ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Caffeine, Complexes with Benzoic Acid and Benzoate Ion. T. Higuchi and D. A. Zuck. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 132.) Data from previous studies of the interaction of benzoic acid and caffeine at 0° C. were extended by experiments at 15° C. and 30° C., the partition coefficients of benzoic acid between water and skellysolve-C being used to determine the concentration of benzoic acid and the quantity of benzoic acid complex. Equilibrium constants for the formation of a complex from 1 molecule of caffeine with 1 molecule of benzoic acid and for reaction of this with a further molecule of benzoic acid are of the same order and experimental results agree with the calculated values. The formation of the equimolecular complex is exothermic (3000 cal./mole) corresponding to the formation of a single hydrogen bond. The higher entropy change on the formation of the second complex suggests that it may be a highly ordered triangular type of molecular compound. The degree of interaction between caffeine and sodium benzoate was determined by adding an excess of caffeine to water and a number of solutions containing different concentrations of sodium benzoate at 0°, 15° and 30° C. After shaking for 24 hours and allowing the solids to settle, the concentration of caffeine was determined spectrophotometrically. Results are not in agreement with the formation of an equimolecular complex and one containing 2 benzoate ions to each caffeine molecule. The possible formation of a complex consisting of 2 caffeine molecules to one benzoate ion could not be excluded since the effect of different concentrations of caffeine on the interaction was not investigated.

G. B.

Caffeine, Interactions with Aspirin, *p*-Hydroxybenzoic Acid, *m*-Hydroxybenzoic Acid, Salicylic Acid, Salicylate Ion and Butyl *p*-Hydroxybenzoate. T. Higuchi and D. A. Zuck. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 138.) An excess of aspirin was placed in a number of 125-ml. glass-stoppered bottles with varying amounts of caffeine, 100 ml. of 0·001N sulphuric acid was added to each to suppress dissociation of the aspirin and decrease hydrolysis and the bottles were shaken for 3 hours at 30° C. or 6 hours at 15° C. Aliquot quantities were removed for the determination of the concentration of aspirin in solution. Similar techniques were applied with salicylic acid, *p*-hydroxybenzoic acid, *m*-hydroxybenzoic acid and butyl *p*-hydroxybenzoate (butyl paraben). Salicylate ion was investigated in the manner described previously for benzoate ion. Thermodynamic constants are given for all compounds studied, and indicate that caffeine forms fairly stable complexes with all of them. The equimolecular caffeine/acid complex seems to predominate, but there is also evidence for the combination of 1 molecule of caffeine with 2 of benzoic acid or 2 of salicylic acid or salicylate with 1 of caffeine. No conclusive evidence was found for the formation of complexes of more than 3 molecular units. *p*- and *m*-Hydroxybenzoic acids show greater ability to form complexes than the *o*-compound, and the esterification of a hydroxyl group as in aspirin or butyl *p*-hydroxybenzoate results in the formation of less stable complexes. The heats of reaction range from 1·5 to 7 kg. cal., probably representing formation of several hydrogen bonds.

G. B.

ANALYTICAL

Morphine, Determination in Opium with the Aid of an Adsorption Column. F. E. Klee and E. R. Kirch. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 146.) The following is a precise and simple method for the assay of opium. 0·5 g. is extracted for 3 hours with dehydrated methanol in a continuous extraction apparatus in which the opium extract passes through an adsorption column of florisil to remove interfering substances. The extract is evaporated to dryness and dissolved in water to produce 200 ml. of solution of which 1 ml., representing about 0·25 mg. of morphine, is mixed with 2 ml. of Folin–Ciocalteu reagent, 3 ml. of saturated solution of sodium carbonate and water to 100 ml. and allowed to stand for 5 hours for the development of the colour. The light absorption is measured at 765 mμ, and the result of the assay is calculated from a curve prepared with standard morphine solutions. Results are slightly higher than those obtained by the U.S.P. method, possibly because of losses during extraction by the longer pharmacopœial process. In experiments in which known amounts of morphine were added to opium, good recoveries were obtained. Meconic acid, narcotine, codeine and papaverine do not interfere. With samples containing lactose, the specified sample size should not be exceeded as longer extraction may be necessary; lactose interferes in the assay when the extraction is prolonged. G. B.

Morphine, Separation from Codeine by Ion Exchange. E. W. Grant and W. W. Hilty. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 150.) Morphine, on account of its phenolic nature is retained on strongly basic anion exchange columns whereas codeine is not. Amberlite XE75 was converted to the hydroxyl form by allowing it to stand overnight in 10 per cent. sodium hydroxide solution, washed with water and packed into columns. A sample of about 0·2 g. of morphine sulphate, dissolved in 15 ml. of water was retained by the column and was not removed by washing with water, methanol, ethanol or acetone. The morphine was completely removed by washing with 50 ml. of 2 per cent. phosphoric acid. Codeine phosphate placed on the column was completely removed by washing with methanol (95 per cent.). For the separation of codeine phosphate and morphine sulphate the mixture was dissolved in 20 ml. of water and passed through the column. Methanol (95 per cent.) was passed through until a total of 100 ml. of eluate was obtained, in which the codeine was determined by titration. The column was washed with water and the morphine removed with phosphoric acid (2 per cent.). The light absorption of this solution was measured at 285 mμ and the content of morphine sulphate calculated from the datum $E_{1\text{ cm.}}^{1\text{ per cent.}} = 39{\cdot}8$. Reagent blank tests were performed in each case. Good recoveries of the alkaloids were obtained and the presence of codeine did not interfere with the exchange between morphine and the resin. G. B.

Polyvinylpyrrolidone, Reactions and Estimation of. S. Camozzo and S. Dal Zotto. (*Ann. Chim. appl. Roma*, 1953, **43**, 113.) This substance in 0·5 and 2·0 per cent. aqueous solution gives precipitates with the following reagents:—palladium chloride 5 per cent., Mayer's, Dragendorff's, Marmé's, Sonnenschein's and Scheibler's reagents, potassium ferrocyanide 10 per cent., potassium ferricyanide 10 per cent., potassium chromate 10 per cent., potassium dichromate 10 per cent., silicotungstic acid 1 per cent., perchloric acid 20 per cent., tannic acid 5 per cent. It gives no precipitate with the following:—chloroplatinic acid 5 per cent., mercuric chloride, zinc iodide 5 per cent.,

potassium thiocyanate 10 per cent., sodium nitroprusside 2 per cent., lead chloride, ferric chloride 5 per cent., uranium nitrate 1 per cent., picric acid 1 per cent. With Bouchardat's reagent it gives no precipitate, but a fine mahogany-red colour, which can be used for estimation. Polyvinylpyrrolidone is soluble in methanol and ethanol and in chloroform, but is insoluble in ether, carbon disulphide, carbon tetrachloride, benzene and toluene. It is hygroscopic. The sample used in the tests was purified by precipitating a concentrated ethanolic solution with benzene and drying *in vacuo* over sulphuric acid to constant weight. The colorimetric estimation was carried out in a Pulfrich photometer on the mahogany-red colour produced by iodine. To prepare the curve of standardisation 1 ml. of Bouchardat's solution (iodine 2 g., potassium iodide 4 g., distilled water to make 100 ml.) is placed in a 100 ml. graduated flask; from 2 ml. to 20 ml. of aqueous solution of polyvinylpyrrolidone containing 1 g./l. is added, made up to 100 ml., mixed, allowed to stand 20 minutes, and the colour compared in 5 mm. cells with a solution of 1 ml. of the iodine reagent in 100 ml. of water, using filter S55. Up to 14 mg. the colour follows the Lambert-Beer law, but above that the curve is no longer a straight line. Another method is to precipitate with silicotungstic acid and determine the excess of the latter with titanous chloride. In a 20-ml. flask put from 2 to 20 mg. of the product and 5 ml. of solution of silicotungstic acid (15 g. of silicotungstic acid, 500 ml. of 2N hydrochloric acid and distilled water to 1 l.) Make up to 20 ml., filter bright, take 10 ml. of the filtrate, add 0·5 ml. of titanous chloride, 10 per cent., and read the colour in a Pulfrich photometer against distilled water, using an S72 red filter. A straight line graph is obtained and the results agree with those obtained with iodine. H. D.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Quercetin and its Glycosides in Leaves of *Vaccinium myrtillus*. C. H. Ice and S. H. Wender. (*J. Amer. chem. Soc.*, 1953, **75**, 50.) The details of the isolation in pure form of quercetin (3:3′:4′:5:7-pentahydroxyflavone) and 5 of its glycosides from the leaves of the "huckleberry", *Vaccinium myrtillus* are reported. Ion exchange resin (Amberlite IRC-50 (H)) and adsorption chromatography was used in the isolation and separation of the flavonoids. The glycosides were identified as quercetin-3-arabinoside; *iso*quercitrin (quercetin-3-glucoside); quercitrin (quercetin-3-rhamnoside); quercetin-3-gluco-glucoside; and apparently a new quercetin rhamnoside, not identical with quercitrin. A. H. B.

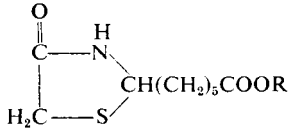
PLANT ANALYSIS

Podophyllotoxin from *Juniperus Spp.*; Savinin. J. L. Hartwell, J. M. Johnson, D. B. Fitzgerald and M. Belkin. (*J. Amer. chem. Soc.*, 1953, **75**, 235.) Because aqueous suspensions of the dried needles of certain junipers caused hæmorrhage and necrosis of Sarcoma 37 in mice, a search for the active principle was instituted. Successive fractionation with different solvents and chromatography was used, and the distribution of biological activity in the fractions was followed by quantitative bioassay with mice bearing Sarcoma 37. In this way crystalline podophyllotoxin was isolated from certain junipers. From one juniper (savin) a new substance called savinin, inactive towards tumours, was also obtained. Certain physical and chemical data, including infra-red and ultra-violet absorption curves, are recorded for savinin. A. H. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Actithiazic Acid, a New Thiazolidone Antibiotic. W. M. McLamore, W. D. Celmer, V. G. Bogert, F. C. Pennington, B. A. Sobin and I. A. Solomons. (*J. Amer. chem. Soc.*, 1953, **75**, 105.) The antibiotic actithiazic acid, a new *streptomyces* antibiotic effective *in vitro* against *Mycobacterium tuberculosis* was isolated by solvent extraction of the fermentation broth and finally purified by recrystallisation from methanol. It is shown to be (-)2-(5-carboxypentyl)-4-thiazolidone (I)



(I)

by means of degradation and synthetic studies which are described. A. H. B.

Adrenaline and Noradrenaline in the Human Fœtal Adrenals and Aortic Bodies, Determination of. K. Niemineva and A. Pekkarinen. (*Nature, Lond.*, 1953, **171**, 436.) Adrenaline and noradrenaline have been assayed chemically in human fœtal adrenals and in the Zuckerkandl's bodies. During the last 3 months of the intrauterine period the total catechols of the adrenals averaged 71 μg. and for the aortic bodies 40 μg. and 11 μg., respectively. This is less than the corresponding amount in normal adult adrenals. The greater part of the catechols consisted of noradrenaline, especially in the aortic bodies. G. F. S.

Glucose, Action of Insulin on the Intestinal Absorption of. M. Lourau. (*C.R. Acad. Sci. Paris*, 1953, **236**, 1376.) Insulin was administered to 2 groups of guinea-pigs. One group also received glucose intramuscularly so as to produce blood-sugar levels similar to those in a control group of guinea-pigs which received no insulin. There was no significant difference between the rate of intestinal absorption of glucose in the control group and in the treated animals having comparable blood-sugar levels. The rate of absorption in the group receiving insulin only was rather less than that indicated by extrapolation of the linear graph relating speed of absorption to logarithm of the blood-sugar level, obtained in previous work. Therefore the hypoglycæmic effect of the insulin was sufficient to account for the increased rate of absorption and there was no evidence that insulin increased the permeability of the mucosa to glucose. Since insulin is known to increase the permeability of the cell membranes in the peripheral tissues, it seems that there is more than one mechanism which regulates the entry of glucose into the cells. G. B.

Œstrogenic Diols, Partition Chromatography of. E. O. Haenni, J. Carol and D. Banes. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 167.) Following the observation that œstrogenic diols may be separated by partition chromatography using sodium hydroxide solution on a celite 545 column, the effects of varying alkali concentration, tightness of packing, mobile solvent, flow rate and temperature were investigated. The use of 0·4N sodium hydroxide on the column with benzene as the mobile phase and a flow rate of 2 to 3 ml./minute are recommended. Separation is more efficient when the temperature is raised but, in collecting the fractions, allowance must be made for the change in partition

coefficient. Details of a short column method for the separation of œstradiols and dihydro-equilins from dihydro-equilenins are given. For the separation of œstradiols, dihydro-equilins and dihydro-equilenins on a long column the first 185 ml. of eluate (plus 14 ml. for each degree below 25° C. or minus 8 ml. for each degree above) is rejected, the œstradiol being eluted in the following 225 ml. (plus 16 ml. for each degree below 25° C. or minus 5 ml. for each degree above). The next 320 ml. (plus 24 ml. for each degree below 25° C. or minus 15 ml. for each degree above) contains the α-dihydro-equilin. The dihydro-equilenins may be removed from the column with ether as solvent. Partition coefficients between the phases used in the columns were determined for β-œstradiol and α-dihydro-equilin and shown to be in agreement with figures deduced from the chromatographic experiments. The partition coefficients for α-œstradiol, β-dihydro-equilin and β-dihydro-equilenin were also determined on the columns. G. B.

Pituitary Antidiuretic Peptide and Similar Urinary Peptide, Isolation of, by Paper Chromatography. G. C. Arneil and H. E. C. Wilson. (*Lancet*, 1953, **264**, 568.) The unusual peptide which occurs in the urine of patients with nephrosis has been found to be present also in the urine of patients with œdema from other causes and of those who have sustained acute trauma. Excessive antidiuretic activity has been found in the blood of patients with nephrosis and it was thought that the peptide might be associated with antidiuretic activity. It was also observed that diuresis occurred after administration of corticotrophin and it seemed possible that this might be due to a lessening of the production of pituitary antidiuretic hormone. 8 commercial pituitary extracts were therefore investigated to see whether they contained a similar peptide and if so whether it had antidiuretic activity. Two-dimensional paper chromatographs were prepared from the extracts, using phenol ammonia and butanol acetic acid as solvents. The nephrosis peptide has a high R_F in phenol and a very low R_F in butanol acetic acid. The positions of the amino-acids were ascertained by the ninhydrin reaction, and the part containing the peptide was eluted with water. The urinary peptide was isolated in essentially the same way. Tests on rats showed that the eluates from the pituitary extracts had strong antidiuretic activity. In 22 similar tests on the eluates from the urine of nephrotic patients, a well-marked antidiuretic response was obtained in only 3 instances. In 8 of the 22 tests, convulsions occurred shortly after injection. The urinary peptide therefore differs from the pituitary peptide although it gave the same amino-acids after hydrolysis and resembled it in its behaviour on chromatography and electrophoresis. The same urinary peptide was obtained from the urine of two normal people after antidiuresis had been induced by nicotine. It is suggested that the urinary peptide is the form in which pituitary antidiuretic hormone is excreted. H. T. B.

3:5:3′-Triiodothyronine; Isolation from Thyroid Gland and Synthesis. J. Gross and R. Pitt-Rivers. (*Biochem. J.*, 1953, **53**, 645.) The unknown radioactive component previously detected in extracts of thyroid gland, plasma, tissues and fæces of animals which had been injected with radioactive iodide or thyroxine labelled with radioactive iodine, has been identified as a 3:5:3′ triiodothyronine. Large-scale extraction of ox thyroid glands followed by tryptic digestion yielded a fraction which when submitted to paper chromatography showed spots attributable to the unknown fraction. Elution from large numbers of filter papers yielded still impure material, which could be demonstrated to be acidic, and which was shown by the ninhydrin reaction to contain

an amino group. Its position on the chromatograms compared with the positions of a number of thyroxine peptides indicated that it was likely to possess a diphenyl ether structure; this was supported by the purple colour obtained with diazotised sulphanilic acid. The possibility that this substance might be diiodothyronine having already been excluded by Gross *et al.*, the synthesis of triiodothyronine was attempted. Moniodination of 3 : 5-diiodothyronine with iodine in the presence of ammonia gave triiodothyronine as the main reaction product. The latter was purified by crystallisation as the hydrochloride from aqueous hydrochloric acid, and by partitioning on a column of kieselguhr, using butanol/chloroform and 0·5 N aqueous sodium hydroxide. The synthetic substance behaved chromatographically on paper identically with the radioactive unknown substance. D- and DL-triiodothyronines were obtained similarly. The possibility that triiodothyronine may be derived from thyroxine as an artefact of alkaline hydrolysis was excluded by control experiments. The knowledge of its chemical properties gained during its synthesis made it possible to isolate triiodothyronine in a pure stable form from thyroid gland. J. B. S.

BIOCHEMICAL ANALYSIS

α-Œstradiol in Œstrogenic Mixtures, Colorimetric Determination of, using Partition Chromatography. E. O. Haenni, J. Carol and D. Banes. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 162.) In preparing extracts of œstrogenic mixtures all but traces of ketosteroids may be eliminated by the method of Carol and Rotondaro, employing the Girard-T reagent. The resulting extract may contain α- and β-dihydro-equilenins, α-dihydro-equilin and œstradiols, as well as impurities which cause interference in assays by charring during the iron-Kober reaction. Dihydro-equilin and œstradiols may be separated from this extract by partition chromatography on a column of celite 545 using 0·4N sodium hydroxide on the column and benzene as the mobile solvent. The first fraction of the eluate, containing most of the non-œstrogenic impurities is rejected, the following fraction containing œstradiols and α-dihydro-equilin being collected and the content of α- and β-œstradiols determined by the spectrophotometric method described in a previous paper. Small amounts of α-dihydro-equilin may be ignored, but for larger quantities a correction should be made. For the determination of α-dihydro-equilin a similar method is used, but the iron-Kober reagent is diluted with 45 per cent. of its volume of N hydrochloric acid, 1 to 3 hours before use, and the optical density of the solution is measured at 472 mμ at intervals until the maximum colour has been reached. A quantity of pure α-dihydro-equilin is submitted to the reaction concurrently and the content of the sample is calculated in terms of the standard. Collaborative tests in 6 laboratories indicate that the method is satisfactory. G. B.

Steroid Hormones, Quantitative Determination of, with 2 : 4-Dinitrophenylhydrazine. A. G. Gornall and M. P. Macdonald. (*J. biol. Chem.*, 1953, **201**, 279.) Steroid hormones are determined quantitatively as 2 : 4-dinitrophenylhydrazones in extracts of urine, blood plasma or tissues, which have been prepared by methods usually considered satisfactory for steroid hormone assay. Suitable aliquots of the extracts to be analysed containing 1 to 20 μg. of cortisone are first evaporated to dryness under nitrogen and the residue dissolved in methanol; 2 : 4-dinitrophenylhydrazine in acid methanol is added and the mixture heated at 60° C. for 90 minutes. The cold solution is made alkaline,

suitably diluted and the optical density is measured against a suitable blank at 475 mμ. Δ^4-C_3-steroid ketones react quantitatively within 5 minutes at 20° C. to give hydrazones which show a peak absorption in alkaline solution at 450 mμ. Heating for 90 minutes at 59° C. causes, in addition, the complete reaction of C_{20} ketones in a dihydroxyacetone side chain, but only partial reactions in monohydroxy side chains. Other ketones react slightly. Molar extinction coefficients for a large range of ketosteroids have been determined under both sets of conditions and the reactivity of the ketone estimate. A C_{21}-hydroxyl group causes a specific shift in the wavelength of the absorption maximum for the hydrazone of the C_{20} ketone. J. B. S.

CHEMOTHERAPY

Amithiozone (Thiacetazone) Analogues from Aralkyl Ketones. W. L. Nobles and J. H. Burckhalter. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 176.) A series of 18 thiosemicarbazones of ketones was prepared by heating the ketone and thiosemicarbazide dissolved in dilute ethanol, with the addition of a few drops of a mineral acid. Satisfactory yields were obtained by heating in a water-bath for 15 to 20 minutes, the thiosemicarbazone being precipitated during the reaction or on cooling. Compounds of the type $C_6H_5{\cdot}C(CH_2R){:}NNH{\cdot}CS{\cdot}NH_2$ were prepared where R = –H, $–CH_3$, $–NHCOCHCl_2$, $–NH_2$, $–CH_2N(CH_3)_2$, $–OCOCH_3$, $–NH{\cdot}CO{\cdot}CH_3$, or $–CH_2{\cdot}CO_2H$, the phenyl group being *p*-chloro, *p*-nitro, *p*-phenyl, *p*-methoxy or 3:5-dinitro substituted or replaced by 2-thienyl. All these compounds are related to *p*-acetamidobenzaldehyde thiosemicarbazone (thiacetazone) and some also show a structural resemblance to chloramphenicol. The vinylog, $C_6H_5{\cdot}CH{:}CH{\cdot}C(CH_2){:}NNH{\cdot}CS{\cdot}NH_2$ and 3 ring-substituted derivatives were also prepared. Of the 10 compounds submitted to *in vitro* tests against the β-benzoylpropionic acid derivative, 1 was inactive and 8 others showed a certain amount of activity, while *p*-chloroacetophenone thiosemicarbazone was 4 times as active as 4:4′-diaminodiphenylsulphone. G. B.

Chloramphenicol Analogue. DL-**2-Dichloroacetamido-1-(4-pyridyl)-1 : 3-propandiol.** S. van der Meer, H. Kofman and H. Veldstra. (*Rec. Trav. chim. Pays-Bas*, 1953, **72**, 236.) Several attempts to prepare the pyridine analogue of chloramphenicol failed because of a tendency to intermolecular quaternisation but the synthesis was successfully accomplished in the following stages. The *O*-*p*-toluenesulphonyl derivative of 4-acetylpyridine was converted to the diacetal of 4-(ω-aminoacetyl) pyridine with potassium ethoxide. Reaction of an ethanolic solution of this compound with benzoic anhydride yielded a stable benzoyl derivative from which the corresponding ketone was obtained by heating with hydrochloric acid. 4-(α-Benzoylamino-β-hydroxypropionyl) pyridine was formed by heating with formaldehyde in the presence of sodium bicarbonate and ethanol, and reduced to the corresponding alcohol with aluminium *iso*propoxide. The benzoyl group was removed by hydrolysis with hydrochloric acid and the pyridine analogue of chloramphenicol, DL-2-dichloroacetamido-1-(4-pyridyl)-1 : 3-propandiol obtained by reaction with methyl dichloroacetate at room temperature. This product and a number of intermediate compounds and derivatives were tested *in vitro* against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurum* and *Eberthella typhi*. The pyridine analogue of chloramphenicol showed only 1 to 3 per cent. of the activity of chloramphenicol against *S. typhimurum* and *E. typhi*. G. B.

Thiazole Carboxylic Acid Hydrazides, Tuberculostatic Activity of. H. C. Beyerman and J. S. Bontekoe. (*Rec. Trav. chim. Pays-Bas.*, 1953, **72**, 262.) Thiazole-2-carboxylic acid hydrazide was prepared by reaction of the ethyl ester of the corresponding acid with hydrazine acetate, and the 5-carboxylic acid hydrazide was obtained from the corresponding methyl ester. Thiazole-4-carboxylic acid hydrazide was made by the hydrogenation of ethyl 2-bromothiazole-4-carboxylate in the presence of Raney nickel. Thiazole-2-carboxylic acid hydrazide proved to be of the same tuberculostatic activity as the corresponding pyridine derivative, picolinic acid hydrazide, both *in vitro* and *in vivo*. Thiazole-4-carboxylic acid hydrazide was 1/5th as active against BCG and inactive against type D–328 tubercle bacilli. Thiazole-5-carboxylic acid hydrazide, analogous to the inactive nicotinic acid hydrazide was also inactive *in vitro* against type D–328 bacilli and showed only 1/25th of the activity of the 2-substituted isomer against BCG. Thiazole does not give an analogue of *iso*nicotinic acid hydrazide, since the sulphur atom takes the place of the α position of pyridine. G. B.

PHARMACY

NOTES AND FORMULÆ

Hexamethonium Chloride (Methium Chloride). (*New and Nonofficial Remedies, J. Amer. med. Ass.*, 1953, **151**, 385.) Hexamethonium chloride is hexamethylenebis(trimethylammonium chloride), and occurs as a white, crystalline, hygroscopic powder with a faint odour, m.pt. 289° to 292° C. with decomposition, very soluble in water, soluble in ethanol, and practically insoluble in chloroform and ether; the *p*H of a 3 per cent. solution is 5·0 to 6·5. The diperchlorate obtained by adding a neutral solution of sodium perchlorate to an ethanolic solution of hexamethonium chloride melts at 261° to 265° C., after washing with ethanol and ether and drying at 105° C. for 4 hours. When dried in an Abderhalden-pistol drier over phosphorus pentoxide at 100° C. and 5 mm. Hg for 4 hours, the loss in weight does not exceed 1·5 per cent. It contains 25·4 to 26·5 per cent. of chloride and 72·6 to 75·5 per cent. of hexamethonium ion. The hexamethonium ion is assayed by refluxing with acetic anhydride, dissolving the product in acetic acid, treating with a solution of dry mercuric acetate in acetic acid and titrating with 0·1 N perchloric acid, using a solution of crystal violet in glacial acetic acid as indicator, and titrating to the blue-green end-point. The 0·1 N perchloric acid is prepared by mixing 70 to 72 per cent. perchloric acid with glacial acetic acid and adding acetic anhydride. G. R. K.

Probenecid (Benemid). (*New and Nonofficial Remedies, J. Amer. med. Ass.*, 1953, **151**, 298.) Probenecid is *p*-(dipropylsulphamyl)benzoic acid and occurs as a white, odourless, crystalline powder, m.pt. 198° to 200° C., soluble in acetone, ethanol, dilute alkalis, and dilute sodium bicarbonate solution, and insoluble in water and dilute acids. When reduced with Raney nickel in alkaline solution, the product yields no colour on diazotisation followed by treatment with sulphamic acid and *N*-(1-naphthyl)ethylenediamine (distinction from caronamide). A 0·001 per cent. solution in ethanol exhibits ultra-violet absorption maxima at about 2250 and 2480 Å. ($E^{1\ \text{per cent.}}_{1\ \text{cm.}}$, about 336). Probenecid loses not more than 0·1 per cent. of its weight when dried at 105° C. for 4 hours; it yields not more than 0·1 per cent. of ash and complies with limit tests for halides, sulphate, heavy metals, and free acid. It contains 95·0 to 105·0 per cent. of probenecid when determined by measuring the absorption

of a 0·001 per cent. ethanolic solution at 2480 Å., and 4·81 to 5·01 per cent. of nitrogen when determined by semimicro Kjeldahl. Probenecid is used to increase and prolong the plasma concentration of penicillin, sodium aminosalicylate, and other substances, and to promote the elimination of uric acid in the interval treatment of chronic gout. G. R. K.

PHARMACOLOGY AND THERAPEUTICS

Adrenaline and Noradrenaline, Effect of, on the Activity of Isolated Preparations of the Gut from the Fœtal Guinea-pig. A. F. Munro. (*Brit. J. Pharmacol.*, 1953, **8**, 38.) While adrenaline abolishes the tone and rhythmicity of the longitudinal muscle of the small intestine it contracts the sphincters. Adrenaline and noradrenaline have been shown to cause contractions of isolated segments taken from the two ends of the small intestine of the fœtal guinea-pig while an intervening region was either relaxed or not affected. Atropine potentiated the contraction and reversed the inhibitory responses of adrenaline. In both the fœtal and adult terminal ileum, adrenaline caused a dual response, large doses causing contraction and small doses relaxation. Adrenaline reduced or abolished both the contraction and relaxation caused by noradrenaline in segments from the fœtal ileum. It is suggested that in the fœtal guinea-pig differentiation of function between sphincter and duodenal muscle is as yet incomplete. G. F. S.

Adrenaline and Noradrenaline Infusions, Effects of, on Respiration in Man. R. F. Whelan and I. M. Young. (*Brit. J. Pharmacol.*, 1953, **8**, 98.) The influence of adrenaline and noradrenaline on the respiratory pattern and gaseous exchange has been examined in conscious human subjects. An infusion of adrenaline or noradrenaline increased the tidal volume, respiratory minute volume and lowered the alveolar carbon dioxide. Both compounds had similar activities in these actions. The respiratory rate was usually increased. Adrenaline increased the oxygen consumption by an average of 32 per cent. while noradrenaline caused no significant change. It is suggested that the hyperventilation is independent of any general increase in the metabolic rate. G. F. S.

Anti-*Jk*^{b}. A New Blood-group Antibody. G. Plaut, E. W. Ikin, A. E. Mourant, R. Sanger and R. R. Race. (*Nature, Lond.*, 1953, **171**, 431.) This paper reports the finding of the antibody corresponding to the antigen and gene *Jk*^{b} in the serum of a female, a multipara who had had two miscarriages. The existence of an allelomorphic gene *Jk*^{b} in the *Kidd* human blood-group had hitherto been assumed. The existence of this anti-body now promotes the *Kidd* system to fourth place in the order of "usefulness" of the 9 blood group systems. G. F. S.

Anticurare Agents. A. R. Hunter. (*Brit. med. J.*, 1953, **1**, 640.) In view of the fatalities which have occurred after the use of neostigmine as an antidote to the respiratory depressing action of curarising drugs, its dangers and the methods available for their elimination were investigated. The compound was given intravenously together with atropine to 50 patients who had been curarised with various myoneural blocking agents and anæsthetised with nitrous oxide and oxygen and a supplement. As the primary danger is cardiac inhibition, counts of the pulse rate were made every 15 seconds after the injection at first and at intervals of a minute subsequently. The effects on the pulse

rate of giving the atropine before the neostigmine, and of giving a sympathomimetic drug in addition, were also determined. In all patients there was a progressive slowing of the pulse rate to a minimum during the first 5 to 10 minutes. The minimum rate depended upon the initial rate; a rate of 120 tended to drop to 70 to 100 while a relatively slow pulse might drop to 40 to 50. Atropine is widely believed to cause a slowing of the pulse by medullary stimulation before its peripheral accelerating action occurs, but doses of up to 1/50 grain at various intervals before the neostigmine produced acceleration immediately the drug reached the heart and there was no change in the pattern of the response to neostigmine even when the latter was given as much as 19 minutes after the atropine. The injection of 30 mg. of ephedrine or 10 to 20 mg. of methedrine with the atropine and neostigmine increased the initial cardiac acceleration but the pattern of the subsequent slowing was unaltered. The use of a vasopressor is therefore unnecessary. On the assumption that a rate of 50 indicated a dangerous degree of bradycardia, it is suggested that 1/50 gr. of atropine should be used when 2·5 mg. of neostigmine is given. Since it is impossible to forecast the exact degree of bradycardia which will be produced by a dose of neostigmine, the patient must be watched for 10 minutes after the injection and atropine given intravenously if necessary. H. T. B.

Antimalarials, Field Trials of, in West Africa. L. J. Bruce-Chwatt and H. M. Archibald. (*Brit. med. J.*, 1953, **1**, 539.) Field trials were carried out in West Africa on the 4 antimalarial drugs chloroquine sulphate, camoquin, pyrimethamine and azacrin (2-methoxy-6-chloro-9-(5′-diethylamino-2′-pentyl) amino-3-aza-acridine). 120 African schoolchildren, aged 5 to 10 years and all of them naturally infected with malaria, were divided into 5 groups, one group receiving no treatment to act as a control, while one of the drugs to be tested was administered to each of the other 4 groups. Dosage given was half that usual for adults. In the chloroquine sulphate and pyrimethamine groups the drug was given in two different regimens. Of those given chloroquine sulphate, half received 0·75 g. in a single dose on the first day, while the other half were given 0·75 g. on the first day followed by 0·25 g. on each of the 2 following days. Of the pyrimethamine group, half received a single dose of 25 mg. on the first day, and the other half had 25 mg. on the first day followed by 25 mg. the next day. The camoquin group were given a single dose only of 0·4 g. The azacrin group received 0·2 g. on the first day, followed by 0·1 g. on each of the 2 following days. Daily blood examinations were carried out and the parasite rate, parasite density and any other malariometrical indices were recorded. All 4 antimalarials were found to be good schizonticides for *Plasmodium falciparum.* The clearance times for trophozoites of *P. falciparum* were similar although camoquin and azacrin appeared to act more rapidly than the other two. None of them is an effective direct gametocide for *P. falciparum.* Infections with *P. malariæ* were too few to give comparable results, but chloroquine and camoquin seemed to act more rapidly than pyrimethamine and azacrin. Two infections of *P. ovale* mixed with *P. falciparum* disappeared within 24 hours of giving camoquin and azacrin. Of the 4 drugs used, pyrimethamine appeared to be the best tolerated, since no complaints of nausea or headache were made. Of the chloroquine sulphate group only 2 children complained of nausea, and 4 of 23 given camoquin complained of headache and nausea within 4 hours of taking the drug. Azacrin was well tolerated on a dose of 0·2 g. but 0·3 g. caused nausea, stomach pains and vomiting in 7 of 10 children. H. T. B.

Blowpipe Dart Poison from Borneo. J. A. Robinson and H. W. Ling. (*Brit. J. Pharmacol.*, 1953, **8**, 79.) Blowpipe dart poison used by the nomadic Pĕnan of Borneo has been shown to contain a cardiac toxic substance resembling ouabain. In the cat and rabbit it had 20 per cent. and in the mouse 33 per cent. of the toxicity of crystalline ouabain, which suggests that it is not ouabain itself. It has also been shown to differ from aconitine. G. F. S.

Butazolidine, Effect of, on the Excretion of Water and Electrolytes. J. Green and P. O. Williams. (*Lancet*, 1953, **264**, 575.) Preliminary studies of the urinary excretion of sodium and potassium after the intramuscular injection of 1 g. of butazolidine indicated a prompt reduction in the volume of urine and a steep fall in the urinary sodium level. 4 healthy volunteers were then placed on a diet low in sodium and potassium and were given every 2 hours 250 ml. of water and a cachet containing 1 g. of sodium chloride and 0·2 g. of potassium chloride. The daily intake was 2 l. of water, 9 g. of sodium chloride and 4 g. of potassium chloride. After 1 day for stabilisation and 1 day as a control period, 3 of the volunteers were given 1 g. of butazolidine intramuscularly. The fourth volunteer served as a control. Samples of urine were collected 2 hourly during the day and the overnight urine was pooled. The average retention in the 12 hours following the injection was 766 ml. of water, 2·4 g. of sodium, 0·4 g. of potassium and 3·56 g. of chloride. Retention of water and sodium chloride is therefore not merely an occasional toxic effect of butazolidine but a normal result of its use. Œdema does not therefore indicate intolerance and should be controllable by restriction of the sodium intake and perhaps by the use of mercurial diuretics. The effect appears to be due to reabsorption of sodium, and not to a true antidiuretic effect.
H. T. B.

Chelating Agent, Effect of, on Urinary Lead Excretion. J. B. Sidbury, J. C. Bynum and L. L. Fetz. (*Proc. Soc. exp. Biol., N.Y.*, 1953, **82**, 226.) A study of the effectiveness of disodium calcium ethylenediamine tetra-acetate, administered by the oral and intravenous route, on 7 patients with symptoms of lead poisoning, was undertaken. Intravenously, 1 g. was given to 5 adults on the first day, followed by 2 g. daily for a total of 5 treatment days. The doses were subdivided and each administered in 250 ml. of 5 per cent. glucose solution given over 1 hour by infusion. 2 children were given 30 mg./kg. in glucose solution twice daily. Orally both adults and children received 30 mg./kg. twice daily. 24-hour urine collections and whole blood specimens were obtained for lead analysis before, during and after the treatment period. Intravenous administration produced a 10- to 40-fold increase in urinary lead excretion on the first day. Subsequent values were generally lower, but never less than 3 times the observed pretreatment level. After oral administration the rise in excretion was more gradual, with a maximum on the third and fourth day, and the effect was less than that of the intravenous method. Side reactions were minimal with the dosages employed. It is concluded from the results that the drug is the most effective agent yet proposed for the treatment of plumbism. J. R. F.

***dl-cyclo* Hexyloxy-α-phenylethylamines, Morphine-like Properties of.** A. McCoubrey. (*Brit. J. Pharmacol.*, 1953, **8**, 22.) Evidence is presented that the analgesic effect of these compounds is more nearly related to that of morphine and amidone than to that of phenacetin. Three related amines,

m- and *p-iso*propyloxy- and *p*-ethoxyl-α-phenylethylamine were not analgesic and their pharmacological properties were different from those of the *cyclo*hexyl ethers and morphine. G. F. S.

3:3-Dithienylalkenylamines, Analgesic and Other Properties of. A. F. Green. (*Brit. J. Pharmacol.*, 1953, **8**, 2.) A series of 3:3-dithienylalkenylamines have been tested for analgesic, antihistamine and atropine-like properties. 3-Dimethylamino, 3-ethylamino, 3-diethylamino, 3-pyrrolidino and 3-piperidino-1:1-di-2′-thienylbut-1-ene hydrochlorides showed on the rat and the rabbit analgesic activities between 0·6 and 1·0 times that of morphine, but only 0·1 to 0·4 times the activity of morphine in the dog. Keele has shown these compounds to be less active than morphine in man, which suggests that analgesic activities in man and dog are similar. Toxicity tests in mice showed these compounds to be between 0·4 to 0·8 times as toxic as amidone. In mice they caused analgesia, hyperexcitability, mydriasis and respiratory depression leading to death. Respiratory depression was related to analgesic activity. None of these compounds had a mydriatic activity greater than 0·005 times that of atropine in the mouse or an antihistamine activity greater than 0·1 times that of mepyramine on the isolated guinea-pig ileum. While they had a local anæsthetic action on the eye they caused corneal damage. In some respects the actions of these compounds are more closely related to pethidine than morphine. G. F. S.

Ergometrine Intravenously to Prevent Post-partum Hæmorrhage. J. D. Martin and J. G. Dumoulin. (*Brit. med. J.*, 1953, **1**, 643.) The results in 1000 consecutive obstetric patients given ergometrine intravenously were compared with those obtained in 1000 patients delivered previously who did not receive ergometrine. The volume of blood from the vagina and perineum was measured. Episiotomy under local analgesia was performed if a perineal laceration seemed imminent. The dose of ergometrine in the treated series was 0·5 mg. given intravenously with the crowning of the baby's head. On completion of the third stage 0·5 mg. of ergometrine was given intramuscularly. The intramuscular injection was also given in the control series. Blood losses of 20 fl. oz. or more occurred in 13·1 per cent. of the controls but only in 1·2 per cent. of the treated patients. Secondary post-partum hæmorrhage occurred in 3 of the control patients and in 8 of the treated group. Manual removal of the placenta took place in 11 controls and 30 of the treated group. The duration of the third stage was much less in the treated group and the results confirm the view that there is a direct relation between length of the third stage and blood loss. The treated group showed a slightly higher hæmoglobin level than the controls on the 4th or 5th day of the puerperium. Timing of the injection is of the utmost importance. Intravenous ergometrine acts within 1 minute and should produce a tonic uterine contraction and separation of the placenta while part of the baby still occupies the uterine cavity. The disadvantage of the procedure is the need for an assistant to be present to give the injection at the right moment. H. T. B.

Hyaluronidase, New Inhibitors of. M. Fabinyi-Szebehely, L. Hahn and J. Szebehely. (*Brit. J. Pharmacol.*, 1953, **8**, 30.) 4 triphenylmethane derivatives, polycondensed trihydroxytricarboxytriphenylmethane, polycondensed hexahydroxytricarboxytriphenylmethane ("trigentisic acid") and two polycondensed heptahydroxytricarboxytriphenylmethanes, as well as two

diphenylmethane derivatives, polycondensed dihydroxydicarboxydiphenylmethane and polycondensed tetrahydroxydicarboxydiphenylmethane ("digentisic acid") have been found to have a strong inhibitory effect *in vitro* on hyaluronidases and have been tested for their inhibitory effect *in vivo* by a method based on the action of hyaluronidases on the rate of absorption of urethane injected subcutaneously in mice. All of the compounds possessed marked *in vivo* activity, trigentisic acid being the most active. All 6 compounds had an anti-inflammatory action reducing the artificial œdema produced by the injection of egg white in rats. Toxicity tests showed trigentisic acid to be the least toxic in mice and a daily dose of 8 g. produced no ill effects in human volunteers. G. F. S.

Isoniazid in Pulmonary Tuberculosis. A. J. Proust, E. G. Beacham and H. S. Allen. (*Med. J. Aust.*, 1953, **1**, 179.) 20 patients, of whom 19 were clinically resistant to streptomycin and *p*-aminosalicylic acid, and 18 had far advanced and 2 moderately advanced pulmonary tuberculosis, were treated with isoniazid, 0·15 to 0·2 g. daily, for periods of 21 to 25 weeks. 16 noted a definite improvement in appetite and feeling of well-being. 17 patients gained weight over 2 lb. during the period, the average gain being 12·4 lb. 2 patients showed no gain and 1 lost 0·5 lb. Of 17 patients with significant fever prior to therapy, 11 showed a marked change after treatment, and of 18 patients who produced sputum before treatment, 7 showed no change, 7 showed a definite decrease in amount, and in 4 sputum production was eliminated. The toxic effects of the drug were minimal, but the development of resistant strains of the bacteria has necessitated the addition of streptomycin, *p*-aminosalicylic acid or viomycin to the treatment in almost all cases. J. R. F.

Isoniazid in Treatment of Pulmonary Tuberculosis. Second Report of the Tuberculosis Chemotherapy Trials Committee to the Medical Research Council. (*Brit. med. J.*, 1953, **1**, 521.) Results are reported of 3 months' treatment of 364 patients at 40 hospitals. 142 patients received streptomycin (1 g. daily) with isoniazid (100 mg. twice daily), 102 received streptomycin (1 g. daily) with sodium aminosalicylate (5 g. four times daily), and 120 received isoniazid (100 mg. twice daily) alone. Patients were allocated to one of the three courses of treatment by random selection, and divided into three main groups: acute rapidly progressive disease of recent origin (excluding bilateral disease between the ages of 15 and 30), other forms suitable for chemotherapy, and chronic disease considered unlikely to respond to chemotherapy. Details are tabulated of general clinical conditions, weight and temperature changes, sedimentation rates, radiographic appearances and bacillary sensitivity. Judging solely from the results at 3 months, the combination of streptomycin and isoniazid was found clinically to be the most effective of the three treatments studied, although its superiority to streptomycin and sodium aminosalicylate was not great. Clinical improvement was marked, especially in weight increase, with isoniazid alone, but an important defect in this treatment is the frequent and rapid emergence of bacterial resistance to the drug. After the 3 months' treatment, bacillary resistance to isoniazid was found in 62 per cent. of culture-positive patients treated with isoniazid alone, compared with only 13 per cent. in patients treated with streptomycin and isoniazid. Bacillary resistance to streptomycin was found in 11 per cent. of these cases. For this reason, it is suggested that none of the 3 drugs should be used by itself. Similarly, a combination of 2 of these drugs administered to a patient in whom the infecting bacteria are already resistant to one of the two appears to be tantamount to giving the other alone,

and resistance to the second drug rapidly appears. Wherever possible, the drug sensitivity of the infecting organisms should be assessed before chemotherapy is started so as to avoid an unsuitable combination of drugs, and also on its completion in case more chemotherapy is required later. H. T. B.

Morphine, Antidiuretic Action of. N. J. Giarman, L. R. Mattie and W. F. Stephenson. (*Science*, 1953, **117**, 225.) Morphine sulphate was administered to male adult rats and the pooled urinary output in its natural state or after chemical or physical treatment was dialysed, concentrated to the original volume and assayed for antidiuretic activity in rats. Morphine consistently produced urine with a distinct antidiuretic action although neither pethidine nor methadone did so. Heating the urine under a reflux condenser or in an open vessel for 10 hours at 60° C., heating under a reflux condenser with hydrochloric acid (10 per cent.) for 3 hours at 100° C. or heating for 30 minutes at 100° C. in 2N sodium hydroxide destroyed the antidiuretic principle. Treatment with sodium sulphite at room temperature gave inconsistent results. Similar properties are reported for the antidiuretic hormone of the pituitary gland. Morphine injected into hypophysectomised rats has no antidiuretic effect and this together with the foregoing results suggests that the antidiuretic substance produced by morphine is the antidiuretic hormone which is released by the stimulation of the optico-posterior lobe of the pituitary gland by morphine. G. B.

Nicotinamide, Pharmacological Effects of Massive Doses of. F. Bergmann and L. Wislicki. (*Brit. J. Pharmacol.*, 1953, **8**, 49.) A method is described for the assay of nicotinamide in blood, based on a reaction with alkaline hydroxylamine and ferric chloride after deproteinisation with trichloracetic acid. It was shown that nicotinamide injected intravenously was distributed very rapidly throughout the extracellular fluid but was absorbed only slowly by the tissue cells. Studies of the effects of massive doses of nicotinamide in cats, dogs and rabbits showed that it produced a marked hyperglycæmia and oliguria preceded by a period of anuria which appeared to be due to an action on the tubules. Intravenous injections of very high doses (0·5 to 1·0 g. kg.) caused a fall in blood pressure and an increase in the rate and amplitude of the respiratory movements. G. F. S.

Phenylbutazone (Butazolidine) in Chronic Arthritis. L. Cudkowicz and J. H. Jacobs. (*Lancet*, 1953, **264**, 223.) Courses of phenylbutazone, given either intramuscularly or orally, and ranging from 4 months to a few days, were administered to 34 patients with rheumatoid arthritis and 11 with osteo-arthritis. The daily dose was varied according to the response of the patient but never exceeded 1 g. per day. In rheumatoid arthritis phenylbutazone appears to increase mobility and muscle power, and reduces pain to a significant degree. The changes in erythrocyte sedimentation rate were inconclusive and there were no significant changes in white-cell counts. Of the 11 patients with osteo-arthritis 7 showed objective improvement, and, subjectively, 8 had relief from pain. Toxic effects occurred in 22 pateints and were often quite severe. They included abscess at site of injection, gastro-intestinal symptoms, reactivation of peptic ulcer, melæna, salt and water retention with œdema, and rash. It is concluded that phenylbutazone has a place in the management of chronic joint disease in which the outstanding feature is pain; but careful selection and constant supervision of patients are necessary. A past history of peptic ulceration, or the presence of hypertension, chronic bronchitis and emphysema, or valvular heart disease with a past episode of failure are absolute contra-indications since cardiac failure may follow salt and water retention.

S. L. W.

Phenylindanedione as an Anticoagulant. M. Toohey. (*Brit. med. J.*, 1953, **1**, 650.) Phenylindanedione was used as an anticoagulant in 68 patients, most of them suffering from coronary thrombosis or deep venous thrombosis. Prothrombin levels of 10 to 20 per cent. of normal were aimed at and the usual dosage was 100 mg. twice during the first 24 hours followed by 50 mg. twice during the second 24 hours. The full effect is not produced for 36 to 48 hours and only rarely should these doses be exceeded. Only 6 failed to reach an adequate prothrombin level in 48 hours and in only 1 was the level about 40 per cent. Further dosage was based on the response after 36 to 48 hours. In 44 patients the daily maintenance dose was between 75 and 100 mg. and in 5 it was more than 150 mg.; the daily dose was given in 2 equal amounts, morning and evening. While dosage must be based on prothrombin determinations, the amount required is influenced by the weight and condition of the patient. Heavy patients require more, while acutely ill patients, especially those severely shocked, require less until their condition improves. Frail patients also require less. Renal function is particularly important in determining dosage since deficient function causes delayed excretion. No toxic effects were noticed in any of the patients. If an antidote is required, vitamin K_1, even by mouth, has a dramatic effect within 8 to 20 hours. When once a patient's maintenance dose has been ascertained, prothrombin determinations need only be made 2 to 3 times a week. H. T. B.

Procainamide and Hexamethonium Bromide; Combined Use in Controlled Hypotension. A. A. Mason and J. F. Pelmore. (*Brit. med. J.*, 1953, **1**, 250.) In a series of 50 unselected surgical cases an average dose of 65 mg. of hexamethonium bromide was given intravenously, preceded by 1 g. of procainamide intravenously in 2 doses. In a control series an average dose of 95 mg. of hexamethonium bromide alone was given. In a further series of 17 cases in which hexamethonium bromide also failed to produce a sufficient fall in blood pressure, the subsequent injection of 1 g. of procainamide produced a quick response. If it is deemed advisable to use the two drugs together, as, for example, with ventricular arrhythmias and maxillo-facial operations, they may be employed without toxic effects. The blood pressure does fall, however, more than with either used separately, and with smaller doses. In other words, the drugs show a true potentiation of each other's hypotensive effects, and when employed together they must be used with extreme caution. Failure of hexamethonium bromide alone seems to occur with the greatest frequency in patients under the age of 30, and it may be anticipated that procainamide will be required most often in this age group. In an addendum the authors report on a further 150 cases, in which the pulse rate has almost invariably been reduced following injection of procainamide. They used the drug in doses of 0·5 to 1 g. in those cases in which the pulse rate remained higher than 110. S. L. W.

Proguanil, Isolation of a Metabolite with High Antimalarial Activity. A. F. Crowther and A. A. Levi. (*Brit. J. Pharmacol.*, 1953, **8**, 93.) It has been suggested that proguanil is modified in the body to form a product which is responsible for antimalarial activity. This paper reports the isolation of a highly active metabolite from the urine of rabbits and from human volunteers which has been shown to be 4:6-diamino-1-*p*-chlorophenyl-1:2-dihydro-2:2-dimethyl-1:3:5-triazine. It was found to have an activity about 10 times that of proguanil against *P. gallinaceum* in the chick and to be active against both the erythrocytic and exoerythrocytic forms. Methods are described for its isolation and identification and a method of synthesis is to be published. G. F. S.

Pyrimethamine in Acute Malaria. T. Wilson and J. F. B. Edeson. (*Brit. med. J.*, 1953, **1**, 253.) The authors report on the use of pyrimethamine in the treatment of 126 patients with acute malaria in Malaya. 80 patients had previously untreated acute falciparum malaria. A single dose of 50 mg. of the drug failed to cure 2 out of 15 patients; a dose of 100 mg. (in 2 doses of 50 mg. each) failed to cure 4 out of 39; and a total dose of 300 mg. (100 mg. on the first day and 50 mg. daily for the next 4 days) failed to cure 7 out of 26. Apart from the failures the action on fever and symptoms was usually slow. The results in 24 proguanil-resistant patients treated with 100 mg. of pyrimethamine were so similar as to suggest that resistance to proguanil does not affect the response to pyrimethamine. It is concluded that pyrimethamine must be regarded as unsuitable for the treatment of acute malaria due to Malayan strains of *P. falciparum*. 11 patients with acute vivax malaria were treated with the 50 mg. single dose and 11 with the 100 mg. dose. Disappearance of parasites was slow, but there were no failures. S. L. W.

Terramycin for Infections in Diabetic Patients. J. B. Walker. (*Lancet*, 1953, **264**, 521.) Infections associated with diabetes are frequently caused by mixtures of organisms, and terramycin, in view of its wide range of activity, is therefore particularly suitable for treating them. The author used terramycin in the treatment of a total of 93 infectious conditions among 70 patients. The majority were infections of the feet varying in severity from simple cases to those that were frankly gangrenous, but other types of infection were treated, including boils and carbuncles, urinary tract infections, respiratory infections and wound infections. Dosage was generally 0·5 g. thrice daily, with or immediately after meals, the total amount required averaging 10 g. In the series of foot infections treated, swelling and pain subsided rapidly, with marked improvement in 48 hours. Toxicity appeared to be low, 11 cases of diarrhœa, one severe, being reported in the 93 treatments. There appears to be little risk of disturbing the diabetic control, and no change of diet was required. Relapse when it occurred was due to arteriosclerosis, but by keeping the infection controlled an active life for the patient could be prolonged. Sensitivity tests seem to be unnecessary, or even misleading, since the organisms were found frequently to be more sensitive clinically than laboratory tests had indicated. Development of resistance to the antibiotic was not observed. H. T. B.

Thiobarbituric Acids, Ultra-short-acting. E. E. Swanson and K. K. Chen. (*Proc. Soc. exp. Biol., N.Y.*, 1953, **82**, 212.) A comparison of four *N*-methyl thiobarbituric acids with sodium 5-allyl-5-(1-methylbutyl)thiobarbiturate and thiopental was made using rats, rabbits, cats and dogs. All were potent anæsthetics by intravenous injection. The duration of anæsthetic and hypnotic action for the *N*-methylated derivatives was found to be shorter than that of the two non-methylated compounds, and less cumulative action was also observed when one-half of the AD50 was injected intravenously. Rabbits and rats were found to be more sensitive to the *N*-methylated derivatives. In cats, all 6 compounds had approximately the same AD50 and the same LD50. 2 of the *N*-methylated derivatives produced less hiccough, sneezing and coughing in the anæsthetised cats than the 2 non-methylated compounds, while the other 2 *N*-methylated derivatives were found to be free from these effects. Dogs showed less sensitivity to the *N*-methylated derivatives, which did not inhibit vagal response but induced slight hypothermia and tachycardia in the anæsthetised animals following an AD50. J. R. F.

Triethylene Melamine in Malignant Disease. E. Paterson, P. B. Kunkler and A. L. Walpole. (*Brit. med. J.*, 1953, **1**, 59.) Clinical trials were carried out with enteric coated tablets, after experiments in dogs had shown that they provided a much more constant response than plain tablets or capsules. In Hodgkin's disease, 0·2 to 0·3 mg./kg., as a single dose, or divided, was suitable for treatment and produced results similar to nitrogen mustards. It proved inadvisable to use triethylene melamine in patients with a low initial polymorphonuclear leucocyte count or to repeat the treatment unless the count was fully restored. A dosage of 0·1 to 0·2 mg./kg., repeated at intervals of several weeks gave results probably superior to radiotherapy in chronic lymphoid leukæmia, although results in myeloid leukæmia were disappointing. No beneficial effects were observed in tumours originating in epithelial or connective tissue. Oral administration of enteric coated tablets simplifies treatment compared with intravenous injections as used previously, but hæmatological controls are essential because the hæmopoietic system is highly sensitive to triethylene melamine. G. B.

Triiodothyronine, Biological Activity of. E. G. Tomich and E. A. Woollett. (*Lancet*, 1953, **264**, 726.) Experiments to determine the relative potencies of 3:5:3′-L-triiodothyronine and L-thyroxine were conducted by various methods. A comparison of the effectiveness of the two substances in preventing goitre in thiouracil-treated rats showed L-triiodothyronine to be 7·4 times as active as L-thyroxine. A comparison by a mouse-anoxia method gave an activity ratio of 4·5:1 in favour of L-triiothyronine. Assessments based on the oxygen consumption rates in rats showed L-triiodothyronine to be 5·1 times as active as L-thyroxine (or 5·3 times as active when the drugs were given by mouth). During the course of absorption and excretion studies on rats and cats with L-triiodothyronine it was found that recoveries measured by the Blau isolation technique were similar to those obtained with thyroxine. If exogenous thyroxine is deiodinated to triiodothyronine in the body those who have been using the Blau method for recovering thyroxine from animal tissues may well have been estimating triiodothyronine alone or a mixture of both substances. S. L. W.

3:5:3′-Triiodothyronine, Physiological Activity of. J. Gross and R. Pitt-Rivers. (*Biochem. J.*, 1953, **53**, 652.) The activity of 3:5:3′-triiodothyronine relative to thyroxine has been determined by the gastric prevention test in rats and the L-form found to possess 5 times the activity of L-thyroxine. D-Triiodothyronine showed only 7 per cent. of the activity of the L-form, whereas DL-triiodothyronine has about 57 per cent. of the activity of the L-form. Administered orally 3:5:3′-L-triiodothyronine has only about 86 per cent. of the activity exhibited by an equivalent dose given subcutaneously. A daily dose of 1 μg. in thyroid-ectomised rats was sufficient to maintain a rate of growth equivalent to that obtained with 5 μg. daily of L-thyroxine. Large doses of triiodothyronine reduced the rate of growth, causing at the same time enlargement of the kidney cortex, heart and adrenal glands. L-Triiodothyronine was more effective than L-thyroxine in preventing enlargement of basophil and degranulation of acidophil cells in the pituitary gland of the thiouracil-treated rat. It also prevented enlargement of the pituitary gland in the thyro-parathyroid-ectomised rat. It is suggested that 3:5:3′-L-triiodothyronine is the peripheral thyroid hormone and that thyroxine is its precursor. J. B. S.

BACTERIOLOGY AND CLINICAL TESTS

Bis*iso*quinolinium Salts, Antibacterial Activities of. H. O. J. Collier, M. D. Potter and E. P. Taylor. (*Brit. J. Pharmacol.*, 1953, **8**, 34.) A series of polymethylene bis*iso*quinolinium salts, with chain lengths of 8 to 14, 16, 18 and 20 methylene groups have been prepared and tested for antibacterial activity *in vitro* against *Streptococcus pyogenes*, *Str. fæcalis*, *Staphylococcus aureus*, *Vibrio choleræ*, *Salmonella typhi*, *Shigella shigæ*, *Sh. flexneri*, *Pseudomonas pyocyanea* and *Mycobacterium phlei*. While they showed appreciable activity none was as active as cetrimide. Antibacterial activity increased with chain length but so did toxicity. They were not considered to be of sufficient activity to be useful against bacterial infections in man. G. F. S.

Polymyxin, Absorption of, by Bacteria. A. V. Few and J. H. Schulman. (*Nature, Lond.*, 1953, **171**, 644.) Experiments are described concerning the absorption of polymyxin E by washed suspensions of several Gram-negative and Gram-positive bacteria; the release of soluble cell constituents containing free purines and pyrimidines, initiated by the presence of the antibiotic, was followed concurrently by examination of the absorption maximum at 260 mμ. An assay process was developed, based upon the fact that the polymyxins form stable unimolecular films at the air/water interface when spread upon 70 per cent. (w/w) ammonium sulphate solution. Details of the absorption experiments are given for polymyxin E for 6 organisms together with absorption isotherms, the final solutions being assayed for residual polymyxin and for the presence of released cell constituents absorbing at 260 mμ. Determinations of the sensitivities of the organisms by streaking 24-hour broth cultures upon nutrient agar containing increasing quantities of polymyxin E showed that *Ps. denitrificans*, *B. subtilis* and *E. coli* were all inhibited by 5 μg./ml., whereas *S. aureus*, *P. vulgaris* and *Str. fæcalis* were not inhibited by 100 μg./ml. The initial absorption process was complete at a polymyxin concentration in the supernatant layer of 100 to 150 μg./ml., after which further absorption was slight. Of 4 strains of *E. coli* investigated, 3 showed agglutination phenomena which were maximal at supernatant concentrations of 150 μg./ml. For highly resistant organisms, polymyxin does not significantly increase the release of material showing absorption at 260 mμ, although with sensitive bacteria the presence of polymyxin caused a rapid release of cellular material, complete at low concentrations of polymyxin in the supernatant liquid. With *B. subtilis* the absorption of polymyxin and release of material showing absorption at 260 mμ was rapid and unaffected by incubation times between 5 and 60 minutes. R. E. S.

Tubercle Bacilli; Variations in Virulence Effected by Tween 80 and Thiosemicarbazone. H. Bloch and H. Noll. (*J. exp. Med.*, 1953, **97**, 1.) Tubercle bacilli were grown in the presence of concentrations of tween 80 ranging from 0·05 to 2·1 per cent., and equal numbers of viable bacteria from these cultures were compared in infection experiments in mice, the average survival time of the mice being used as a criterion of the virulence of the bacilli. Reduction of virulence was slight in bacterial suspension from cultures with tween 80 ranging from 0·05 to 1·0 per cent., but was considerable in cultures with 2·1 per cent. Bacteria grown in the presence of 2·1 per cent. of tween 80 gave rise to the same number of colonies, *in vitro*, as bacteria grown in ordinary media,

(ABSTRACTS *continued on p.* 568).

LETTER TO THE EDITOR

The Melting Point of 4:4′-Diaminodiphenyl sulphone (Dapsone)

SIR,—4:4′-Diaminodiphenyl sulphone is required by the British Pharmaceutical Codex to have a melting point as defined in the British Pharmacopœia 1953, page 703, lying between 176° C. and 179° C. (B.P.C. 1949, Supplement 1952, page 19), and material conforming to these limits has been manufactured over a number of years without difficulty. Recently, however, samples have been encountered melting sharply in the region of 180·5° C.; these high melting temperatures were not associated with any known modifications in the method of preparation and it is hardly possible that they represent a purer product. Thus, on frequent occasions, samples having melting points in the region of 178° C. exhibited, after grinding, melting points in the region of 180·5° C., and on a few occasions the same effect was obtained by leaving samples melting at about 178·5° C. in an oven at 50° C. for several days. On the other hand, recrystallisation of the high melting material from water and isolating at 5° C. caused the melting point to fall from 180·5° C. to 178·5° C. and it remained at this level on repeated recrystallisation; recrystallisation from methanol had the same effect.

We conclude from these observations that 4:4′-diaminodiphenyl sulphone can be obtained in at least two forms, melting respectively at about 178·5° C. and 180·5° C.; each of these melting points represents material of a high degree of purity, but the material having the higher melting point does not conform strictly to the requirements of the B.P.C. Monograph.

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(ABSTRACTS *continued from p.* 567).

but their oxygen uptake was increased. Virulent bacteria grown in the presence of high amounts of tween 80 decolorised methylene blue in a test in which organisms from the same virulent strain but cultured without tween 80 did not reduce the dye (a positive methylene blue test is typical of non-virulent tubercle bacilli). Essentially the same changes occurred when virulent tubercle bacilli were grown in the presence of 0·5 μg./ml. of *p*-formacetanilide thiosemicarbazone; this amount was not sufficient to prevent the growth of bacteria or reduce the number of viable cells in a culture, but it reduced the virulence of bacteria considerably and rendered them capable of decolorising methylene blue. Cord factor, a lipid constituent of virulent bacteria which is toxic for mice, was shown to be present in filtrates from cultures of virulent bacteria when the media contained 2 per cent. tween 80, but could not be recovered from culture filtrates containing 0·05 per cent. On the other hand, no toxic material could be extracted from bacteria grown in the presence of 0·5 μg./ml. of *p*-formacetanilide thiosemicarbazone. S. L. W.