. The only substance found to interfere in the concentrations used was copper; the danger of laboratory contamination in the determination of 1 μg. amounts of phosphorus was slight. R. E. S.

FIXED OILS, FATS AND WAXES

***Apium graveolens*, (Karaffs') Seed Oil.** M. O. Farooq, M. Kiamuddin and S. M. Osman. (*Rec. Trav. chim. Pays-Bas*, 1953, **72**, 135.) Oil was obtained by extraction of the seed with light petroleum and saponified with ethanolic potassium hydroxide to remove resins and unsaponifiable matter. The fatty acids were separated by a lead salt method into a solid fraction (52·5 per cent.) and a liquid fraction (47·5 per cent.). The fractions were esterified separately with methanol and fractionated to obtain an estimate of the composition. The major component, petroselinic acid was isolated as colourless shining crystals, m.pt. 29° to 30° C. The total acids consisted of resin acids 7 per cent., palmitic acid 11·7 per cent., petroselinic acid 41·1 per cent., oleic acid 30·5 per cent., and linoleic acid 9·7 per cent. The oil also contained 17 per cent. of fat and 6·4 per cent. of unsaponifiable matter. The composition of this oil differed from that of the English equivalent plant, *Apium graveolens* L. which is reported to contain palmitic acid 3 per cent., petroselinic acid 51 per cent., oleic acid 26 per cent., and linoleic acid 20 per cent. G. B.

Wool Alcohols in Water-in-Oil Emulsions. J. Tiedt and E. V. Truter. (*J. appl. Chem.*, 1952, **2**, 633.) The emulsifying properties of wool fat and its components were investigated. In this work the emulsifying power is expressed as the water number which is the maximum weight of water taken up by the material expressed as ml./100 g. It was measured as follows. Distilled water, in 0·3 ml. quantities, was added from a burette to an accurately weighed 5·0 g. sample of liquid paraffin containing 5 per cent. of the emulsifying agent to be studied. The mixture was well stirred, the additions being continued until it was no longer possible to emulsify any further quantity of water. End-points were recorded when, in fluid emulsions, a droplet of water could be seen at the bottom of the vessel, or in stiffer emulsions, when slippage occurred. In all instances the values obtained were reproducible to within 5 per cent. and were usually more accurate. Wool fat was chromatographed on alumina to remove the more polar constituents, and the resulting product was shown to have a very low water number. In order to determine the extent to which the emulsifying power of wool fat is influenced by the free acids, the water numbers of 4 fractions obtained from the mixed acids were measured. The results indicate that the esters of wool fat are incapable of forming emulsions by themselves, and that the ability to hold water in the form of a water-in-oil emulsion is due, almost entirely, to the free alcohol content. Of the constituents, the most powerful emulsifying agents are members of the $\alpha\beta$-glycol series. A decrease in the emulsifying power is invariably observed when *iso*cholesterol is mixed with another surface-active species or with its esters. It is concluded that the intensifying action of esters of cholesterol and octadecanol upon their respective alcohols is attributable to the additional rigidity of the interfacial film which results, partly from the association into ester alcohol pairs owing to hydrogen bonding, and partly from the adhesion between the hydrocarbon chains, the effect of which increases exponentially with the length of the acid chain in the ester. A. H. B.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Digitalis Glycosides, Paper Chromatography of. G. Vastagh and J. Tuzson. (*Pharm. Zentralh.*, 1953, **92**, 88.) An analytical separation of the glycosides of *Digitalis lanata* and partially degraded glycosides can be effected by paper chromatography. Upward chromatography was used, with 3 different types of

paper. The mixed solvent was prepared from purified alcohol-free chloroform, and was allowed to stand for 16 hours before use to allow droplets to settle out. The paper was first washed with water, saturated with ethyl acetate, and dried. The position of the spots was shown by spraying with a chloroform solution of trichloracetic acid, allowing to dry in air, and heating for 3 minutes at 90° to 100° C., the marks being observed in ultra-violet light. Fluorescence of the paper may be eliminated by a preliminary spraying with 5 per cent. aqueous phenol solution, drying, and heating for 5 minutes at 80° C. before treating with trichloracetic acid. The genuine digilanids remain in the neighbourhood of the starting line, and cannot be sharply separated. R_F values are as follows:

Paper	Digitoxin	Gitoxin	Digilanide	Solvent—Ethyl acetate: chloroform:water
Macherey and Nagel 214 ..	0·972	0·599	0·085	10:8:5
Unknown origin	0·981	0·624	0·104	10:8:5
Schleicher and Schüll 1574 ..	0·989	0·924	—	10:10:5

G. M.

Sarmentosides, Separation of. F. Reber and T. Reichstein. (*Pharm. Acta Helvet.*, 1953, **28**, 1.) Crude sarmentoside A crystallisate, from the seeds of *Strophanthus sarmentosus* A.P.DC., was acetylated and treated chromatographically. 4 products were obtained—the acetates of sarmentoside A (yield 33·3 per cent.), sarmentoside C (10·0 per cent.), sarmentoside D (1·3 per cent.) and sarmentoside E (3·4 per cent.). Melting points, absorption spectra, and optical rotations are given for these products, and also for the de-acetylated products. By treatment with Girard and Sandulesco's reagent, the crude sarmentoside A crystallisate was separated into an aldehyde-free fraction (about 28 per cent.) and an aldehyde fraction (about 71 per cent.). The latter fraction appeared however to have been damaged by the treatment, and it was not found possible to recover more than traces of crystalline products from it. G. M.

Sarverogenin, Reactions of. D. H. H. Taylor. (*J. chem. Soc.*, 1952, 4832.) Sarverogenin, a cardiac aglycone occurring in several species of *Strophanthus* including the seeds of *S. amboënsis*, *S. courmontii*, *S. sarmentosus*, and *S. intermedius*, was analysed and the analyses agree with the formula $C_{23}H_{30}O_7$ rather than $C_{23}H_{32}O_7$, the formula ascribed to sarverogenin by Buzas *et al.* (*Helv. chim. Acta*, 1950, **33**, 465). The reactions of sarverogenin are recorded, including the degradation by ozonolysis and periodic oxidation. Some properties of the degradation products are reported. A. H. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Amino-acids, Ion Exchange Behaviour of. M. E. Carsten and R. K. Cannan. (*J. Amer. chem. Soc.*, 1952, **74**, 5950.) The measurements of the exchange equilibria of a representative group of neutral amino-acids with ion exchange resins were made as a basis for the rational use of ion exchange columns in the chromatography of amino-acids. Equilibria were measured in (*a*) the exchange of amino-acid cations for hydrogen ions on 2 sulphonic acid resins, (*b*) the exchange of amino-acid anions for chloride ions on a quaternary amine resin, (*c*) the binding of amino-acid dipolar ions by the hydrogen form of the sulphonic acid resins. The affinity of the amino-acids for a resin was found to

increase with the size of the side chain of the amino-acid, but was depressed by the introduction of a hydroxyl group. From the equilibrium data exchange constants were calculated for 7 amino-acids and theoretical retardation volumes computed. A few chromatograms of proline and hydroxyproline are also reported. These provide data for testing the validity of theoretical relations between exchange equilibrium and retardation volumes under a range of conditions of load and of flow rate. A. H. B.

Amino-acids of Urine, Ion Exchange Chromatography of. M. E. Carsten. (*J. Amer. chem. Soc.*, 1952, **74**, 5954.) The urine is first perfused through a large column of the acid form of a sulphonated resin. All cations, including the ampholytes, are retained while the anions and the neutral constituents are discarded in the effluent. The ampholytes, together with the weak bases, are recovered from the column by elution (eluate I) with 2 M ammonia, while the cations of the stronger bases are retained. After concentration *in vacuo*, an aliquot of eluate I is passed through column II which is composed of a quaternary base resin in its basic form. This column retains the ampholytes and rejects the weak bases. The former are recovered by elution (eluate II) with 1 M hydrochloric acid. After concentration, *in vacuo*, eluate II was subjected to elution chromatography on the hydrogen form of a sulphonated resin (column III) using 2 and 4 M hydrochloric acid as the eluent, to separate the single amino-acids which were identified by paper chromatography and by specific tests. The results obtained for simulated urine, normal urine and pathological urine are given. The ampholyte fraction contained no salts, 4 per cent. of the urea of urine, 2 per cent. of the uric acid, less than 45 per cent. of the creatinine and creatine, while the amino-acids were essentially recovered. A. H. B.

Antidiuretic and Oxytoxic Substances Liberated by the Digestion of Plasmatic Proteins with Pepsin. H. Croxatto, L. Barnafi, G. Rojas, A. Reyes and I. Infante. (*Nature, Lond.*, 1953, **171**, 82.) Hydrolysis of hypertensinogen or a second hydrolysis of extracts of pepsitensin with pepsin at *p*H 2·5 produces a substance which lacks hypertensive effect, but which shows instead an intense antidiuretic action in water-loaded rats. The substance is similar chemically and pharmacologically to vasopressin after it has been treated with sulphydrilic substances such as thioglycollate and thiosorbitol. The antidiuretic substance liberated by pepsin is soluble in methanol, ethanol and acetic acid. It is absorbed by "Norit" and eluted with acetic acid from which solution it is readily precipitated by the addition of either ether or benzene. It is not destroyed by boiling in acid solution, is not precipitated by trichloroacetic acid, and is dialysable through cellophane membranes. It is resistant to the prolonged action of pepsin, but is destroyed by chymotrypsin. Norit adsorbates eluted with acetic acid and precipitated with ether produce at a dose of 10 mg. per rat (200 g.) in the hyperhydrated rat, an effect comparable with that of 500 to 1000 μ of vasopressin. Also present in the same extracts is a second substance having marked oxytoxic activity on both guinea-pig and rat uterus. This substance is stable to pepsin and trypsin, but is destroyed by chymotrypsin. It is suggested that these facts confirm previous deductions about the chemical similarity of hypertensin and of hormones from the neurohypophysis and also that probably these hormones are bound to certain blood proteins. J. B. S.

Bacitracin A, Amino-acid Content of. L. C. Craig, W. Hausmann and J. R. Weisiger. (*J. biol. Chem.*, 1952, **199**, 865.) Bacitracin A (1 g.) was hydrolysed with 200 ml. of 6N hydrochloric acid for 24 hours at 110° C. in a

sealed evacuated tube, the excess of hydrochloric acid then removed under reduced pressure, the residue dissolved in water and this solution used for quantitative analysis on the ion exchange column, using the technique of Moore and Stein. (*J. biol. Chem.*, 1951, **192**, 663). The results obtained were supported by a preparative separation by counter-current distribution of all of the amino-acids from the hydrolysate of a cruder preparation of the antibiotic. The recovery of the known residues amounted to only 87·4 per cent. of the weight of peptide taken, but despite this discrepancy, the data approximate to single molar ratios for each amino-acid except aspartic acid and *iso*leucine which are present in twice the molar proportions of the others. Of the amino-acids found, phenylalanine, glutamic acid, and ornithine were found to belong to the D series, asparatic acid was racemic, leucine, *iso*leucine, cysteine, histidine, and lysine had the configuration of the L series. A. H. B.

Bacitracin, Effects of Surface-active Agents on. C. B. Bruce and L. Mitchell. (*J. Amer. pharm. Ass. Sci. Ed.*, 1952, **41**, 654.) Combinations of bacitracin with 6 surface active agents were tested by the *in vitro* paper disc method against *Micrococcus flavus.* Cationic and non-ionic detergents increased the effect of the antibiotic, whereas anionic detergents opposed it. The concentration of surface-active agent used was insufficient to exhibit any anti-bacterial activity itself. In further tests against *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Neisseria catarrhalis* the effect of bacitracin was enhanced by benzethonium chloride. Possibly the enhanced activity is due to greater penetration of the antibiotic through the medium in the presence of a surface-active agent. It is suggested that combinations of cationic quaternary ammonium compounds possessing strong antiseptic properties with bacitracin might be useful for the local treatment of infections. G. B.

Cephalosporin N: a New Type of Penicillin. E. P. Abraham, K. Crawford, G. G. F. Newton, H. S. Burton and C. W. Hale. (*Nature, Lond.*, 1953, **171**, 343.) Evidence is presented which indicates that cephalosporin N, the substance (or group of substances) obtained from a species of *Cephalosporium*, and possessing activity against a number of Gram-positive and Gram-negative organisms, is a new type of penicillin. The reasons for believing that the antibiotic is a penicillin are: (1) it was inactivated by preparations of the enzyme penicillinase in high dilution, and, like benzylpenicillin, it stimulated the adaptive production of penicillinase by suspensions of *Bacillus cereus*; (2) it was rapidly inactivated at room temperature in aqueous solution below *p*H 4 or above *p*H 9, and also at *p*H 7 in the presence of heavy metal ions such as those of copper, lead and tin; (3) acid hydrolysis of purified material resulted in the liberation of carbon dioxide. On addition of mercuric chloride to the hydrolysate a mercaptide was precipitated, and decomposition of the latter yielded the hydrochloride of *iso*propylidenepenicillamine. Cephalosporin N differs strikingly from the common penicillins in its hydrophilic character and its antibacterial activity. It shows antibacterial activity of the same order of magnitude against a number of Gram-positive and Gram-negative bacteria.

A. H. B.

Chloramphenicol, Mode of Action of. E. D. Bergmann and S. Sicher. (*Nature, Lond.*, 1952, **172**, 931.) The inhibition caused in *E. coli*, wild type, by chloramphenicol, can be fully reversed by indole and DL-tryptophane, less easily by DL-phenylalanine and L-tyrosine, but not reversed by anthranilic acid, indicating that chloramphenicol interferes with the synthesis of indole from anthranilic acid. With the anthranilic acid mutant of *E. coli* (121–35), small

quantities of anthranilic acid reverse the inhibition caused by chloramphenicol, whereas larger doses inhibit growth. With the indole mutant (19–2), which is also inhibited by chloramphenicol, the inhibition is reversed by indole. With the tryptophane (19–2), phenylalanine (M83–5) and tyrosine (83–9) mutants, the inhibition is only reversed by the respective amino-acids if it is caused by a low concentration of chloramphenicol. The hypothesis that *N*-formylanthranilic acid is the intermediate in the synthesis of indole from anthranilic acid has been tested, and *N*-formylanthranilic acid found to be inactive. The inhibition of *E. coli*, wild type, by chloramphenicol is overcome in the presence of anthranilic acid, methionine and vitamin B_{12}. Folic acid and *p*-aminobenzoic acid were both inactive when used with anthranilic acid and methionine. The inhibition in the anthranilic acid mutant was also reversed by the addition of vitamin B_{12} or methionine. J. B. S.

Folic Acid, Leucovorin, Vitamin B_{12}, and the Excretion of *N'*-Methylnicotinamide. L. S. Dietrich, W. J. Monson, and C. A. Elvehjem. (*J. biol. Chem.*, 1952, **199**, 765.) The determination of the comparative effects of folic acid, synthetic *citrovorum* factors (leucovorin), and vitamin B_{12} on the excretion of *N'*-methylnicotinamide (I) in the rat is studied. Folic acid-deficient rats lacked the ability to methylate efficiently and excrete as (I) large doses of nicotinamide compared with control animals. A single injection of 10 μg. of folic acid produced an immediate and significant increase in the excretion of (I) in the folic acid-deficient rat receiving 140 mg./kg. of body weight of nicotinamide per day. Under similar conditions, the administration of 10 μg. and 100 μg. of synthetic *citrovorum* factor was without effect. Vitamin B_{12}-deficient rats receiving 140 mg./kg. of nicotinamide per day excreted significantly lower levels of (I) compared with control animals. Under similar conditions, the administration of 4 μg. of vitamin B_{12} produced no significant increase in the level of (I) excreted until approximately 1 week after supplementation. The data presented indicate an apparent difference on the point of action of vitamin B_{12} and folic acid.

A. H. B.

Insulin, Maximum Molecular Weight of. J. M. Creeth. (*Biochem. J.*, 1953, **53**, 41.) The sedimentation and diffusion characteristics of solutions of various samples of crystalline insulin have been investigated in the *p*H range 7 to 8; the variation of diffusion coefficient with concentration and *p*H confirmed the evidence of dissociation obtained from previous work. Temperature variation within the range 14° to 30° C., had no apparent effect on the dissociation equilibrium. The substance was apparently stable in the range *p*H 7·0 to 7·4 and at concentrations greater than 0·3 g./100 ml.; under these conditions, the sedimentation constant was 3·12 and the diffusion coefficient about 8·2. Using the values for the sedimentation and diffusion constants, the Svedberg equation gave the molecular weight values of 34,800, if $\bar{v} = 0{\cdot}735$ or 36,700 on the older figure, $\bar{v} = 0{\cdot}749$. R. E. S.

Insulin, Partition Chromatography of. R. R. Porter. (*Biochem. J.*, 1953, **53**, 320.) A description is given of liquid two-phase systems using a variety of glycol ethers, water and organic and inorganic solutes found to be suitable for partitioning proteins. Many of the systems could be adapted for partition chromatography and the difficulties inherent in the technique and its application are described. 3 such systems containing water, ethyl and butyl cellosolves and sodium or potassium phosphate have been used for the partition chromatography of insulin which, when thus prepared from crude or crystalline material, was of

uniform activity of about 34 I.U./mg. Recovery from the columns appeared to be nearly 100 per cent. Under the conditions of the chromatography the insulin samples used behaved as a single entity. It is suggested that the method could be used as a method of insulin assay, measurement of the area under the peak of a chromatogram graph giving the insulin content of the sample used.

R. E. S.

Nicotinic Acid and Related Compounds, Metabolism of. K. K. Reddi and E. Kodicek. (*Biochem. J.*, 1953, **53**, 286.) The work deals with the differentiation and estimation of various metabolites of nicotinic acid and related compounds in the urine of man and the rat by employing chromatographic techniques recently developed for both tertiary and quaternary nicotinyl compounds; the procedure did not detect *N*′-methyl-2-pyridone-5-carbonamide. Human oral doses of nicotinic acid appeared in the urine in decreasing order as nicotinuric acid, *N*′-methyl-nicotinamide and nicotinamide; nicotinic acid itself appeared in the urine only when flushing of the skin and other vasodilatory symptoms occurred. Nicotinic acid was excreted consistently by the rat. Nicotinamide,, given to human subjects and to rats caused only an increased excretion of *N*′-methylnicotinamide and of nicotinamide, although rat urine contained, in addition, an increased amount of nicotinic acid; nicotinuric acid was not excreted by either of the species. After ingestion of L-tryptophan, human urine showed an increase in the excretion of *N*′-methylnicotinamide, while rat urine had in addition an increased output of nicotinic acid. Quinolinic acid given to normal rats resulted in an increased excretion of *N*′-methylnicotinamide and nicotinic acid, while rats deficient in nicotinic acid showed no such rise. Urine from undosed human subjects contained a small amount of nicotinamide and of *N*′-methylnicotinamide; from undosed rats, urine contained *N*′-methylnicotinamide, nicotinamide and nicotinic acid. R. E. S.

Nisin, Purification and Nature of. N. J. Berridge, G. G. F. Newton and E. P. Abraham. (*Biochem. J.*, 1952, **52**, 529.) A sample of the antibiotic nisin was resolved by counter-current distribution between solvents into at least 4 active polypeptides named nisin A, B, C, and D. Nisins A and B showed similar activity against *Streptococcus agalactiæ*, although nisins C and D were about one-fifth as active as A and B. Nisins A, B, and C contained leucine and/or *iso*leucine, valine, alanine, glycine, proline, aspartic acid, histidine, lysine, methionine, lanthionine and a cystathionine or *allo*-cystathionine, the latter being present in larger amount by weight than most of the other amino-acids; nisin D contains glutamic acid but no valine or methionine. Various commercial preparations of nisin were similar in amino-acid composition, but some batches contained glutamic acid and serine, which were absent from nisins A, B, and C. Nisin and subtilin, although distinct, show general similarities in their amino-acid composition and also in their physical and antibacterial properties; nisin, however, contains no tryptophan and subtilin no methionine.

R. E. S.

Œstrogens, Paper Partition Chromatography of. H. Heusghem. (*Nature, Lond.*, 1953, **171**, 42.) A method has been devised for the separation and purification of the small amounts of natural œstrogens normally present in biological fluids. Spots on paper chromatograms representing 5 μg. are readily detected after spraying with 10 per cent. antimony trichloride in chloroform and heating for 5 minutes at 90° to 100° C. The following colours are obtained: brown for œstrone, yellow for œstradiol, and pink for œstriol. The limit of

sensitivity is about 1 μg., though this can be increased by examination under ultra-violet light. Preliminary chromatograms run with a single alkaline aqueous solvents by the ascending method gave good separations for small quantities of steroid, though with larger amounts streaky movements were obtained. Variable R_F values were, however, noted with biological extracts. More satisfactory separations were obtained using a descending partition technique on Whatman paper with 0·5N ammonium hydroxide and a mixture of chloroform (1 part) and benzene (9 parts). A movement of 30 cm. took place at room temperature in 2 to 3 hours and, after drying the paper, the spots were detected with antimony trichloride as described above. The following R_F values are reported: œstrone (0·89), œstradiol (0·78) and œstriol (0·02). A qualitative elution of the hormones from the paper is possible using a mixture of chloroform and acetone.

J. B. S.

Retinene$_2$ and Vitamin A$_2$. H. R. Cama, P. D. Dalvi, R. A. Morton and M. K. Salah. (*Biochem. J.*, 1952, **52**, 542.) Rats fed on a diet free from carotenoids were found to convert retinene$_2$ to vitamin A$_2$ which was found in the liver within 24 hours, pure vitamin A$_2$ being recovered. Vitamin A$_2$ was moderately well stored by rats, but was not wholly satisfactory as a substitute for vitamin A$_1$. Vitamin A was obtained by the reduction of retinene$_2$ using the Ponndorf and lithium aluminium hydride methods. The spectroscopic properties of vitamin A$_2$ are given in detail and a study of the antimony trichloride colour reaction of vitamin A$_2$ is recorded. The structure of vitamin A$_2$ and retinene$_2$ is discussed, particularly with reference to light absorption data and it is concluded that there is strong evidence that vitamin A$_2$ is 3-dehydrovitamin A$_1$.

R. E. S.

Retinene$_2$, Preparation and Properties of. H. R. Cama, P. D. Dalvi, R. A. Morton, M. K. Salah, G. R. Steinberg and A. L. Stubbs. (*Biochem. J.*, 1952, **52**, 535.) Previous work on this subject was repeated on a larger scale, details being given of the preparation of retinene$_2$ from ling cod-liver oil and from pike-liver oil. Ling cod liver oil contains vitamin A$_1$ and vitamin A$_2$ in the ratio 1:8 to 1:10, sterol-free unsaponifiable matter in contact with manganese dioxide and light petroleum giving a mixture of retinene$_1$ and retinene$_2$ which can be separated by chromatography on watered alumina. Pike-liver oil contained more vitamin A$_2$ than vitamin A$_1$, the unsaponifiable fraction after oxidation containing the two retinenes which could be separated. Liver oil obtained from mixed large fresh-water fishes of Lake Nyasa contain up to 15 per cent. of mixed vitamins A (A$_2$:A$_1$ about 1:3); manganese dioxide oxidation yielded mixed retinenes. Retinene$_2$ is an aldehyde $C_{20}H_{26}O$: one preparation showed m.pt. 61° C., but subsequent preparations melted sharply at 77 to 78° C. Light absorption data are recorded for a number of solvents: the antimony trichloride colour test is greenish-blue $\lambda_{max.}$ 740 mμ. The material obtained agreed in properties with retinene$_2$ obtained from fresh-water fish retinas. R. E. S.

Retinene$_2$, Properties of. H. R. Cama, P. D. Dalvi, R. A. Morton and M. K. Salah. (*Biochem. J.*, 1952, **52**, 540.) A study of some reactions of amines and acids with retinene$_2$ has been made. With aliphatic amines, retinene$_2$ formed spectroscopic analogues of acid and alkaline indicator yellow$_2$; aromatic amines yielded compounds which after saponification showed a new broad absorption band having $\lambda_{max.}$ 560 mμ. Retinene$_2$ reacted with concentrated sulphuric acid to give coloured unstable products having absorption peaks close to 570, 525 and 470 mμ. The mechanism of the various reactions is examined and the bearing of the results on the chemistry of vision is discussed. R. E. S.

Vitamin B_{12f}, Properties and Distribution of. V. J. Lewis, D. V. Tappan and C.A. Elvehjem. (*J. biol. Chem.*, 1952, **199**, 517.) Vitamin B_{12f}, previously isolated from rat fæces, was chromatographed on columns of activated alumina mixed with Filter-Cel (2:1), to remove the vitamin B_{12f} from the vitamin B_{12f} preparations. The vitamin B_{12f} preparations were also passed through ion-exchange resins. The vitamin was not held by anion exchange columns, and cation exchanges absorbed it so strongly that none could be detected in the effluent, which indicates a strongly basic group in the molecule. It was shown to be a cyanide complex by paper chromatographic studies. It is completely inactive in promoting growth in the rat and chick, but does possess growth-stimulating properties in *Lactobacillus leichmannii*, *Escherichia coli*, and *Euglena gracilis*. Excretion and storage in the rat was investigated. Orally administered vitamin B_{12f} is excreted primarily by way of the fæces while, when injected, elimination is principally through the kidneys. A definite increase in vitamin B_{12} activity of liver was noted with injected vitamin B_{12f}, but, when orally administered, only a slight increase took place. By paper chromatography a compound with an R_F value corresponding to vitamin B_{12f} was obtained from the fæcal matter of the cow, sheep, pig, horse, chicken, guinea-pig and man, but no vitamin B_{12f} was detected in rat or beef liver nor in reticulogen. The results are discussed and data presented which suggest that vitamin B_{12f} is identical with pseudovitamin B_{12}, despite the difference in their absorption spectra. A. H. B.

BIOCHEMICAL ANALYSIS

Adrenal Cortex, Quantitative Paper Chromatography of Reducing Steroids of. V. Schwarz. (*Biochem. J.*, 1953, **53**, 148.) A method is given for the quantitative evaluation of adrenal extracts in which a mixture of cortical steroids is resolved into its components by paper chromatography and the chromatogram is treated with arsenomolybdate reagent. 2 paper chromatographic methods were used; with the first the more polar and the less polar steroids were resolved on separate strips, one being run in toluene/propylene glycol, the other in benzene/formamide; in the second method the solvent systems described by Bush (*Biochem. J.*, 1952, **50**, 370) were used, enabling all the biologically active steroids to be separated from each other on one strip and in a much shorter time. The chromatograms were treated with arsenomolybdate reagent, steroids containing a ketol side chain or having an $\alpha\beta$-unsaturated 3-keto structure quantitatively reducing the reagent to molybdenum blue; the blue zones of the developed chromatogram corresponding to the various cortical steriods were eluted and the blue complex determined in a photoelectric colorimeter. About 5 to 100μg. of reducing steroid could be determined with an accuracy of $\pm$ 10 per cent. Details of procedure are given, together with results obtained for 2 synthetic mixtures and 2 adrenal extracts. R. E. S.

Calcium in Serum, Colorimetric Determination of. M. U. Tsao. (*J. biol. Chem.*, 1952, **199**, 251). The selective colour reaction of alkaline earth metals with an alkaline solution of 2:3:4-tryhydroxybenzoic acid has been used as a basis for the estimation of serum calcium. The intensity of the violet colour produced is measured at 620 mμ and is linear with concentration in the range 5 to 18 mg. per cent., a range which covers the extreme values of pathological hypo- and hyper-calcæmia. Calcium is first precipitated on a semi-micro scale

as oxalate, the reaction being brought to completion rapidly by heating at 56° C. for 15 minutes. The precipitate is separated by centrifuging and is dissolved in hot 4N sulphuric acid, cooled and treated with a saturated solution of 2:3:4-trihydroxybenzoic acid. The solutions are mixed whilst the tube is cooled to 0° C., made alkaline by the addition of 9N sodium hydroxide and immediately sealed off from the atmosphere with a small quantity of molten wax. These precautions are essential to ensure a relatively stable colour, which reaches its maximum intensity in 5 minutes, after which time measurements are made. This method compares favourably with the usual volumetric method and possesses the advantage that duplicate analyses can be carried out on 1 ml. of serum in 1 hour. J. B. S.

Citric Acid in Biological Fluids, Determination of. R. H. Ettinger, L. R. Goldbaum and L. H. Smith. (*J. biol. Chem.*, 1952, **199**, 531.) The method depends upon the conversion of the citric acid to pentabromoacetone which is determined photometrically. A simplified oxidation-bromination procedure is introduced, and the reaction of the pentabromoacetone with alkaline pyridine solution is described for the production of colour. The resulting photometric procedure measures amounts of citric acid in the range of 2 to 40 mg. and is accurate to ± 5 per cent. It possesses a high degreee of specificity and may be used on a variety of biological materials. A. H. B.

Coumarins, Paper Chromatography of. T. Swain. (*Biochem. J.*, 1953, **53**, 200.) The R_F values on filter paper chromatograms of a number of coumarins and related cinnamic acids in various solvents have been determined. In most cases well-defined round spots were obtained, especially with the solvent systems containing acids. The compounds were best detected on the chromatogram by their fluorescence in ultra-violet light. Tables are given of the chromatographic properties of 19 coumarins and related cinnamic acids in 20 different solvent systems. The results obtained with different solvent systems and the colour reactions with various spray reagents are discussed in relation to the structure of the compounds. It was considered that the colours produced by the chromogenic sprays considered in conjunction with the R_F values in different solvent systems made it possible to classify and in some cases identify coumarins occurring in natural extracts. R. E. S.

Hyaluronidase, Assay of. R. Jaques. (*Biochem. J.*, 1953, **53**, 56.) A biological assay of hyaluronidase is described in which use is made of the early spreading activity of highly diluted hyaluronidase preparations in the skin of freshly killed guinea-pigs, the activity being compared with that of a standard preparation. Details of the method of assay are given. Tests performed 2 months apart showed reproducible and consistent estimates of potency. Using 12 animals per assay and choosing those skin sites which showed the smallest variation, statistical analysis showed the average limits of error to be less than 30 per cent., thus making it possible to distinguish easily between twofold differences in activity. R. E. S.

Hyaluronidase, Correlation between Methods for Assay of. J. H. Humphrey and R. Jaques. (*Biochem. J.*, 1953, **53**, 59.) 5 different preparations of testicular hyaluronidase, one of streptococcal and one of staphylococcal origin were compared by an accurate skin-diffusion assay and by viscosimetric and turbidimetric methods. With the testicular preparations the results of the 3 methods agreed well, provided that the potency was measured in terms of a reference preparation of enzyme, and that the pH and ionic strength of the

solvents were approximately physiological. A highly purified substrate was used and the enzymes were stabilised with gelatin rather than with gum acacia; the use of gum acacia gave relative potencies which were dependent on the turbidity end-point chosen and hence on the time taken to reach this end-point. The activities of the two bacterial enzymes showed a close but not a complete correlation between the 3 tests, although there was no evidence that single enzymes were present or that their action was identical with that of the testicular preparations. R. E. S.

Iron in Blood Plasma or Serum, Determination of. W. N. M. Ramsay. (*Biochem. J.*, 1953, **53**, 227.) A method for the determination of iron in serum or plasma, based on the replacement of the metal-combining globulin of the plasma with the co-ordinating reagent 2:2′-dipyridyl, is described. Plasma or serum is heated with 2:2′-dipyridyl reagent (0·075 per cent. in *p*H 5 acetate buffer containing 0·1 per cent. of hydroxylamine hydrochloride) on a water bath for 5 minutes during which the ferrous dipyridyl complex is formed and the proteins are coagulated; after cooling and filtering the intensity of the pink colour is measured at 520 mμ or using Ilford 604 or 624 filters. The standard deviation of a single determination from the mean was found to be 2·8 per cent.; experiments with known amounts of added iron gave recoveries of 83 to 110 per cent. Results obtained were higher by some 30 to 60 μg./100 ml. than those previously published. The plasma iron content of individuals varied considerably, values ranging from 112 to 180 μg./100 ml. being obtained on a single person. It was found that the oral intake of ferric hydroxide in the presence of ascorbic acid caused a considerable increase in the plasma iron content. R. E. S.

Khellin in Blood, Ultra-violet Spectrophotometric Estimation of. F. G. Soloni and J. F. Márquez. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 20.) The following method is suitable for the determination of khellin in the blood of patients being treated with the drug. A small sample only is required and the assay can be completed within 15 minutes. 2 ml. of oxalated or heparinised blood is stirred with 20 ml. of ethanol (96 per cent.) and the precipitate removed by centrifuging. The ethanolic extract, after evaporation to 7 ml. is passed through a chromatographic column of alumina and silica to remove chromogens. The column is washed with ethanol and the ultra-violet absorption of the solution (total volume 12·5 ml.) is determined at the absorption maximum of 250 mμ. From the figure is subtracted the result of a blank test performed on khellin-free blood from the same individual, or if this is not possible 0·300 is subtracted from the optical density reading. The concentration is calculated by comparison with standard solutions of khellin. Under the best working conditions the error does not exceed 1 mg./l. G. B.

Œstrogens, Fluorescence Reactions of. J. W. Goldzieher, J. M. Bodenchuk and P. Nolan. *J. biol. Chem.*, 1952, **199**, 621). The production of fluorescence by the action of sulphuric, phosphoric, and formic acids on œstrone, œstradiol-17α, œstradiol-17β, and œstriol was studied. From the results it is apparent that the concentration and chemical structure of the œstrogen, the acid used, and the wavelength of the exciting radiation influence the resulting fluorescence spectrum. Sulphuric acid was found to be the most potent of the 3 agents tested for the production of fluorescence, except in the case of œstradiol-17α, with which phosphoric acid gave most intense fluorescence. Excitation by 436 mμ radiation produced maximum fluorescence. The application of the method to quantitative measurement, is discussed.

A. H. B.

Potassium in Blood Serum, Determination of. J. M. Barry and S. J. Rowland. (*Biochem. J.*, 1953, **53**, 213.) It was found that the low accuracy of many methods of estimating serum potassium based on the sodium cobaltinitrite procedure was due mainly to 3 factors. Firstly the dilution of the serum with water led to as much as 20 per cent. of the potassium remaining unprecipitated owing to the solubility of potassium sodium cobaltinitrite; secondly insufficient time was allowed for the precipitation of the potassium; and thirdly some of the precipitate was dissolved during washing. Experimental details of the investigations are given, together with the final revised method in which the potassium is precipitated as potassium sodium cobaltinitrite by adding a sodium cobaltinitrite reagent to the serum in a centrifuge tube; after washing with aqueous ethanol, the precipitate is dissolved in water and the cobalt content is measured from the intensity of the green colour produced on adding choline chloride and potassium ferrocyanide solutions. The standard deviation of replicate determinations did not exceed 1 per cent. of the mean value while the results showed that the recovery of added potassium was satisfactory.

R. E. S.

Pyrogens, Assay by the Febrile Response in Rabbits. D. M. Tennent and W. H. Ott (*Analyst*, 1952, **77**, 643). The presence of pyrogens in antibiotics and in solutions for parenteral injection are detected in rabbits. This paper describes important factors influencing the response of rabbits to pyrogens normal temperature, body weight, individual sensitivity, excitation and tolerance phenomena. These are minimised by careful selection of the rabbits and measuring from the results of preliminary sensitivity tests the response to 0·1 mg. of a prepared pyrogen standard obtained from a culture of *Pseudomonas* grown in synthetic media. Of particular importance was the observation of tolerance developing after a single dose of pyrogen. Maximum tolerance occurred in 7 to 10 days after a single injection of pyrogen, recovery being complete in about 3 weeks. Routine samples which are pyrogenic are quantitatively assessed by injecting a series of dilutions of them into several groups of rabbits and comparing them with the responses of similar groups given the standard pyrogen solution. The febrile response in °C. is a linear function of the log dose. Using 6 groups of 3 rabbits, half for the unknown and half for the standard, the slope was 0·78 and the standard error for the assay result ± 0·22 log dose.

G. F. S.

Vitamin B_{12} in Human Serum, Determination of Activity of. H. L. Rosenthal and H. P. Sarett. (*J. biol. Chem.*, 1952, **199**, 433). Detailed studies of the preliminary treatment of human serum necessary for microbiological determination of vitamin B_{12} activity with *Lactobacillus leichmannii* are described. B_{12} active material was measured microbiologically by the method of Thompson *et al.* as modified by Register and Sarett (*Proc. Soc. exp. Biol., N.Y.*, 1951, **77**, 837) using crystalline vitamin B_{12} as a standard. Crystalline vitamin B_{12} added to serum in concentrations of the same order of magnitude as that found in the serum can be quantitatively recovered, the recovery ranging from 88 to 110 per cent. of the added material. The conditions necessary to destroy the activity of vitamin B_{12} by heating in alkaline solution were examined in order to determine whether the measured B_{12} activity was due mainly to vitamin B_{12} or to some other microbiologically active material. Vitamin B_{12} added to serum remains stable at *p*H 5 to 8 after 30 minutes at 100° C., but is partially inactivated at *p*H 8 to 10 and completely deactivated above *p*H 10·5 under the same time-temperature conditions. Vitamin B_{12} in an extract of serum at *p*H 11 is stable for at least 30 minutes between 0° C. and 37° C., but is rapidly inactivated at higher temperatures.

The rate of inactivation at *p*H 11 and 100° C. is rapid, 90 per cent. being inactivated in the first 10 minutes. Determination of vitamin B_{12} activity in 24 samples of normal human serum showed a variation from 0·08 to 0·42 mμg./ml. of serum (average 0·20 mμg./ml.). Little variation occurred in the serum level of B_{12} activity throughout the day. Alkali-stable vitamin B_{12} activity determined in samples of pooled serum represented 0 to 9·4 per cent. of the total activity (average 3·4 per cent. of total activity). Dialysis experiments showed that vitamin B_{12} activity in normal serum is firmly bound; limited amounts of added vitamin B_{12} are also bound by the serum and cannot be removed by dialysis. J. B. S.

CHEMOTHERAPY

4-Aminobenzamides and 4-Aminobenzenesulphonamides as Antitubercular Agents. P. Truitt, G. Sammons and D. Zachary. (*J. Amer. chem. Soc.*, 1952, **74**, 5961.) The preparation of series of 1-(4-nitrobenzoyl)- and 1-(4-aminobenzoyl)-4-alkylpiperidines, and 1-(4-nitrobenzene-sulphonyl)-, 1-(4-acetylaminobenzenesulphonyl)- and 1-(4-aminobenzenesulphonyl)-4-alkylpiperidines and 1:2:3:4-tetrahydroquinolines is described. Most of the compounds were inactive in the antitubercular test in the presence of bovine serum, but 1-(4-aminobenzoyl)-4-(1-octyl)-piperidine was active at 0·078 mg. per cent. and was also active in the presence of serum. This compound was also amœbistatic at 1 : 500 dilution and showed moderate activity against *Neisseria catarrhalis*, *Streptococcus hæmolyticus*, and *Brucella suis*. A. H. B.

Chaulmoogric Acid, a Pyrimidine Analogue of. T. D. Heyes and J. C. Roberts. (*J. chem. Soc.*, 1952, 4935.) A general synthesis for ω-(2:6-dimethyl-4-pyrimidyl) alkane-1-carboxylic acids is presented. The compound, 12-(2 : 6-dimethyl-4-pyrimidyl) dodecane-1-carboxylic acid was prepared as an analogue of chaulmoogric acid in which the cyclopentenyl radical of chaulmoogric acid has been replaced by the 2:6-dimethyl-4-pyrimidyl radical. It possessed a tuberculostatic activity of 5, while the corresponding undecane-1-carboxylic acid had an activity of 1. A. H. B.

Emetine, Analogues of. J. M. Osbond, J. D. Fulton and D. F. Spooner. (*J. chem. Soc.*, 1952, 4785.) Analogues of emetine were prepared, including 2-(1:2:3:4-tetrahydro-6:7-dimethoxy-2-methyl-1-*iso*quinolylmethyl)-1-(1:2:3:4-tetrahydro-6:7-dimethoxy-1-*iso*quinolyl)-butane and -pentane, which can be regarded as derived from the emetine formula by the fission of one bond. None of the compounds prepared was active in concentrations up to $1:10^4$ *in vitro* against *E. histolytica*, whereas emetine under similar conditions was active at $1:10.^6$ A. H. B.

Hydroxamic Acids, Tuberculostatic. N. P. Buu-Hoï, N. Dat Xuong and N. Hoang Nam. (*C.R. Acad. Sci. Paris*, 1953, **256**, 635.) A number of hydroxamic acids, R·CO·NHOH, were synthesised. 5-Chlorosalicylhydroxamic acid was prepared by adding a solution of 10 g. of methyl 5-chlorosalicylate in 70 ml. of ethanol to a solution of hydroxylamine in ethanol, shaking and filtering. The product was isolated by acidifying to *p*H 2 with acetic acid and precipitation with water. 5-Bromosalicylhydroxamic acid was made similarly. 5-Benzylsalicylhydroxamic, 3:5-dichlorosalicylhydroxamic and 3:5-dibromosalicylhydroxamic acids were obtained as above, but boiling for 15 minutes and allowing to stand at room temperature for 24 hours before adding the acetic acid. The most promising compound appeared to be 5-chlorosalicylhydroxamic acid, which is not very toxic; the sodium salt is sufficiently soluble in water for use in intravenous injections. G. B.

Methonium Series, Ganglion-blocking Properties of. R. Wien, D. F. J. Mason, N. D. Edge and G. T. Langston. (*Brit. J. Pharmacol.*, 1952, 7, 534). A study has been made of the ganglionic blocking properties of a series of 16 homologous compounds in the alkamethonium series in which the methyl groups were successively replaced by ethyl groups on the two quaternary nitrogen atoms. Also studied were γ-phenoxypropyltriethylammonium and its trimethyl homologue. The compounds were tested for their effect in paralysing transmission through the superior cervical ganglion of the cat, on the peristaltic reflex of the isolated guinea-pig intestine as well as for neuromuscular blocking activity using the phrenic nerve diaphragm preparation of the rabbit. The potency of all the compounds was considerably altered by replacement of ethyl for methyl groups on the quaternary nitrogen atoms. In the alkamethonium series increase in chain length from 4 to 6 carbon atoms or partial replacement of methyl by ethyl groups increased potency. Replacement of all the methyl groups reduced activity. In the tetramethylene compounds the compound tetramethylene-1:4 bis-diethylmethylammonium bromide was as active as hexamethonium and had an affinity for the superior cervical ganglion relative to its effect on the intestine. Two homologues of tetraethylammonium, the diethyldimethyl- and ethyltrimethyl-ammonium compounds showed marked nicotine-like properties. The structure-action relationships of compounds in this series is discussed.

G. F. S.

PHARMACY

GALENICAL PHARMACY

Phenolated Calamine Lotion. G. L. Stanko and H. G. DeKay. (*Amer. J. Pharm.*, 1952, **124**, 328.) When phenol is added to calamine lotion prepared with 2 per cent. polyethylene glycol 400 monostearate as described in the U.S.P. XIV, a marked decrease in viscosity, followed by clumping of the particles into large aggregates and separation of the preparation, occurs. Similar results are obtained when 1 per cent. sodium stearate gel is used instead of the polyethylene glycol ester. It is suggested that phenol acts by disrupting micelle formation. The following formula was satisfactory: calamine 8 per cent., zinc oxide 8 per cent., glycerin 2 per cent., tween 80 0·5 per cent., sodium alginate (2 per cent.) 5 per cent., and veegum magma (5 per cent.) 25 per cent. The combination of veegum and sodium alginate is recommended because film formation with alginate alone may be excessive. This lotion is unaffected by the incorporation of 1 per cent. of liquified phenol, 1 per cent. of thymol, 1 per cent. of thymol with 0·5 per cent. of menthol, 1 per cent. of camphor with 0·5 per cent. of menthol, and 5 per cent. of benzocaine. The use of methylcellulose or carboxymethylcellulose did not give satisfactory results. Phenol in the lotions may be determined by diluting, centrifuging and measuring the colour produced in the supernatant liquid after reaction with copper sulphate, hydrogen peroxide and ammonium hydroxide.

G. B.

NOTES AND FORMULÆ

Antazoline Phosphate (Antistine Phosphate). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1952, **150**, 1220.) Antazoline phosphate is 2-(*N*-benzylanilinomethyl)-2-imidazoline phosphate and occurs as a white, odourless, crystalline powder, with a bitter taste, m.pt. 194° to 198° C. with decomposition, soluble in water, sparingly soluble in methanol, and almost insoluble in benzene and ether; *p*H of a 2 per cent. solution about 4·5. A solution gives a flocculent

pink precipitate with ammonium reineckate, a yellow precipitate, which melts between 155° and 159° C., with trinitrophenol, a red colour, which rapidly becomes dark green, with nitric acid, and a precipitate of the free base, which melts at 114° to 118° C., with sodium hydroxide. A 0·001 per cent. solution exhibits an ultra-violet absorption maximum at about 2420 Å [$E^{1\ \text{per cent.}}_{1\ \text{cm.}}$, 414 ± 5]. When dried at 105° C. for 4 hours it loses not more than 0·5 per cent. of its weight; sulphated ash, not more than 0·2 per cent. It contains 97·0 to 103·0 per cent. of antazoline phosphate, assayed by measuring the absorption of a 0·001 per cent. solution at 2420 Å, and 70·8 to 75·2 per cent. of antazoline, determined as the picrate. G. R. K.

Chloromethapyrilene Citrate (Chlorothen Citrate). (*New and Nonofficial Remedies, J. Amer. med. Ass.*, 1952, **150**, 1221.) Chloromethapyrilene citrate is *NN*-dimethyl-*N*′-(2-pyridyl)-*N*′-(5-chloro-2-thenyl)ethylenediamine citrate and occurs as a white almost odourless substance, m.pt. 116° to 118° C., very slightly soluble in ether, soluble in ethanol (about 1 in 40) and in water (about 1 in 20); a 1 per cent. solution is clear and colourless and has a *p*H of 3·9 to 4·1. It yields a dipicrate which melts at 150° to 152° C. after drying at 105° C. A 0·001 per cent. solution in ethanol exhibits absorption maxima at 2420 Å [$E^{1\ \text{per cent.}}_{1\ \text{cm.}}$, about 410] and at 3050 Å [$E^{1\ \text{per cent.}}_{1\ \text{cm.}}$, about 89]. It loses not more than 0·1 per cent. in weight when dried over phosphorus pentoxide for 18 hours, and yields not more than 0·05 per cent. of sulphated ash. It contains 98·5 to 101·5 per cent. of chloromethapyrilene citrate, determined by measuring the absorption of a 0·001 per cent. solution in ethanol at 2420 Å. It is also assayed for citrate by electrometric titration with 0·1N sodium hydroxide and for nitrogen by a semi-micro Kjeldahl method; the content of citrate is 38·8 to 40·0 per cent. and of nitrogen, 8·50 to 8·70 per cent. Chloromethapyrilene citrate is a histamine antagonist. G. R. K.

Propylhexedrine (Benzedrex). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1952, **150**, 1220.) Propylhexedrine is *N*α-dimethyl*cyclo*hexane-ethylamine and occurs as a clear colourless liquid with a characteristic amine-like odour, b.pt 202° to 206° C., very slightly soluble in water, soluble in dilute acids, ethanol and ether. It is distinguished from adrenaline, ephedrine and phenyl-ephrine by the copious white granular precipitate produced on the addition of mercuric chloride to an aqueous suspension. An aqueous suspension also gives a brown precipitate with iodine and a picrate which melts at 108° to 110° C. It yields not more than 0·1 per cent. of ash and contains 98·0 to 102·0 per cent. of propylhexedrine; it is assayed by titration in ethanolic solution with sulphuric acid, using methyl red as indicator. It is used as a vasoconstrictor. G. R. K.

Starch Derivative Dusting Powder. M. Andersen and V. Würtzen. (*Dansk Tidsskr. Farm.*, 1953, **27**, 25.) A starch ester preparation suitable for replacing talc as dusting powder is prepared by the following procedure. 100 g. of maize starch is treated with 5 g. of potassium hydroxide in 20 g. of absolute ethanol, followed by 5 g. of epichlorhydrin in 10 g. of absolute ethanol. The mixture is warmed to 40° C. and allowed to dry in the course of about 2 hours. This treatment is repeated, alkali is removed by washing with water, and the material is dried at 40° C., 2 per cent. of magnesium oxide being then added. The preparation, which does not swell with water, was tested by injection into the peritoneal cavities of rats, and was found to be greatly superior to talc in freedom from granuloma, necrosis and foreign body reactions, while the absorbability was good. G. M.

PHARMACOGNOSY

Digitalis, Diurnal Variations in Glycoside Content of. R. Hegnauer. (*Pharm. Weekbl.*, 1953, **88**, 69.) The author reviews and discusses the conflicting evidence regarding the variation of glycoside content of digitalis at different hours of the day. In many cases the activity has been calculated on the basis of the dry weight of the leaves, which is considerably higher after a period of illumination than after a period of darkness. Metabolic products tend to accumulate in the leaves during daylight, while the water content is lowered. Both effects cause an increase in the percentage of dry matter, while the first also results in an increase in the absolute amount of dry matter. Thus owing to variations in the accumulation and translocation of reserve materials, the percentage of glycosides in the leaf may alter without any alteration in the actual quantity present. On the other hand, the percentage of glycosides would remain unaltered if the glycoside accumulation, translocation or splitting ran parallel with the changes in the reserve material in the leaf. In practice it would appear desirable to harvest the leaves at midday, since although the relative glycoside content may not be greater, yet the absolute yield will be increased, and it is certain that the percentage of glycosides is not lower at this time. G. M.

Solanaceæ, Diurnal Variation in Alkaloidal Content of. R. Hegnauer. (*Pharm. Weekbl.*, 1953, **88**, 106.) There is no agreement between different investigators regarding the optimum period of the day at which solanaceous drugs should be harvested. The author reviews the evidence but is unable to come to any definite conclusion. Modern knowledge of the biology of these plants suggest that at least 4 processes may be involved—the accumulation of root alkaloids in the leaves, the diurnal variations in the content of dry matter in the leaves, synthesis of alkaloids in the leaves themselves, and migration of alkaloids out of the leaves. These may be influenced by weather conditions the age of the plant and of the particular leaves, and the characteristics of the species. Thus the relative alkaloidal content of a leaf which is analysed at a particular point of time is determined by the synergistic or antagonistic interplay of numerous factors. In practice it does not appear justified to insist on harvesting in the morning, since the differences which have been found are small and it is quite possible that they were due merely to a drop in the content of dry matter during the night. Tests made with cut (halved) leaves are not conclusive since they may be affected by traumatic effects, and moreover it is known that the alkaloidal concentration is greatest in the midrib and stalk. G. M.

Strophanthus Seeds, Cardiac Aglycones of. I. E. Bush and D. A. H. Taylor. (*Biochem. J.*, 1952, **52**, 643.) A report is given on a preliminary investigation using paper chromatography of the distribution of cardiac aglycones in various *Strophanthus* species. The chromatography of seed extracts was carried out with aqueous methanol as the stationary phase; for the development of the spots a 1 per cent. solution of 3:5-dinitrobenzoic acid in 0·5N 50 per cent. v/v aqueous methanolic potassium hydroxide was suitable, giving a purple colour. *S. hispidus* contained strophanthidin as the main constituent while *S. sarmentosus* samples were divided into two types, one containing sarmentogenin as the only genin present linked to a deoxy sugar, the other type containing sarverogenin as well. Detailed results of chromatographic data obtained on other *Strophanthus* species are given, the results are discussed, and a classification of the genus *Strophanthus*, on purely chemical grounds, is proposed. R. E. S.

Vein Islet Numbers in Species Distinction. C. W. Ballard and R. Bebarfald. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 13.) The chief difficulty in the determination of vein islet numbers appears to be the clearing of dense leaves so that the ultimate branches of veins can be properly observed. The use of sodium hypochlorite solution should be avoided as it oxidises lignin, causing the smaller veins to disintegrate and resulting in a low vein islet count. It is recommended that specimens should be cleared with chloral hydrate solution and then treated with phloroglucinol followed by hydrochloric acid. This stains the lignin in the small veins and removes calcium oxalate. The material is again cleared in chloral hydrate and mounted for observation in a mixture of equal quantities of glycerol, ethanol and water. When the leaves of a species are of uniform size such as in buchu, senna or coca the vein islet numbers may aid in determining the species, but in larger leaves variations due to position, environment and size impair its usefulness. In the genus *Datura* the differentiation of species by vein islet counts is difficult if not impossible. G. B.

PHARMACOLOGY AND THERAPEUTICS

Adrenaline Antagonists. A. Fleckenstein. (*Brit. J. Pharmacol.*, 1952, **7**, 553.) A study has been made of the anti-adrenaline activities of various substances on the perfused vessels of the rabbit's ear. The exact concentration of each substance which reduced the effect of adrenaline to 1/10th of its initial effect, was determined. The speed of recovery of sensitivity after stopping perfusion of the anti-adrenaline compound was found to give the best indication of activity. Ergot alkaloids, regitine, and dibenamine, regarded as specific adrenaline antagonists, were active at very low concentrations and recovery to normal sensitivity was very slow. The antihistamine compounds promethazine and phenindamine acted in low concentrations but were quickly washed out. Many of the compounds antagonised the constrictor actions of histamine in this preparation, but true antihistamine compounds were active at much lower concentrations and had a much more prolonged action. These observations are explained by "specific" inhibition being due to a preference for a certain type of receptor with the formation of a close and stable combination.

G. F. S.

Adrenal Medulla, Hormones of. A. S. Outschoorn. (*Brit. J. Pharmacol.*, 1952, **7**, 605.) The results of changes in the adrenaline and noradrenaline content of the adrenals of rats treated with three compounds, insulin, morphine and tetrahydro β-naphthylamine, known to cause a release of the adrenal medullary hormones, are described. All 3 compounds caused depletion of adrenaline after some hours, but no significant depletion of noradrenaline occurred although there was a tendency for depletion with insulin. During the first 2 to 4 hours after injection of the drugs, the noradrenaline content tended to increase. In anæsthetised cats, collection of adrenal venous blood showed large amounts of active substances to be released after stimulation of the adrenal medullæ by electrical excitation of the cut splanchnic nerves, or injection of acetylcholine, or potassium chloride into the stump of the cœliac artery. The hormone released during stimulation was mainly adrenaline with smaller amounts of noradrenaline The proportion of adrenaline to noradrenaline did not depend on the method or time of stimulation in this species. In the resting gland the venous effluent concontained small amounts of adrenaline and sometimes larger amounts of noradrenaline. There is a species difference to the rat which may be able to synthesise noradrenaline better than adrenaline when its medullary resources

are heavily taxed, whereas the cat may have no special ability to synthesise noradrenaline quicker. G. F. S.

Antihistamines, Protective Action of, in the Anaphylactic Microshock of the Guinea-pig. P. Armitage, H. Herxheimer and L. Rosa. (*Brit. J. Pharmacol.*, 1952, 7, 625.) Guinea-pigs were sensitised to egg albumin and 21 days later exposed to ærosolised antigen, the preconvulsion time being determined. The protective effect of a number of antihistamine compounds—antazoline, chlorcyclizine, chlortrimeton, diphenhydramine, mepyramine, promethazine and tripelennamine—as well as atropine, procaine and procaine amide was examined. The degree of protection depended upon the dosage and while protection was seen with all the antihistamines it was never complete except with very high doses which would be toxic to man. Determination of the doses beyond which there was no significant increase in protection showed considerable differences between the substances tested. Atropine and procaine had only weak antihistamine actions while the protective action of procaine was doubtful.

G. F. S.

Cortisone Desensitisation, Influence of Carbohydrate Metabolism on Bacterial Allergy. J. W. Cornforth and D. A. Long. (*Lancet*, 1953, **264**, 160.) Albino guinea-pigs, rendered sensitive to tuberculin by the injection of BCG vaccine were used. A number of substances were injected prior to tuberculin and the response used as a measure of allergic sensitivity. Single subcutaneous injections of adenosine diphosphate diminished or abolished sensitivity, whereas single subcutaneous injections of adenosine triphosphate prevented desensitisation by alloxan, cortisone acetate and dehydroascorbic acid, although desensitisation by glucose-1-phosphate was unaffected. Single subcutaneous injections of insulin did not significantly affect sensitivity, but prevented desensitisation by alloxan and dehydroascorbic acid, and increased sensitivity in animals treated with cortisone acetate, the desensitising action of glucose-1-phosphate being unaffected. The compensatory phase following insulin hypoglycæmia was associated with desensitisation, thought to be due to naturally liberated cortisone. Depressed sensitivity due to growth hormone was unaffected by injection of reduced glutathione, adenosine triphosphate or insulin or by ascorbic acid deficiency. Growth hormone had a synergistic action with cortisone acetate or alloxan. It is suggested that cortisone facilitates oxidation of ascorbic acid to dehydroascorbic acid, which inactivates phosphoglucomutase and increases the tissue concentration of glucose-l-phosphate which is concerned in desensitisation. Adenosine triphosphate and insulin oppose this by stimulation of hexokinase activity, removing phosphate which otherwise would be used to form glucose-l-phosphate from glycogen. This effect is enhanced by cortisone which influences the formation of glucose from protein. Adenosine triphosphate and growth hormone cause breakdown of glycogen to glucose-l-phosphate, due, in the latter case, to inhibition of hexokinase activity. G. B.

Dextran, an Improved Type of. A. M. Boyd, F. Fletcher and A. H. Ratcliffe. (*Lancet*, 1953, **264**, 59.) Molecules in intravenous solutions for use as blood volume restorers should not be so large as to cause undesirable reactions nor so small as to be readily lost to the circulation by excretion or diffusion into the tissue fluids. A number of types of dextran solution, prepared by hydrolysis and fractionation to remove large molecules, or by intensive fractionation to obtain the desired molecular weight range were tested in an effort to obtain a substance easy to administer, free from undesirable side-effects

and capable of maintaining a blood level equivalent to 50 per cent. of the amount infused, after 24 hours. These requirements were met by a preparation obtained by intensive fractionation "narrow fraction C," of predominating molecular weight 120,000 to 250,000, administered as a 6 per cent. solution in normal saline solution. Using this material in intravenous drip it was possible to maintain the blood pressure through the most severe operations and it was rarely necessary to continue the infusion after completion of the operation. 20 per cent. of the volume infused was retained in the blood after 5 days. In hæmorrhage, dextran may be given simultaneously with blood, but the use of gallamine should be avoided as hæmatomata may occur. G. B.

***NN′*-Dibenzylethylenediamine Penicillin: a New Repository Form of Penicillin.** A. P. Fletcher and C. R. Knappett. (*Brit. med. J.*, 1953, **1**, 188.) 1 ml. of a suspension of *NN′*-dibenzylethylenediamine penicillin containing 600,000 units, was administered by intramuscular injection to each of 19 human subjects, and penicillin serum levels were subsequently determined by the *Sarcina lutea* plate technique, using penicillin dilutions in serum to allow for the influence of protein-bound penicillin. The serum penicillin levels were well maintained, an average of 0·042 unit/ml. being observed 5 days after the injection. Results indicated that even with injections at 14-day intervals a summation of effects might occur. Owing to the insoluble nature of the preparation and the long duration of action, measurable penicillin levels appeared only intermittently in some subjects. This form of repository penicillin may be of use in the treatment of venereal disease, since default from treatment is common, and in the prophylaxis of hæmolytic streptococcal infection following rheumatic fever. Unfortunately the low intermittent penicillin levels produced by *NN′*-dibenzylethylenediamine penicillin provide optimum conditions for the emergence of resistant organisms and for sensitisation of the patient to penicillin. G. B.

β-(Diphenyl-methoxy)ethyltrimethylammonium bromide, Cough-alleviating Effect of. L. A. Hahn and H. Wilbrand. (*Arch. int. Pharmacodyn.*, 1952, **91**, 144.) The effect of this substance on the normal threshold for cough was compared with that of codeine in a series of 7 volunteers. The method employed for determining the cough threshold was as follows. The subject breathed through a system of tubes fitted with valves to prevent expiration into the system; ammonia vapour was added in desired quantities to the air to be inhaled and the amount of ammonia vapour causing distinct closure of the glottis was employed as an indicator as the threshold of the cough reflex. The individual pre-medication threshold was determined for each volunteer. The drugs were administered in tablets and cough syrups, the contents of which were unknown to the volunteers. In doses of 10 mg. and 20 mg. the effect on the cough threshold of the quaternary compound and of codeine phosphate was approximately the same. A combination of the two substances (10 mg. of each) tended to be less effective than either of the individual components. The quaternary salt was also more effective alone than in combination with methadone. The depressant effect on the respiration was observed from the use of the quaternary salt. S. L. W.

Hyaluronidase and Salicylates. T. D. Day. (*Nature, Lond.*, 1953, **171**, 268.) White mice were given sodium salicylate (1 per cent.) in their drinking water for 48 hours and, during this period 2 injections of 0·5 ml. of a 1 per cent. solution of sodium salicylate were administered intraperitoneally. The mice were killed and fascia from each, moistened with sodium salicylate solution was fastened

as an occluding membrane to the end of a glass tube. The membrane was immersed in the same solution and more of the solution passed through under a pressure of 4 cm. of water. The rate of flow was measured before and after the perfusion of a solution of hyaluronidase in 1 per cent. sodium salicylate solution. Control tests were made with fascia from mice which received no salicylate, using 0·85 per cent. sodium chloride solution in the *in vitro* tests. The increased rate of flow brought about by the hyaluronidase was unaffected by the presence of salicylate. Probably the inhibition of the spreading action *in vivo* by salicylates is due to their influence on some of the other factors involved. G. B.

DL-Methadone. C. Y. Sung, E. L. Way and K. G. Scott. (*J. Pharmacol.*, 1953, **107**, 12.) A study has been made of the tissue distribution, urinary and fæcal excretion, rate of metabolic change and the tissue binding of DL-methadone in normal rats and rats made tolerant to this compound. Normal and tolerant rats did not differ greatly in concentrating DL-methadone in their tissues, but tolerant rats excreted a greater amount of a metabolic product of the compound. *In vitro* studies showed that liver slices from tolerant rats altered the compound less rapidly than liver from normal rats while muscle homogenates bound it to the same extent in both. Adrenal hypertrophy and a possible decrease in thyroid activity accompanied tolerance, the significance of which is discussed. G. F. S.

Neuromuscular Junction, Antagonistic Actions at. M. J. Dallemagne and E. Philippot. (*Brit. J. Pharmacol.*, 1952, 7, 601.) Decamethonium and *d*-tubocurarine block the neuromuscular junction in fundamentally different ways and each of these substances is able to modify the action of the other. The authors confirm the observations of Hutter and Pascoe that decamethonium is able to restore neuromuscular transmission in the cat which has been blocked by tubocurarine. A similar antagonism has also been shown between tubocurarine. and amyltrimethylammonium, a compound having similar properties to decamethonium. It is noted that tubocurarine is not antagonised by decamethonium and *vice versa* in the dog where, the authors suggest, decamethonium apparently acts like tubocurarine, its blocking effects being alleviated by adrenaline and neostigmine. G. F. S.

Œstrogens, Bioassay of. W. H. Lawrence and C. W. Chapman. (*J. Amer. pharm. Ass. Sci. Ed.*, 1952, **41**, 624.) Adult ovariectomised rats for use in the tests were primed by intraperitoneal doses of 2-aminopyridine œstrone sulphate, and groups of 15 to 20 were used at each dose level of the reference preparation and substance under test. Dilutions of a suspension of the commercial material and of a stock solution of 2-aminopyridine œstrone sulphate were administered daily for 3 consecutive days directly into the stomach of the animal by means of a syringe and catheter. Vaginal smears were obtained twice during the 4th and 5th days, examined microscopically and scored +, ± or −. The potencies, in terms of 2-aminopyridine œstrone sulphate were determined graphically and calculated in terms of sodium œstrone sulphate, one of the principal constituents of commercial preparations, by the use of the factor 0·84. 2-Aminopyridine œstrone sulphate is suitable for use as a reference preparation since it is qualitatively similar to sodium œstrone sulphate, but is stable and non-hygroscopic. The method of assay involving oral administration is recommended because in clinical use the conjugated œstrogens are given by mouth. Partial destruction in the liver following administration by mouth may

result in incorrect assessments being obtained in tests by injection. In 8 commercial preparations results varied from 46·4 to 116·7 per cent. of the stated potency, and only 2 products possessed the œstrogenic activity claimed on the label. G. B.

Polymixins, A, B and E, Toxicity of. P. N. Swift and S. R. M. Bushby. (*Lancet*, 1953, **264**, 110.) Polymixin E was administered to 53 patients, 250,000 units/kg. for adults or 10,000 to 12,500 units/kg. for children being injected intramuscularly every 4 hours. 32 patients received similar treatment with polymixin B. No proteinuria was observed, in contrast to the proteinuria and renal damage caused by polymixins A and D. Possibly a difference in molecular configuration between the polymixins permits or prevents passage through the glomerulus and consequent damage to the tubule on resorption. Local reactions occurred in 8 patients treated with polymixin E, and 25 with polymixin B, while pyrexia was noted in 4 on polymixin E and 21 on polymixin B. Both produced a reaction at the site of injection, but polymixin E caused less pain even when twice the dose was given. Incidence of paræsthesia was about the same for both forms. The more highly purified form of polymixin B caused less pyrexia and malaise than the earlier impure material. The side-effects of polymixin B and E were not serious, the most troublesome being pain persisting for some hours at the site of injections, and this should not prevent their use in treatment. G. B.

Pyrimethamine (Daraprim), Antimalarial Properties of. L. H. Schmidt and C. S. Genther. (*J. Pharmacol.*, 1953, **107**, 61.) A systematic evaluation has been made of pyrimethamine (2:4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine) against *P. cynomolgi* infections in the rhesus monkey. It was effective in small doses in checking the development of both pre-erythrocytic and late exoerythrocytic stages and is a highly effective suppressive when administered at weekly intervals. Against erythrocytic infections it is active in extremely small doses, but comparatively large doses are required for control of infections in all cases. The drug is most effective when given in multiple daily doses and its slow action suggests that it will be unsatisfactory in the treatment of falciparium infections. Its mode of action is identical with that of chlorguanide. Both the sexual and asexual forms rapidly develop resistance to pyrimethamine and these are equally resistant to chlorguanide but susceptible to chloroquine. The greatest value of pyrimethamine would appear to be as a routine suppressive, its slowness of action and potentiality to induce resistance being serious limiting factors in the treatment of active infection. G. F. S.

Pyrimethamine (Daraprim), Pharmacological Properties of. L. H. Schmidt, H. B. Hughes and I. G. Schmidt. (*J. Pharmacol.*, 1953, **107**, 92.) Studies of the absorption, excretion, metabolism and tissue distribution of pyrimethamine (2:4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine) in the rhesus monkey have shown that it was absorbed slowly but completely from the gastro-intestinal tract and peak plasma levels were usually attained in 2 days. Accumulation in the plasma did not occur. The drug was extensively metabolised, only 20 per cent. being recovered from the urine and even this contained metabolic products. The drug was localised in the tissues, particularly in the lung, liver, kidney and spleen. Toxicological studies in the monkey and the rat have shown that pyrimethamine was less innocuous than was formerly supposed. Daily doses equivalent to 190 mg./kg. were fatal to rats and 100 mg./kg. markedly suppressed growth. The high dose for 3 weeks

(ABSTRACTS *continued on p. 404*).

on ignition should be reduced to 0·1 per cent.; the present limit of 0·5 per cent. is unnecessarily high.

Progesteronum. A limit for residue on ignition should be included.

Thiamine Hydrochloridum. The present fluorimetric assay with the possibility of wide limits of error is unsatisfactory for the control of this substance to a purity of "not less than 98·0 per cent." Chemical assays for nitrogen or chlorine content or a spectrophotometric assay would be better.

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2. Hersant, *J. Pharm. Pharmacol.*, 1953, **5**, 135.
3. Cama and Morton, *Analyst*, 1953, **78**, 74.

(ABSTRACTS *continued from p. 397*).

produced skin lesions, marked leucopenia and neutropenia and exhaustion of the myeloid elements of the femoral bone marrow. The monkey was more sensitive, repeated daily doses of 5 mg./kg. being invariably fatal and deaths occurred with 2·5 mg./kg., which suggests that pyrimethamine is at least 16 times as toxic as chloroquine or chlorguanide. Toxic effects in the monkey followed two distinct patterns. In one case a dose of 40 mg./kg. was followed by severe chronic convulsions and death. In all other cases there was a slow progressive intoxication characterised by muscular weakness, malaise, anorexia, diarrhœa and bronze pigmentation of the skin. The drug produced a marked leucopenia and agranulocytosis. There was an extreme atrophy of the spleen and the lymph follicles, lesions in the adrenal cortex and kidney calyx. The most striking effects were on the bone marrow which showed a tremendous reduction in cellularity especially in the myeloid elements. It is emphasised that the toxic characteristics of pyrimethamine are wholly unlike those of chloroquine and chlorguanide and the drug presents a serious potential hazard to the human user. It is concluded that the general use of such a toxic drug is not justified until rigidly controlled studies in man demonstrate that it has unique antimalarial properties conveying a wide margin of safety. G. F. S.

BACTERIOLOGY AND CLINICAL TESTS

Phenylethyl Alcohol, Selective Action of. B. D. Lilley and J. H. Brewer. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 6.) Organisms were grown in infusion broth with and without serum, to which various concentrations of phenylethyl alcohol ($C_6H_5{\cdot}CH_2{\cdot}CH_2{\cdot}OH$) had been added. Phenylethyl alcohol exerted a greater inhibitory effect against Gram-negative than against Gram-positive bacteria and the most useful concentration for use as an aid to the isolation of certain organisms was 0·25 per cent. In plate cultures on agar medium, the separation of *Diplococcus pneumoniæ* II from *Proteus mirabilis* J2, *Streptococcus* C–203 from *P. mirabilis* J2 and *Streptococcus* C–203 from *Pseudomonas æruginosa* K1 was accomplished in the presence of 0·25 per cent. of phenylethyl alcohol. The inhibitor caused changes in colony size and degree of hæmolysis especially when different kinds of blood were used in the agar medium, but organisms retained a recognisable morphology and did not appear to change genetically. Phenylethyl alcohol was applied successfully as an ærosol instead of being incorporated in the medium. G. B.

(ABSTRACTS *continued on p. 406*).

of ring structures. Sometimes the atoms of the ring are printed external to the ring itself, e.g., p. 194 benzene, p. 161 coumarone. Furthermore, there is a lack of consistency in presentation, e.g., benzyl acetate is printed as $C_6H_5{\cdot}CH_2{\cdot}C_2H_3O_2$ (p. 109) whereas ethyl acetate is presented more correctly as $CH_3{\cdot}COOC_2H_5$ on p. 385. The section on steroisomerism (p. 596) is particularly weak and somewhat misleading because of attempts at simplification and compression of material. However, these are only minor blemishes. Possibly the presence or absence of certain entries might be questioned. Nevertheless, when it is considered that the editor was faced with the great difficulty of keeping entries and information within the bounds of one volume, and yet giving adequate coverage and detail to keep the book valuable as a reference work, he and his collaborators must be congratulated on the overall result.

This new edition of "Kingzett" certainly fulfils the aims of its first author and founder in being a reliable reference work and a source of interesting and valuable information to layman and expert alike. Its quality will ensure that the book retains its international reputation and its place on the shelves and desks of all those who wish to be acquainted with any aspect of applied chemistry.

A. H. BECKETT.

(ABSTRACTS *continued from p. 404*).

Quaternary Ammonium Compounds with Bactericidal Properties. H. Sturm, E. Konermann, R. Aeschbacher and R. Gradmann. (*Industr. Engng Chem.* (*Anal.*), 1953, **45**, 186.) A number of quaternary ammonium compounds derived from such triethanolamine monoalkyl ethers as octyl, dodecyl, hexadecyl and octadecenyl are described. These quaternary ammonium salts dissolve readily in water, forming clear solutions with excellent foaming qualities. The benzyl bromide derivatives crystallise well and are not hygroscopic. They were tested for antibacterial power and the benzyl bromide salt of the triethanolamine monododecyl ether produced the best results. The results of bacteriostasis tests, incompatibility with soap tests and toxicity tests are also given. A. H. B.

Sodium Propionate, Inhibition of Growth of *Streptococcus fæcalis* by. C. H. Hill. (*J. biol. Chem.*, 1952, **199**, 329.) Inclusion of acetate as well as propionate ions in cultures of *Str. fæcalis* causes a complete reversal of the inhibition due to propionate, and it is therefore suggested that the effect of the latter may be to block the synthesis of acetate *via* the oxidative decarboxylation of pyruvate. It is further suggested that this blocking of acetate production may be brought about by combination of propionate with coenzyme A, which is essential for acetate production from pyruvate, as reported for *Clostridium kluyveri* by Stadtman. Pantothenic acid, a constituent of coenzyme A, when added to the medium increases the resistance of the organism to propionate, and the absence of pantothenate did not influence growth in the basal medium, indicating that *Str. fæcalis* must be capable of synthesising pantothenate and coenzyme A. The synthesis of pantothenic acid by *Str. fæcalis* was confirmed by assaying cultures with the medium of Skeggs and Wright (*J. biol. Chem.*, 1944, **156**, 21) with *Lactobacillus arabinosus*. J. B. S.