ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Ergot, North American, Alkaloids of. G. A. Svoboda, G. Shahovsky, A. T. Oliver, E. Diller, F. Barton and A. J. Barnes, Jr. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 257.) Rye, durum wheat and barley ergots from the Minnesota region were defatted with light petroleum and extracted with thiophene-free benzene. The extract was evaporated *in vacuo*, the amorphous base extracted with ether and the alkaloids precipitated with phthalic acid. Recrystallisation from acetone yielded material which was shown by identification of degradation fragments and by paper chromatography to be a mixture of ergotamine and ergocristine phthalates in solid solution. X-ray measurements, crystallographic data, optical rotation and counter-current distribution analyses in ethyl acetate/acetic acid (10 per cent.) indicated the presence of a mixture of phthalates of variable composition. Ergometrine was extracted from the samples as maleate. The mixed phthalate was fractionally crystallised from benzene, the ergotamine fraction being recrystallised as ethanesulphonate from acetone and the ergocristine fraction as phthalate from acetone. Similar results were obtained when Canadian and Japanese ergot of rye was examined. Samples of Spanish ergot did not yield any crystalline phthalate. G. B.

***Rauwolfia serpentina* Benth, Two New Alkaloids from.** N. Neuss, H. E. Boaz and J. W. Forbes. (*J. Amer. chem. Soc.*, 1954, **76**, 3234.) Two indole alkaloids were isolated by chromatography of the oleo-resin fraction from *Rauwolfia serpentina* on acid-washed alumina using benzene-chloroform mixtures as an eluant. The physico-chemical data for "alkaloid F," $C_{21}H_{24}N_2O_3$, and for "alkaloid A," $C_{22}H_{26}N_2O_4$ are recorded. "Alkaloid F" was identical in every respect (X-ray patterns, infra-red spectra, m.pt. and mixed m.pt.) with ajmalicine and *py*-tetrahydroserpentine of Klohs (Klohs *et al.*, *J. Amer. chem. Soc.*, 1954, **76**, 1332). "Alkaloid F," ajmalicine of Klohs (*loc. cit.*) and -δ yohimbine of Weisenborn (Weisenborn *et al.*, *Chem. Ind.*, 1954, **73**, 375) represent an anhydrous form of the *py*-tetrahydroserpentine of Bader (Bader and Schwarz, *Helv. chim. Acta*, 1952, **35**, 1594). The similarity of the physica properties of "Alkaloid A," substance I of Popelak (Popelak *et al.*, *Naturwissenschaften*, 1953, **40**, 625) and reserpine of Weisenborn (*loc. cit.*) and Schlittler (Schittler *et al.*, *Experimentia*, 1954, **10**, 133) indicates that these substances are identical. A. H. B.

ANALYTICAL

Amino-acids, Colorimetric Estimation of, on Paper Chromatograms. F. A. Isherwood and D. H. Cruickshank. (*Nature, Lond.*, 1954, **174**, 123.) The principle of the method is that the amino-acids are quantitatively converted on the paper into their yellow 2:4-dinitrophenyl derivatives by treatment with 2:4-dinitro-fluorobenzene, and these derivatives serve both to locate the position of the amino-acid and as a basis for the subsequent colorimetric estimation. A large excess of 2:4-dinitro-fluorobenzene is necessary

to ensure complete conversion of the amino-acids to their derivatives on the paper, which results in the formation of a large excess of 2:4-dinitrophenol. The latter, which would interfere with the colorimetric determination, is removed by dissolving the mixture in 91 per cent. w/w sulphuric acid and extracting this with benzene. In this strongly acid medium the dinitrophenyl amino-acids ionise as mono-acid bases and are insoluble in benzene. The acidic solution is then diluted to 30 per cent. and extracted with a mixture of *tert.*-amyl alcohol and benzene, and the extracted dinitrophenyl amino-acid finally transferred to an aqueous solution of sodium bicarbonate for colorimetric estimation. The amino-acids investigated were glycine, alanine, phenylalanine, and aspartic and glutamic acids. Quantities of the order of 6 μg. (as 3 μl. of 0.02M) of the amino-acids can be estimated to within $\pm$ 5 per cent. and the range of the method is from 0·5 μg. to 20 μg. A. H. B.

Benzylpenicillin in Crystalline Sodium Salts, Determination of, by Infra-red Spectroscopy. J. B. Jensen. (*Acta chem. scand*, 1954, **8**, 393.) A method is described for the compression at high pressure of finely ground potassium bromide to give discs with 70 to 80 per cent. transmittancy in the wavelength range 7 to 15 μ. The addition of a substance to the bromide before compression enables recordings of the infra-red spectrum of the substance in the solid state to be made; such recordings may be applied to quantitative determinations. The method was applied to the determination of benzylpenicillin in crystalline sodium salts, the measurement being referred to the specific maximum at 14·33 μ which is characteristic of benzylpenicillin. A. H. B.

Dicophane (*o, p'* and *pp'* isomers), Rhothane and Methoxychlor, Separation and Identification by Paper Chromatography. L. C. Mitchell. (*J. Assoc. off. agric. Chem., Wash.*, 1954, **37**, 530.) Dicophane, rhothane, and methoxychlor were separated by paper chromatography using soya bean oil as the immobile, and aqueous acetone as the mobile solvent. Dimethylformamide as the immobile and light petroleum as the mobile solvent were used for the separation of *o, p'* and *pp'* dicophane from each other and from rhothane and methoxychlor. Rhothane moved faster than methoxychlor in the dimethylformamide-petroleum system, whereas the reverse was true with soya bean oil-aqueous acetone. A. H. B.

Digoxin Preparations, Assay of. D. Banes. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 355.) For the estimation of digoxin, 5 ml. of an ethanolic solution containing about 0·25 mg. was evaporated on a water bath and cooled *in vacuo*. 4 ml. of freshly prepared alkaline solution of *m*-dinitrobenzene was added, the mixture allowed to stand for 15 minutes with frequent shaking, and measurements of the light absorption made at 615 mμ, using the reagent as a blank, until a maximum value was reached. The maximum was attained in 25 to 30 minutes at 22 to 25° C., and readings remained within 2 per cent. of the maximum value for the following 5 minutes. Only slight deviations from Beer's law were observed in the range 0·1 to 0·3 mg. The concentration of digoxin was calculated by reference to the absorbancy produced from a standard sample at the same time. The concentration of alkali being critical, it was necessary to remove stearic acid from tablets by a preliminary extraction procedure. Gitoxin, in concentrations of less than 8 per cent. in the sample, gave rise to no appreciable interference. For the determination of gitoxin in the presence of digoxin, a separation was carried out on a Celite column, using ethanol (50 per cent.) as the immobile solvent and *iso*-octane as mobile solvent. The quantity of

gitoxin was calculated from measurements of absorbancy at 530 mμ after treatment with Windaus-Schwarte reagent (sulphuric acid-ferric chloride solution). G. B.

Salicylic and Acetylsalicylic Acids, Spectrophotometric Determination of. R. B. Tinker and A. J. McBay. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 315.) Chloroform is used as solvent, since the absorption is greater and more stable in chloroform solutions than in ethanol or water. The following method is accurate to within 0·2 per cent. Shake a sample of crushed tablets of acetylsalicylic acid with chloroform, allow to stand and filter. Dilute the filtrate to obtain an approximately 0·36 per cent. w/v solution of acetylsalicylic acid, and determine the optical density at 308 mμ, the absorption maximum for salicylic acid, against chloroform as a blank. Dilute the solution 100-fold and repeat the determination at 278 mμ, the absorption maximum for acetylsalicylic acid. The content of acetylsalicylic and salicylic acid may be calculated with the aid of equations which take into account the absorption due to each component at both absorption maxima. G. B.

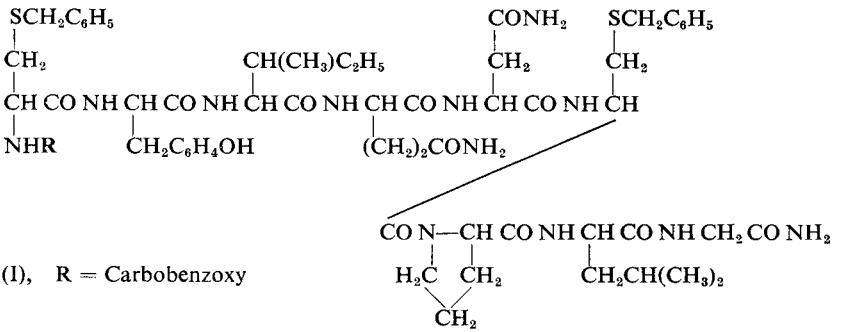
Salts, Slightly Soluble, Determination of, by Ion Exchange. E. Brochmann-Hanssen. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 307.) The following method may be employed. To a sample of slightly soluble salt and a weighed quantity of activated ion exchange resin, add water and shake on a mechanical shaker until the ion exchange reaction is complete. Filter and wash the resin with hot water. Titrate the filtrate and washings with 0·1N sodium hydroxide to determine the quantity of acid liberated from the sparingly soluble salt. With some compounds, hot water may be used or heat applied during the shaking process so as to complete the ion exchange reaction in a reasonable time. Dowex 5–X_2 resin, activated by treatment with 5N hydrochloric acid and washed with water may be used. This method has been applied successfully to a number of salts, using methyl red indicator for the titration of strong acid and phenolphthalein for mandelic, citric and salicylic acids. In titrating phosphoric acid, the first end-point (*p*H 4·5) was sharper than the second, but more liable to interference from common impurities such as chloride and sulphate. Potentiometric titration was successfully applied in this case. For the determination of calcium salts, ion exchange was more rapid than precipitation as oxalate and titration with permanganate, and more rapid than the ether extraction method for calcium mandelate. G. B.

Sodium *p*-Aminosalicylate, Potentiometric Method of Assay. J. R. Stockton and R. Zuckerman. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 273.) To 5 ml. of a solution in water, containing about 2 milliequivalents, add 10 ml. of a mixture of equal volumes of ethylene glycol and *iso*propanol, and titrate electrometrically with 0·4 to 0:1N perchloric acid in the ethylene glycol/*iso*propanol mixture. The quantity of sodium *p*-aminosalicylate is calculated from the quantity of perchloric acid required to titrate to the point of inflection of the titration curve. Samples of sodium *p*-aminosalicylate containing decomposition products (sodium bicarbonate and *m*-aminophenol) give titration curves with more than one point of inflection. The quantity of impurities, and of sodium *p*-aminosalicylate, may be calculated from the amounts of perchloric acid solution added at the points of inflection. The method is applicable to solutions of sodium *p*-aminosalicylate and to *p*-aminosalicylic acid. G. B.

Theophylline, Comparison of Spectrophotometric and the Silver Titration Methods for the Determination of. J. P. Comer and W. W. Hilty. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 287.) The theophylline content of samples was determined by measurement of the light absorption of solutions in 0·1N hydrochloric acid, using the datum that the absorbancy of pure anhydrous theophylline in a 0·001 per cent. w/v solution in 0·1N hydrochloric acid, measured in a 1 cm. cell against a 0·1N hydrochloric acid blank is 0·544. The method, applied to anhydrous theophylline, gave results in agreement with theory, whereas the results of the silver salt titration method of the U.S. Pharmacopeia XIV were about 1 per cent. low. Both methods were also applied to aminophylline powder, injection and tablets, to powder, tablets, capsules and suppositories of theophylline monoethanolamine and to capsules of theophylline monoethanolamine with amobarbital. The theoretical content of theophylline was not known accurately in these cases, but there was satisfactory agreement between the spectrophotometric and titration methods, except that the titration method gave variable results for suppositories. The spectrophotometric method was more rapid and capable of greater precision than the titration method. G. B.

ORGANIC CHEMISTRY

Oxytocin, Synthesis of. V. Du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts and P. G. Katsoyannis. (*J. Amer. chem. Soc.*, 1954, **76**, 3115.) A cyclic octapeptide amide (see *J. Pharm. Pharmacol.*, 1954, **6**, 349 (I)) having the hormonal activity of oxytocin was synthesised through the condensation of *N*-carbobenzoxy-*S*-benzyl-L-cysteinyl-L-tyrosine and the heptapeptide amide L-*iso*leucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide to yield the protected nonapeptide amide (I)



(I), R = Carbobenzoxy

followed by reduction with sodium in liquid ammonia and oxidation of the resulting sulphahydryl nonapeptide. The biologically active synthetic material was purified by countercurrent distribution and compared with natural oxytocin as to potency, specific rotation, partition coefficients, amino-acid composition, electrophoretic mobility, infra-red pattern, molecular weight, enzymatic and acid inactivation and chromatography on the resin IRC–50. The synthetic and natural oxytocin were also compared with respect to milk ejection and induction of labour in the human, as well as rat uterus contraction *in vitro*. The crystalline flavianates prepared from the synthetic and naturally occurring material had the same crystalline form, melting point and mixed melting point. All of these compounds afforded convincing evidence of the identity of the synthetic product with natural oxytocin. A. H. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Anticholinesterases, Hydroxypyridine and Hydroxyquinoline Phosphates as. K. J. M. Andrews, F. R. Atherton, F. Bergel and A. L. Morrison. (*J. chem. Soc.*, 1954, 1638.) The preparation of a number of dialkyl phosphoric esters of hydroxypyridines and hydroxyquinolines is described, and their toxicities and activities as inhibitors of "true" cholinesterase *in vitro* are recorded. The moderate anticholinesterase activity of the compounds was enhanced by conversion into quaternary salts. Crystalline 3-(diethoxyphosphinyloxy)-*N*-methylquinolinium methyl sulphate showed *in vitro* the highest activity so far recorded in the literature for a phosphorus compound, namely I 50 (red cell) $= 1{\cdot}5 \times 10^{-10}$. The hydrolysis of this compound was investigated. A. H. B.

2 : 4-Diaminopteridines as Folinic Acid Antagonists. H. O. J. Collier and M. Phillips. (*Nature, Lond.*, 1954, **174**, 180.) A series of 2 : 4-diaminopteridines have been examined as possible folinic acid antagonists. Tests were carried out in a sterile basal medium containing appropriate levels of synthetic folinic acid (5-formyl-5 : 6 : 7 : 8-tetrahydropteroylglutamic acid) sown with a standardised inoculum of washed cells of *Leuconostoc citrovorum*. After incubation the acid produced was titrated with 0·1N sodium hydroxide. Results were expressed in terms of the concentration of diaminopteridine required to inhibit acid production by 50 per cent., from which, an inhibition factor was calculated as the molar ratio of diaminopteridine to folinic acid in the medium. For 6 : 7-di*iso*propyl-2 : 4-diaminopteridine the inhibition factor was constant over a small range of folinic acid concentrations, indicating that inhibition was competitive. Potency as folinic acid antagonists is related to the nature of the 6 : 7-substituents. Peak activity in the dialkyl series was reached in the di-*sec.* butyl and di*iso*propyl derivatives and in the indole series at *N*-*n*-propyl. Activity was slight in 6 : 7-dimethyl- and 6 : 7-diphenyl-2 : 4-diaminopteridine and not evident at all in unsubstituted 2 : 4-diaminopteridines. Omission of one amino group from the 6 : 7-dialkyl type of compound investigated reduced antifolinic acid activity. In this series no correlation was observed between antifolinic acid activity and ability to suppress *Plasmodium berghei* infections in mice. J. B. S.

Nidulin and Nornidulin ("Ustin"), Chlorine-containing Metabolic Products of *Aspergillus nidulans*. F. M. Dean, J. C. Roberts and A. Robertson. (*J. chem. Soc.*, 1954, 1432.) A method for the isolation of nidulin and nornidulin from a strain of *Aspergillus nidulans* is described. Nidulin is a colourless, optically inactive compound, m.pt. 180° C., $C_{20}H_{17}O_5Cl_3$, containing a methoxyl and a hydroxyl group; it readily yields a mono-acetate and a monomethyl derivative. Nidulin was shown to be a methyl ether of nornidulin and the compounds are considered to be chlorinated depsidones. Partial structural formulæ for nidulin and nornidulin are proposed. Nidulin is the first recorded depsidone to be identified as a metabolic product of a fungus grown on a synthetic medium, although compounds of this type are well known as constituents of lichens. A. H. B.

Rutin and Quercetin, Absorption and Metabolism of, in the Rabbit. C. W. Murray, A. N. Booth, F. DeEds and F. T. Jones. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 361.) After the administration of rutin or quercetin by

mouth to rabbits, phenolic substances capable of forming indophenols with 2:6-dichloroquinonechloroimide appeared in the urine. At least 4 substances not present in normal rabbit urine were detected by paper partition chromatography. Crystals of 3:4-dihydroxyphenylacetic acid were isolated from the urine, and shown by mixed melting point, X-ray diffraction and spectrophotometry of the indophenol, to be identical with 3:4-dihydroxyphenylacetic acid prepared synthetically by demethylation of dimethoxyphenylacetic acid. The quantity of the substance found in the urine accounted for about 25 per cent. of the quercetin administered. G. B.

Salicylhydroxamic Acid, Enzymatic Conversion of, to Salicylamide. J. Lowenthal. (*Nature, Lond.*, 1954, **174**, 36.) In the rat salicylhydroxamic acid is partly metabolised to salicylamide. This paper reports the enzymatic transformation *in vitro*. Rat liver extracts were incubated with sodium salicylhydroxamate, then centrifuged and the supernatant investigated by paper chromatography using a descending system with 1·5N ammonium hydroxide: *n*-butanol (1:1) as the solvent system. Under ultra-violet light, salicylic acid and salicylamide show light blue, while salicylhydroxamic acid shows dark blue. The compounds also give characteristic colours when sprayed with ferric ion. The transformation of salicylhydroxamic acid may be brought about either by a direct enzymatic reduction of the hydroxylamino group or by an exchange reaction in which the hydroxylamino group is exchanged with ammonia or the amide group of glutamine, leading to the formation of salicylamide and hydroxylamine or glutamohydroxamic acid respectively. In these experiments no evidence for the formation of hydroxylamine or glutamohydroxamic acid could be obtained. G. F. S.

Tabun, Enzymatic Hydrolysis of. K-B. Augustinsson and G. Heimbürger. (*Acta chem. scand.*, 1954, **8**, 753, 762.) Hydrolysis of tabun (dimethylamidoethoxyphosphoryl cyanide) by blood plasma is an enzymatic process, the enzyme concerned being designated "tabunase." The enzymic activity of tabunase is not specific for tabun, other organophosphorus compounds being hydrolysed as well. The rate of hydrolysis is proportional to plasma concentration. Rabbit plasma is about 10 times more active than human plasma. Of various rabbit tissues examined as an enzyme source, the adrenal glands (cortex) are most active; kidney and liver are almost as active. Tissues known to contain acetylcholinesterase (brain, spinal cord) are weakly active. Hydrolysis of tabun by plasma or tissue tabunase causes immediate liberation of carbon dioxide from the bicarbonate buffer present, due to the liberation of dimethylamidoethoxyhydroxyphosphine oxide. Hydrocyanic acid is also formed. No release of inorganic phosphate or of dimethylamine was observed. Cyanide and dimethylamidoethoxyhydroxyphosphine oxide both exhibit weak inhibitory actions on tabunase, but have no similar action on acetylcholinesterase. Stability of purified tabunase preparations is greatest around *p*H 7 and decreases in both acid and alkaline media. The Michaelis constant for the tabunase of horse plasma (which contains a variety of esterases including cholinesterase) was found to be $3{\cdot}45 \times 10^{-3}$. A preparation free from cholinesterase had a constant $2{\cdot}15 \times 10^{-3}$. The mechanism of the enzyme-catalysed hydrolysis of tabun is discussed. Experiments with pig and bovine tissues suggest that tabun and di*iso*propyl phosphorofluoridate are split by the same enzyme of kidney and adrenal glands. J. B. S.

Thromboplastin Activity of Bovine Thoracic Aorta. L. L. Campbell. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 359.) Extracts of bovine aorta were prepared and prothrombin times determined using Quick's one stage method. An extract of bovine aorta prepared with normal saline and clarified by centrifuging was as effective as rabbit brain or bovine lung thromboplastin. Material prepared in this way was free from blood cells and plasma constituents which are common contaminants of other forms of thromboplastin. It was shown that the extracts may be preserved with 0·5 per cent. of phenol. Aortas were separated into their 3 anatomical layers, when the greatest thromboplastin activity was found in the outer layer and the least in the intima. G. B.

BIOCHEMICAL ANALYSIS

Nucleic Acid Fragments in Tissues, Extraction and Assay of. G. D. Dorough and D. L. Seaton. (*J. Amer. chem. Soc.*, 1954, **76**, 2873.) A method for the extraction, separation, identification and assay of various nucleotides, nucleosides, purines and pyrimidines occurring in tissues in a loosely bound or uncombined state is described. It consists of homogenising an appropriate sample of the tissue for 20 minutes at 0° C. with an excess of *N N'*-dimethylformamide, centrifuging and evaporating the solution, under reduced pressure, to dryness. After a purification step to remove fatty material, the extracted compounds are separated by two-dimensional paper chromatography, using three developing solvents in place of the usual two. The individual spots are identified by reference to known substances chromatographed in an identical way, and by a comparison of the ultra-violet absorption spectra of the eluates of the spots in acidic and basic media with the corresponding spectra of known compounds. The method was applied to an investigation of the nucleic acid fragments present in 9 normal and 4 cancerous Swiss mouse tissues, and the results are tabulated. Data are shown for tissue samples extracted immediately after removal of the tissues from the animal and for tissue samples extracted after an autolysis period of 4 hours at room temperature.

A. H. B.

Vitamin B_{12} in Complex Extracts, Microbiological Assay of. J. Beck. (*Ann. pharm. franç.*, 1954, **12**, 132.) A microbiological method is described which measures vitamin B_{12} independently of other growth factors which may be present in liver extracts. The sample for assay is divided into two parts, one of which is heated in an autoclave in the presence of sulphuric acid to destroy vitamin B_{12} and peptide growth factors. After neutralisation, a known quantity of pure cyanocobalamin is added and the product used as the standard of comparison to determine the activity of the other part of the sample, by a turbidimetric method using *Lactobacillus leichmannii* 7830/313 as the test organism. The sample may then be submitted to alkaline hydrolysis which destroys only vitamin B_{12} activity so that the activity due to peptides may be measured. The method becomes very sensitive if a 3- to 4-hour culture of the organism is employed, the sample being autoclaved separately to release vitamin B_{12} from its conjugated forms before being added to the test culture. The agar diffusion method, employing a mutant strain of *Escherichia coli*, may be employed for cyanocobalamin solutions, but is not suitable for liver extracts. G. B.

CHEMOTHERAPY

Azomethines, Fungistatic Activity of. W. M. Farrow, C. Hanna and F. W. Schueler. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 370.) A series of 6 Schiff's bases derived from aniline or *p*-aminobenzoic acid were tested for fungistatic activity against *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Trichophyton mentagrophytes*, *Sporotrichum Schenkii*, *Candida albicans* and *Aspergillus amstelodami*. The organisms were grown on plates of Sabouraud's agar and the concentration of azomethine determined which, added to the medium, caused 50 per cent. inhibition in the growth of the colonies in 16 days at room temperature. Solutions for the test were prepared with the aid of ethanol or sodium bicarbonate. The fungistatic effect of the constituent aldehydes and amino compounds was also studied. Toxicity tests were carried out by oral administration to mice, and, in the case of the aldehydes, by injection in propylene glycol solution. 3:4-Methylenedioxybenzylidene-4-carboxyaniline, the anil of piperonal and *p*-aminobenzoic acid, showed a high fungistatic activity and low toxicity. Experiments with this compound suggested that the constituent active against *Blastomyces dermatitidis*, *Histoplasma capsulatum* and *Trichophyton mentagrophytes* is the aldehyde liberated by breakdown of the Schiff's base, rather than other possible degradation products such as *p*-aminobenzoic acid, piperonylic acid, *N*-phenyl-3:4-methylenedioxybenzylamine and aniline. This does not seem to be the case for the other organisms which do not show the same pattern of response. G. B.

β-Diethylaminoethyl Esters of Substituted Acetic and Glycidic Acids as Antispasmodics. F. F. Blicke, J. A. Faust and H. Raffelson. (*J. Amer. chem. Soc.*, 1954, **76**, 3161.) Esters of the general formula $C_6H_5CHXCOOCH_2$-$CH_2N(C_2H_5)_2$, in which X represents chlorine, bromine, dimethylamino, piperidino or morpholino, were prepared. In addition, β-dimethylaminoethyl esters of the following acids were synthesised: diphenylchloroacetic, diphenylbromoacetic, diphenylethoxyacetic, diphenyl-(dimethylamino)-acetic, α β-diphenylglycidic and α-methyl-β-phenylglycidic acids. All of the esters, tested against acetylcholine-induced spasm on an isolated intestinal strip, were ineffective in a dilution greater than 1:1,000,000 with the exception of β-diethylaminoethyl diphenylchloroacetate hydrochloride, and the corresponding bromo derivative, which were active in a dilution of 1:50,000,000 and 1:63,000,000 respectively. It is probable that the chloro- and bromo- acetates are rapidly converted to the benzilate and that the high activity found was due to the presence of this latter ester. A. H. B.

Diphenylacetates and *p*-Aminobenzoates of Basic Alkoxyalkanols, as Antispasmodics. F. F. Blicke and J. H. Biel. (*J. Amer. chem. Soc.*, 1954, **76**, 3163.) Diphenylacetates of the following six alcohols were prepared. 1. 2-(2′-diethylaminoethoxy)-ethanol; 2. 1-dimethylamino-3-methoxy-2-propanol 3. 2-diethylamino-3-methoxypropanol; 4. 2-methoxy-3-diethylaminopropanol; 5. 1:3-dimethoxy-4-diethylamino-2-butanol; and 6. 1-diethylamino-3:4-dimethoxy-2-butanol. The maximum effective dilutions of the compounds on the isolated rabbit intestine against acetylcholine-induced spasm were 1:1,500,000; 1:150,000; 1:300,000; 1:1,500,000 and 1:300,000 respectively. A. H. B.

5-Heptyl-2-thiohydantoin, A New Antitubercular Agent. E. Froelich, A. Fruehan, M. Jackman, F. K. Kirchner, E. J. Alexander and S. Archer. (*J. Amer. chem. Soc.*, 1954, **76**, 3099.) Because of the significant therapeutic effect of 5-hexyl-2-thiohydantoin when tested in mice infected with *Myco. tuberculosis* H37Rv., a number of related compounds were prepared. Small structure changes profoundly affected the antitubercular activity. Maximum activity in the 5-alkyl-2-thiohydantoin series was attained with 5-*N*-heptyl-2-thiohydantoin. The effectiveness fell precipitiously as the 5-alkyl chain was lengthened. Doses of the order of 200 to 300 mg./kg./day of 5-*n*-heptyl-2-thiohydantoin were required to protect all mice infected intravenously with *Myco. tuberculosis* from the lethal effects of the disease. This was true also when a strain highly resistant to streptomycin was used to infect the animals. Post-mortem examination of the surviving mice revealed little if any tuberculous pathology. When the drug was given to hamsters (0·1 per cent. in the diet) a therapeutic effect equivalent to that obtained with 0·4 per cent. of *p*-aminosalicylic acid was achieved. Extensive acute and chronic toxicity studies on rodents, dogs and monkeys showed that the drug is well tolerated in these species. A. H. B.

Sulphathiazoles, Nuclear-substituted, Bacteriostatic and Biological Activity of. S. F. Quan, T. C. Daniels and K. F. Meyer. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 326.) 2′-Methylsulphathiazole,3′-methylsulphathiazole,2′-chlorosulphathiazole, 3′-azobenzenesulphathiazole, 2-(3-azo-*o*-nitrophenylsulphanilamido)thiazole and 2-(3-azo-*m*-nitrophenylsulphanilamido)thiazole were tested *in vitro* against *Pasteurella pestis* by a serial broth dilution method. All were at least as active as sulphathiazole. When the experiments were repeated using *Bacillus subtilis* as the test organism, it was demonstrated that the antibacterial activity of all these compounds is antagonised by *p*-aminobenzoic acid, in contrast to 2-nitrothiophene-4-sulphonamide and 2-aminothiophene-4-sulphonamide, the activity of which is unaffected by the presence of *p*-aminobenzoic acid. All the derivatives proved to be more toxic than sulphathiazole, as assessed by injection into the tail vein of mice. None of the derivatives was more effective than sulphathiazole in the treatment of plague, streptococcal and staphylococcal infections in mice. The hypothesis that the bacteriostatic activity of sulphonamides is directly related to resonance and the contribution of the co-planar forms may have to be modified to account for the activity of these nuclear-substituted compounds. G. B.

PHARMACY

NOTES AND FORMULÆ

Body Temperature, Measurement of. L. C. Clark and H. Trolander. (*J. Amer. med. Ass.*, 1954, **155**, 251.) An instrument is described consisting of a thermistor which changes rapidly in electrical resistance with small changes in temperature, a source of current, and a device to measure the flow of current which reads directly in temperature. The temperature-sensitive thermistor element is mounted at the end of a 6-ft. wire and is sealed and waterproof; it is designed to operate linearly from 20° C. (68° F.) to 41° C. (105° F.). The electrical circuit consists of a balanced Wheatstone bridge, the thermistor bead having a resistance of 2,625 ohms at 20° C. and 1,115 ohms at 40° C.; the unit was calibrated in a water bath against a Bureau of Standards thermometer, the readings differing from the standard by less than 0·1° C. The response time of the instrument is related to the thickness of the coating on the thermistor bead.

R. E. S.

Dihydroxy Aluminium Aminoacetate, The Efficiency of, in Phosphate Insolubilisation. R. S. Murphey and D. L. Kendrick. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 304.) 4 tablets of dihydroxyaluminium aminoacetate were powdered and added to 150 ml. of a solution of hydrochloric acid with pepsin, stirred and maintained at 98·6° F. At 10 minute intervals up to 2 hours, a 20-ml. sample was withdrawn and replaced by an equal volume of acid-pepsin solution. The 20 ml. samples and the final solution remaining were each treated with a known excess of potassium dihydrogen phosphate, adjusted to *p*H 6·9, filtered to remove precipitated phosphate, and the filtrate examined spectrophotometrically to determine the phosphate content. Dihydroxyaluminium aminoacetate was more effective in rendering phosphate insoluble than aluminium hydroxide gel, hydrated alumina or aluminium carbonate preparations. Dihydroxy aluminium aminoacetate removed from solution 96 per cent. of its theoretical equivalent of phosphate, compared with only 43 per cent. for hydrated alumina. In one human subject on a normal diet, a substantial reduction in phosphate excretion was achieved with a dosage of 16 tablets of 0·5 g. of dihydroxyaluminium aminoacetate/day. G. B.

Enteric Coatings, Evaluation of, Using the White Rat. F. C. Hammerness and C. H. Waldon. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 357.) White rats on a normal diet were given pills containing a suitable dye (phenylazodiaminopyridine hydrochloride) and the time at which the dye first appeared in the urine was observed. After an interval of 3 days to ensure that the dye had been completely eliminated from the blood of the rats, the experiments were repeated with enteric coated pills. As a result of similar experiments in human subjects, confirmed by X-ray experiments using barium sulphate pills, it was established that an enteric coating can be considered satisfactory if the dye appears in the rats' urine about 3 hours later than for the uncoated pill. Cellulose acetate hydrogen phthalate coatings were generally satisfactory, whereas cellulose acetate stearate coated pills usually passed into the large intestine without disintegrating. G. B.

Methylergonovine Tartrate (Methergine Tartrate.) (*New and Nonofficial Remedies*; *J. Amer. med. Ass.*, 1954, **154**, 834.) Methylergonovine tartrate is *N*-(2-hydroxymethylpropyl)-*d*-lysergamide tartrate containing two molecules of methanol of crystallisation. It occurs as a white to pinkish-tan, odourless, bitter, microcrystalline powder, very soluble in water, freely soluble in ethanol, and very slightly soluble in chloroform and ether; *p*H of a 0·02 per cent. solution, 5·0 to 5·8. It must be protected from light and heat. Aqueous solutions exhibit a blue fluorescence under ultra-violet light and yield a deep blue colour with *p*-dimethylaminobenzaldehyde. When a solution in water is treated with a mixture of glacial acetic acid and ethyl acetate and superimposed on sulphuric acid, a bluish-purple ring appears at the zone of contact. A 1 per cent. solution in water exhibits a specific rotation, at 25° C., of + 50° to + 60°, and a 0.002 per cent. solution in sulphuric acid (1 per cent.) exhibits an ultra-violet absorption maximum at about 310 mμ($E^{1\ \text{per cent.}}_{1\ \text{cm.}}$, about 191), and a minimum at about 270 mμ. Methylergonovine tartrate loses not more than 5·0 per cent. when dried in a vacuum desiccator over phosphorus pentoxide for 4 hours; and yields not more than 0·05 per cent. of sulphated ash. It contains 95·0 to 105·0 per cent. of methylergonovine tartrate when assayed by measuring the absorption, at 310 mμ, of a 0·002 per cent. solution in sulphuric acid (1 per cent. v/v). It also contains 74·5 to 77·5 per cent. of methylergonovine, equivalent to 98·0 to 102·0 per cent. of methylergonovine tartrate, when assayed in the following manner.

A solution in water is treated with ammonia and brine and extracted with ether, the ether is removed, and the residue dissolved in sulphuric acid and back titrated with alkali, bromophenol blue being used as indicator. G. R. K.

Riboflavine, Physiological Availability of, in Relation to the *in vitro* Disintegration Time of Sugar-coated Tablets. D. G. Chapman, R. Crisafio and J. A. Campbell. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 297.) The disintegration time of sugar-coated tablets was measured by a modification of the U.S. Pharmacopeia method. It was found advantageous to use artificial gastric and alkaline juices in the test, and to introduce a rubber disc into the apparatus so as to subject the tablets to a slight rubbing action. In practice, if the tablets did not disintegrate within 30 minutes at 37° C., they were removed from the gastric juice and placed in alkaline juice until disintegration was complete. To determine the availability of riboflavine from tablets swallowed whole, tests on human volunteers were made, the excretion of riboflavine being determined by analysis of the urine. An 8-hour test was more convenient than the 24-hour test, and equally satisfactory. The use of special diets in the test was not necessary. A relationship was established between disintegration times of the tablets and physiological availability of the riboflavine contained in them. In general, tablets exhibiting disintegration times less than 60 minutes were satisfactory sources of riboflavine. Those with longer disintegration times were not satisfactorily utilised by the experimental subjects, except for a few varieties of tablet from which riboflavine could be leached without disintegration. G. B.

PHARMACOGNOSY

***Datura tatula* L., Effect of Chemicals on the Alkaloidal Yield of.** J. L. Beal, B. V. Christensen and A. B. Colby. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 282.) Plants were grown under field conditions, and treated with chemical solutions 7, 8 and 9 weeks from the seedling stage. Samples were harvested at 8, 10 and 13 weeks, the last corresponding to the fruiting stage. In the case of plants treated with zinc insulin, the final harvest was taken at 16 weeks, as the plants matured more slowly. The solutions investigated were (1) naphthoxyacetic acid 0·5 mg., tween 20 2 ml., water to 200 ml., applied to the soil; (2) zinc insulin 0·01 per cent., tween 20 1 per cent., ethanol 0·2 per cent. in Clark and Lubs buffer, *p*H 3 and (3) diethylstilboestrol 0·01 per cent., tween 20 1 per cent., ethanol 0·1 per cent. in water, sprayed on the foliage. Control solutions, omitting the naphthoxyacetic acid, zinc insulin and diethylstilboestrol were also used. At the third harvest, the leaves and stems of plants treated with naphthoxyacetic acid contained a significantly higher concentration of alkaloid than those treated with the control solution, Treatment with zinc insulin solution also increased the percentage of alkaloid at the third harvest. Tween 20 solution appeared to increase the dry weight of the plants. Solutions of diethylstilboestrol did not affect the dry weight or alkaloidal yield of the plants. G. B.

Mustard, Stability of. O. Weis-Fogh. (*Dansk Tidsskr. Farm.*, 1954, **28**, 70.) Whole black mustard does not undergo any appreciable loss of sinigrin on storage, except under conditions of high humidity. The powdered drug is only stable if the humidity of the air is below 60 per cent., defatted material being more sensitive to moisture than the natural powdered drug. Heat treatment at 80° C. for 8 hours does not produce complete destruction of the enzyme. The conclusion is that black mustard seed should be kept in the whole condition, while the powdered seed, preferably not defatted, may be kept for a limited period at a relative humidity of less than 60 per cent.

G. M.

PHARMACOLOGY AND THERAPEUTICS

N-Allylnormorphine, Pharmacology of. C. A. Winter, P. D. Orahovats, L. Flataker, E. G. Lehman and J. T. Lehman. (*J. Pharmacol.*, 1954, **111**, 152.) This paper describes the pharmacological properties of *n*-allylnormorphine (nalorphine), with particular reference to its antagonistic actions of morphine, methadone, pethidine and other analgesic drugs. The intravenous LD50 in mice was 190 mg./kg., but commercial solutions were more toxic (67 mg./kg.) due to the presence of a citrate buffer. Nalorphine in doses up to 30 mg./kg. in dogs and 64 mg./kg. in rats had no analgesic action. Doses of nalorphine previously reported to antagonise morphine in mice and rats were unnecessarily high, and 0·5 mg./kg. of nalorphine counteracted a dose of 2·0 mg./kg. of *dl*-methidone in rats. Nalorphine, in rats, was shown to effectively antagonise a wide range of analgesic drugs chemically related and unrelated to morphine, including codeine, pethidine, diacetylmorphine, dihydromorphinone, *l*-isomethadone, 3-hydroxy-*n*-methylmorphinan, α-1,3-dimethyl-4-phenyl-4-propionoxypiperidine (prisilidine) and *n*-methyl-Δ^6-dehydro*iso*morphinan. The side effects of the analgesic drugs as seen in rats—hypnosis, catalepsy and respiratory depression—were absent or greatly reduced. Nalorphine antagonised the effects of pethidine in rats, dogs and rabbits. It also antagonised the excitatory action of morphine in cats. Nalorphine had only a very weak atropine-like action (1/40,000th the activity of atropine) on the isolated ileum of the guinea-pig and the rabbit. G. F. S.

Antihistamine Potentiation of Pentobarbital Anæsthesia. H. Lightstone and J. W. Nelson. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 263.). A number of antihistamine drugs, including diphenhydramine, tripelennamine, phenindamine, chlorphenamine and bromazine were administered subcutaneously to rats. After 30 minutes, 60 mg./kg. of pentobarbital was given intraperitoneally, and the duration of sleep measured, the end-point being taken as the time of return of the righting reflex. All the drugs tested were found to prolong the sleeping time, but there was no correlation observed between prolongation of sleep and other common side-effects of the antihistaminics. A threshold blood level of pentobarbital was necessary before an altered response could be obtained with antihistamine drugs. In experiments with bromazine, analyses of the tissues suggested that the drug did not interfere with the detoxification of pentobarbital in the liver, but caused an increase in the rate of entry of the barbiturate into the brain. G. B.

Autonomic Ganglion Stimulants. N. Ambache. (*Arch. int. Pharmacodyn.*, 1954, **97**, 427.) It has been possible to show, where a parasympathetic ganglion is a discrete anatomical structure (e.g., the ciliary ganglion), that the responses to drugs are similar to those of a sympathetic ganglion (Perry and Talesnik, *J. Physiol.*, 1953, **119**, 43). Perfusion of such a ganglion can separate "neuronal" from direct actions of drugs on the innervated structures. However, where the ganglia are in close connection with the effector cells, as in the intestinal ganglia, pharmacological means are necessary for differentiating sites of action. Two procedures were used. In the older method, *Clostridium botulinum* toxin type A was injected subperitoneally around a segment of rabbit gut *in vivo* and the responses of this segment when isolated 2 to 4 hours later were compared with those of a similar segment removed from the rabbit just before injection. *m*-Bromophenyl ether of choline, a strong ganglionic stimulant, and nicotine both contracted the control segment, but relaxed the poisoned segment. The

inhibitory effects of both drugs were blocked by ephedrine (used here as an adrenergic blocking agent) and by hexamethonium, suggesting that the drugs were stimulating adrenergic neurones, this action being unmasked by the elimination of cholinergic effects by the toxin. In the second technique guinea-pig ileum was exposed to type D toxin *in vitro* for 5 to 20 minutes. This method is more satisfactory since the same piece of gut can act as control. Also, the type D toxin is thought to be inactive by mouth in man. The nicotine and *m*-bromophenyl ether of choline contractions as well as those of 1 : 1 dimethyl-4-phenylpiperazinium were abolished or reversed after the toxin, while those of muscarine were relatively unaffected. The responses to 2268F and to 5-methyl-furfuryltrimethyl ammonium were first slightly depressed, but subsequently were greater than the control. The toxin also reduced the effects of barium chloride, potassium chloride and eserine, the barium action in small doses being almost entirely "neuronal" in the guinea-pig. G. P.

Chlorpromazine in Neuropsychiatric Disorders. N. W. Wilkelman. (*J. Amer. med. Ass.*, 1954, **155**, 18.) 142 patients received chlorpromazine for periods varying from 2 to 8 months. In general the symptom complex of the psychoneurotic patients was one of anxiety, palpitations of the heart, insomnia, profuse sweating, urinary frequency, decrease or loss of libido, gastro-intestinal disturbances, dizziness, headaches, generalised aches and pains, and, in many cases, an obsessive-compulsive-phobic syndrome A senile group of 27 patients showed all degrees of agitation with behaviour problems, restlessness and insomnia. In out-patients and the agitated senile patients therapy was begun with an oral dose of 75 mg. (25 mg. with each meal), the dosage being gradually increased after the first week of treatment until clinical improvement was obtained or excessive drowsiness occurred. Patients received the drug for 8 to 32 weeks and could be satisfactorily maintained on doses of from 30 to 150 mg. a day. Psychotic patients in hospital were given 25 mg. intramuscularly 4 times daily for the first 2 or 3 days, and then 50 mg. intramuscularly 4 times daily, the patient being maintained in a resting or somnolent state. Of 67 patients with severe anxiety reactions, 56 showed improvement ranging from moderate to complete relief of symptoms; 5 of 6 patients with phobic reactions and 6 of 8 patients with obsessive thinking, obtained moderate to complete relief; 21 of the 27 agitated senile patients became markedly improved; 2 of 6 patients with epilepsy were greatly benefited; and in 6 patients with paralysis agitans no significant change was observed. With short-term therapy the beneficial effect of chlorpromazine lasted for 2 to 4 days after administration was stopped; with long-term therapy (3 to 6 months) the beneficial effects persisted after therapy had been discontinued for 2 to 5 months. The main side-effects included drowsiness (in almost all patients), dryness of mouth (20 per cent.), bad or bitter taste in the mouth (10 per cent.), increased frequency and intensity of dreaming (15 per cent.); jaundice developed in 3 patients, and there was a fall in blood pressure in 7 out of 8 patients after an intramuscular injection of 50 mg. Two-thirds of the patients experiencing drowsiness became tolerant to this side-effect. Although the mode of action of the drug has not been clearly defined, it would appear that it interferes with synaptic transfer of excessive psychomotor excitement between cortical areas and the diencephalon, so that conscious perception of, and pattern of reaction to, personality-disturbing stimuli are altered. S. L. W.

Chlorpromazine in the Management of Severe Pain. M. S. Sadove, M. J. Levin, R. F. Rose, L. Schwartz and F. W. Witt. (*J. Amer. med. Ass.*, 1954, **155**, 626.) When given chlorpromazine with narcotics or sedatives that

previously proved ineffective for relieving pain, 22 of 28 patients obtained satisfactory relief from severe abdominal, bone and neuritis pain associated with malignant lesions. The chlorpromazine was administered in doses of 25 mg. orally from 2 to 4 times daily, or in doses of 25 or 50 mg. intramuscularly 4-hourly. It seems that the enhanced analgesia with chlorpromazine is due, in part, to chlorpromazine's ability to alter the patient's reaction to pain. With the addition of chlorpromazine some patients could be made comfortable while receiving a lower dosage of narcotics, while others could be made comfortable while receiving less potent narcotics than they had previously required. Since chlorpromazine also has anti-emetic properties it serves a dual purpose when nausea and vomiting accompany conditions of pain. Side-effects consist principally of drowsiness, dryness of the mouth, pyrosis, and mild hypotension. Its use is contraindicated in comatose states caused by barbiturates, opiates, alcohol and other central nervous system depressants. S. L. W.

Ergotamine Tartrate and Caffeine in Relief of Migraine. J. R. Graham. (*New Engl. J. Med.*, 1954, **250**, 936.) An alternative means of taking ergotamine tartrate which avoids the inconvenience of injections and the nausea following oral use is the rectal administration of suppositories containing ergotamine tartrate 1 mg. and caffeine alkaloid 100 mg. The suppository is inserted at the beginning of an attack. Some variation in the dosage might be required to suit the needs of individual patients. In 577 attacks of migraine in 100 patients the suppositories produced good results in 423 (73 per cent.), fair results in 101 (17 per cent.) and poor results in 53 (10 per cent.). In only 30 cases (5 per cent.) were unpleasant side-effects reported, and with 2 exceptions these were only mild or moderately severe. The side-effects consisted of nausea or vomiting, muscle cramps, lassitude and prostration. A few patients suffered from nervousness, tremor and insomnia, and in these cases it was necessary to omit the caffeine. A more convenient mode of administration is the rectal use of tablets containing these medicaments, though the results obtained are not so good as with the suppositories. S. L. W.

Ethanol, Metabolism of. L. C. Schroeter and A. G. Zupko. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 270.) To determine the effect of ethanol on the deposition of cholesterol *in vitro*, sections of hog aorta were immersed in 0·47, 0·94, 1·7 and 30 per cent. ethanol. This preliminary treatment did not affect the deposition of cholesterol from a carbon-pigmented suspension, applied after treatment of the sections with a 3 per cent. solution of lead acetate. Groups of rats were fed by stomach tube with 7 ml. of 50 per cent. ethanol, alone or accompanied by 1 g. of glucose or fructose. After 45 minutes the rats were decapitated and samples of kidney were found to contain more citrate than those in an untreated control group. Normal alcoholaemic curves were established after the administration of 0·75 g./kg. of ethanol (25 per cent.) to a number of rabbits. After the distribution of ethanol was complete, its elimination from the blood was an approximately linear function in all cases. Adenosine triphosphate and fructose accelerated the metabolism of ethanol, but chlorophenamine, propantheline and methylpentynol had no significant effect. G. B.

Gentisic Acid, Pharmacological Tests upon Derivatives of. W. E. Moore, F. W. Bope and B. V. Christensen. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 334.) The acute toxicity of 7 esters of gentisic acid was determined by

intraperitoneal injection into mice. 2-Butylaminoethyl gentisate, 3-(1-methylpiperidyl)methyl gentisate, 2-dimethylaminoethyl gentisate and 2-diethylaminoethyl 5-ethoxysalicylate were administered as the hydrochlorides, and 5-diphenylacetylgentisic acid, 5-diethylacetylgentisic acid and 5:5′-digentisyl phthalate as the soluble sodium salts. All these esters were more toxic than gentisic acid. Three of the compounds caused convulsions preceding death, and the remainder showed no anticonvulsant activity in mice treated with leptazol. The four esters of gentisic acid with aminoalcohols were tested for antispasmodic activity on an isolated strip of rabbit intestine. Only 2-diethylamino 5-ethoxysalicylate hydrochloride showed an appreciable effect, which was somewhat less than that of papaverine hydrochloride. Anæsthetic activity was assessed by the time taken for a solution under test to block the motor response to a given electrical stimulus applied to the spinal end of the frog sciatic nerve. 2-Diethylaminoethyl 5-ethoxysalicylate was slightly less active than procaine. The other compounds tested showed only slight anæsthetic activity. G. B.

Hydroxyphenyltrialkylammonium Compounds, Anti-curare Action of, in Avian Muscle. E. W. Pelikan, C. M. Smith and K. R. Unna. (*J. Pharmacol.*, 1954, **111**, 30.) Avian skeletal muscle exhibits a dual response to neuromuscular blocking agents of the decamethonium type. These agents cause a contracture of the muscle, in addition to the usual "depolarisation block" of neuromuscular conduction. Curare-like agents do not produce this contractural response. Since the mode of action of the phenyltrialkylammonium compounds in antagonising the effects of *d*-tubocurarine at the motor end-plate has been considered by some to be a direct effect on the end-plate and by others to be due to an anticholinesterase action, an attempt was made to establish a correlation between anti-curare action and contractural response. White Leghorn chickens were used, the semi-isometric twitch response of the gastrocnemius muscle to single maximal stimuli and short tetani applied to the sciatic nerve being recorded, together with the ischiadic arterial blood pressure. Drugs were injected into either the superficial wing veins or the external jugular veins. With decamethonium-like substances there was little correlation between the dose-effect curves for the degree of neuromuscular blockade and for the contracture of the muscle. Also, tetramethonium induced a contracture in the muscle, although it apparently blocked the muscle twitch competitively rather than by depolarisation, since tensilon could easily reverse it and *d*-tubocurarine increased the degree of block. The *m*-substituted phenyltrialkylammonium compounds when given alone in doses which effectively antagonised *d*-tubocurarine block, increased the twitch response to single stimuli and decreased the tetanic response. Such doses did not produce any contracture. In larger doses they produced both contracture and considerable neuromuscular blockade. Progressive substitution of the *N*-methyl groups by *N*-ethyl groups was accompanied by a decrease in ability to cause contracture. As with mammalian and frog muscle, the anti-curare effects were immediate in onset, unlike that of neostigmine, which developed slowly. Tensilon was the most active agent of the group and its triethyl analogue the least active. In the presence of tetraethyl pyrophosphate no anti-curare effects were demonstrable either with neostigmine or the other phenyltrialkylammonium derivatives. These observations therefore substantiate the view that, like neostigmine, the hydroxyphenyltrialkylammonium derivatives possess anti-curare activity by virtue of an anticholinesterase action. G. P.