ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Amino-acids, Paper Chromatographic Method for the Determination of. A. L. Levy. (*Nature, Lond.*, 1954, **174**, 126.) The method is based upon the conversion of the amino-acids to their yellow 2:4-dinitrophenyl derivatives, followed by the separation of these compounds on a two-dimensional filter-paper chromatogram, and their subsequent elution from the paper and estimation at 360 mμ. Dinitrophenylation is effected quantitatively by stirring an aqueous solution of the amino-acids (20 to 30 μM in 3 ml.) with a slight excess of 2:4-dinitroflurobenzene for 80 minutes at *p*H 9·0 and 40° C., the *p*H being maintained at this value throughout this period by intermittent additions of standard alkali. Excess reagent is then extracted with ether, the solution acidified, and the dinitrophenyl amino-acids extracted with ether. The aqueous solution, which contains dinitrophenyl arginine and α-dinitro-phenyl histidine, is diluted to 10 ml. A 2 ml. aliquot of the ether solution and a 1 ml. aliquot of the water solution are applied to adjacent corners of an 18¼ by 22½ inches sheet of Whatman No. 1 filter paper, which is then irrigated by the ascending procedure with a toluene-chloroethanol-pyridine-0·8N ammonia (5:3:1.5:3) mixture. The chromatogram is dried for 3 to 4 hours at 40° C. and the spots due to dinitrophenyl arginine and α-dinitrophenyl histidine excised. The paper is then run on the second dimension by the descending procedure with 1·5M aqueous phosphate buffer. The spots are cut out, extracted with water and the optical density at 360 mμ measured. The dinitrophenol by-product does not interfere with the chromatography when the condensation is carried out as described above in a solution approximately 0·1M with respect to amino-acids. A. H. B.

Codeine, Chromatographic Determination of. G. C. McElheny, G. DeLamater and R. D. Rands. (*Analyt. Chem.*, 1954, **26**, 819). A chromatographic method of analysis is described which is applicable to opium, to intermediates, and to complex pharmaceutical preparations containing codeine. The procedure is based on the extraction of the opium with a saturated solution of sodium acetate, the nonphenolic alkaloids being isolated as a benzene solution and separated on a column of alumina with the aid of special developing solutions. By the method described thebaine, cryptopine, neopine, papaverine, and narcotine are eluted from the column before codeine and are separated completely from it, but not from one another. After deposition of the alkaloids on alumina from benzene solution a number of developing solutions containing *iso*propanol, chloroform, and benzene are used to elute the alkaloids; the effects of solvent changes on the elution are discussed. Tables are given showing the accuracy and precision as applied to the assay of opium. R. E. S.

Cottonseed Oil, Detection of Trichloroethylene in. G. A. Wiese and C. L. Jesina. (*Drug Standards*, 1954, **22**, 105.) In view of the increasing use of the toxic solvent trichloroethylene in the extraction of cotton-seed oil, the authors suggest that the U.S.P. XV should contain the following test for its presence. Add 2 ml. of A.R. pyridine to 2 ml. of 10 per cent, sodium hydroxide (U.S.P.)

solution in a test tube, which is then placed in a water bath at 90° C. After 5 minutes remove the tube from the bath and immediately add 1 ml. of the oil under test. A pink colour, developing in the pyridine layer within 20 minutes and varying from deep pink to faintly pink, indicates the presence of trichloroethylene. Comparison tubes containing: *a*, pressed cotton-seed oil, *b*, oil containing 1 in 100,000, *c*, 1 in 200,000 and *d*, 1 in 300,000 of trichloroethylene, treated as described above, are used to establish the concentration of the solvent.

J. R. F.

Dihydrostreptomycin, Streptomycin and Framycetin and their Salts, Titration of, in Non-aqueous Media. P. Pénau, E. Saïas and J. Ferdet. (*Ann. pharm. franç.*, 1954, **11**, 740.) The antibiotics were assayed by titration with perchloric acid in the presence of ethylene glycol and acetic acid. Large quantities of sulphate interfered with the colour changes of the indicators, unless previously removed with benzidine. The use of barium acetate for the removal of sulphate was not successful. The quantity of sulphate present in dihydrostreptomycin and streptomycin sulphates was first determined by titration with 0·1 N sodium hydroxide, using thymolphthalein as indicator (1/3 rd. of the sulphate was titrated). Sulphate in framycetin sulphate (an antibiotic from *Streptomyces decaris*) was determined by titration in the presence of pyridine. The antibiotic salts were dissolved in perchloric acid and ethylene glycol. So as to avoid an excess of the reagent, sufficient 0·05 M benzidine in glacial acetic acid was added to precipitate only 95 per cent. of the sulphate present. After allowing precipitation to occur, 2 drops of crystal violet indicator solution were added and the solution titrated with potassium phthalate. The quantity of dihydrostreptomycin, streptomycin or framycetin was calculated, an arbitrary figure being used for the molecular weight of framycetin. When titrating organic acid salts of the antibiotics, the benzidine solution was replaced by glacial acetic acid.

G. B.

Fluoride, Colorimetric Determination of. M. L. Nicholls and A. C. Condo. (*Analyt. Chem.*, 1954, **26**, 703.) Eighteen organic reagents, giving colour reactions with ferric iron in acid solution were studied and the wavelength of maximum absorption was determined; in addition a quantitative study was made of the effect of both *p*H and ferric ion concentration upon the ability of the fluoride ion to cause a fading effect. The fading effect was determined by measurement of the increase in transmittancy of the coloured complex at constant *p*H and constant ferric ion concentration as the fluoride ion concentration was increased. It was found that the ferric ion complexes of 3 reagents, resorcylaldoxime, 5-phenylsalicylic acid, and resacetophenone, gave approximately a 4 per cent. change in percentage transmittancy per 1 p.p.m. of fluoride at a *p*H of 2 to 3. The coloured complex of resorcylaldoxime was unstable on standing but the other two complexes were stable and gave reproducible and sensitive results for fluoride in concentrations from 0 to 6 p.p.m. Foreign ions which formed stable complexes with iron, such as citrate and tartrate, and also a high concentration of aluminium, interfered with the determination.

R. E. S.

Glucose, Microdetermination of. B. Mendel, A. Kemp and D. K. Myers. (*Biochem. J.*, 1954, **56**, 639.) A colorimetric micromethod for the determination of glucose is described, based on the formation of a bluish pink colour when one volume of a dilute solution of glucose is heated with 3 volumes of

96 per cent. w/w sulphuric acid; the intensity of the pink colour is proportional to the concentration of glucose. Details of the method are given together with light extinction curves for the solutions obtained; the final colour is measured at 520 mμ. The reaction is highly dependent on the concentration of sulphuric acid and appears to be specific for glucose, fructose and related carbohydrates. The concentration of glucose in the test solution is read from a standard curve; up to a concentration of 15 mg./100 ml., the relationship between the glucose concentration and colour intensity is linear, an extinction of about 0·160 at 520 mμ. being obtained with a aqueous solution containing 10 mg. glucose/100 ml The glucose content of blood can be determined after the blood has been deproteinised with a trichloroacetic acid solution containing silver sulphate. R. E. S.

***iso*Propanol in Dextran Solution, Determination of.** G. J. Frisone. (*Analyt. Chem.*, 1954, **26**, 924.) The method is a modification of the dichromate oxidation procedure of Stanley (*J. Assoc. off. agric. Chem., Wash.*, 1942, **25**, 693), the time per determination being shorter. By varying the concentration of sulphuric acid and of the potassium dichromate it was found possible to oxidise *iso*propanol to acetone within 5 minutes on a boiling water bath. Excess dichromate was then removed by the addition of sodium hydroxide, and the acetone was distilled into hypoiodite solution; excess iodine remaining was then titrated with standard thiosulphate. The accuracy of the method depended both on the primary distillation of the *iso*propanol from the dextran solution and on the secondary distillation of the acetone from the oxidation solution; the results obtained showed that the first distillation was not less than 98 per cent. complete. In the secondary distillation, analysis of two successive 60 ml. fractions of distillate showed that all the acetone was recovered in the first fraction. R. E. S.

Sulphonamides, Paper Chromatography of. P. Heinänen, L. Tuderman and L. Rämö. (*Farm. Notisblad.*, 1954, **63**, 66.) The method is a development of that previously described by the authors (*Farm. Notisblad.*, 1951, **60**, 84) using *n*-butanol saturated with 3 per cent. ammonia. Ehrlich's reagent is used for the detection of the spots. Sulphanilamide, sulphathiazole, sulphamethazine, sulphadiaizine and sulphacetamide may be separated and determined by extracting the single spots with ethanol and determining spectrometrically at 260 mμ. By this method it is possible to obtain a mean deviation of ± 1 to 3 per cent. G. M.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Carbohydrates, Detection of. R. U. Lemieux and H. F. Bauer. (*Analyt. Chem.*, 1954, **26**, 920.) A slightly alkaline (*p*H 7·2) spray reagent for chromatography is prepared by mixing 4 parts of 2 per cent. aqueous sodium metaperiodate and 1 part of 1 per cent. potassium permanganate in 2 per cent. aqueous sodium carbonate solution. The presence of a periodate- or permanganate-reducing substance on the paper results in the formation of a greenish-yellow spot, usually in a short period of time, the background retaining the permanganate colour for at least 1 hour at room temperature. The reagent can be used for the detection of 2 to 3 μg. of glucose, xylose, ascorbic acid, sorbose, maltose, cellobiose, and olefines; 5 to 8 μg. glucurono-lactone, 3-methylglucose, mannitol, erythritol, ethylene glycol, xylonolactone, and tartaric acid; 10 to 15 μg. of substances which reduce periodate slowly such as β-methyl glucopyranoside, sucrose, trehalose, pentaerythritol, 2:3:4:6-tetramethylglucose, 2:3-butanediol, and lactic acid. R. E. S.

Erisimin, A Cardioactive Glycoside from *Erysimum canescens*, Roth. V. V. Feofilaktov and P. M. Loshkarev. (*Doklad. Akad. Nauk*, SSSR, 1954, **94**, 709.) The properties of erisimin ($C_{29}H_{42}O_9 \cdot 2_2H_2O$), a cardiac glycoside separated from the herb *Erysimum canescens*, are described. It forms colourless needles, m.pt. 168 to 172° C. (decomp.), $[\alpha]_D^{20°C.} = +43{\cdot}48°$ (in ethanol); soluble in ethanol, in methanol and in acetone; insoluble in benzene, in light petroleum and in ether. It gave the characteristic colour reactions of the cardiac glycosides. No methoxy groups are present. The pharmacological action is similar to that of strophanthin; it contains 62,000 frog units per g., and in the cat assay the effective dose was 0·86 to 0·90 mg./kg. The triacetate ($C_{35}H_{48}O_{12}$) had m.pt. 230 to 232° C. The triacetate oxime ($C_{35}H_{49}O_{13}$) formed prismatic crystals with m.pt. 243 to 244° C. and $[\alpha]_D^{20°C} = 52{\cdot}19°$ (ethanol). Hydrolysis of erisimin gave erisimidin ($C_{23}H_{32}O_6 \cdot 2C_2H_5OH$), m.pt. 161 to 164° C., soluble in ethanol and in methanol, less soluble in benzene and practically insoluble in water. By recrystallisation from hot chloroform-benzene, this was obtained free from ethanol with m.pt. 227 to 229° C. On heating with sodium hydroxide solution it formed the *iso*-aglycone, *iso*erisimidin, with m.pt. 210 to 213° C. The aglycone contains a β-oriented lactone ring at the C17 position, an angular CHO group at the C10, a secondary OH group at the C3, and a tertiary β-oriented OH group at the C14 position; the position of a third OH group was not established. The sugar component is of the digitoxose type. E. H.

ORGANIC CHEMISTRY

Analgesics, Stereochemistry of. A. H. Beckett and A. F. Casy. (*Nature, Lond.*, 1054, **173**, 1231.) Certain isomers, (−)-methadone, (−)-ethyl-3-dimethylamino-1 : 1-diphenylbutyl sulphone, and (+)-3-dimethylamino-1 : 1-di (2′-thienyl)-but-1-ene which are the more analgesically active isomers of the respective enantiomorphic pairs, were shown to possess identical configurations related to that of D-(−)-alanine. Evidence is presented indicating that drugs exhibiting high analgesic activity have configurations complementary to that of specific receptor sites through which the pharmacological effect is mediated. The validity of the method by which Bick (*Nature, Lond.*, 1952, **169**, 755) assigned the absolute configuration of morphine as related to L-(+)-alanine is questioned. J. R. F.

Azaserine, a New Tumor-inhibitory Substance, Isolation and Characterisation of. S. A. Fusari, R. P. Frohardt, A. Ryder, T. H. Haskell, D. W. Johannessen, C. C. Elder and Q. R. Bartz. (*J. Amer. chem. Soc.*, 1954 **76**, 2878.) Detailed methods, involving chromatographic techniques used for the isolation of crystalline azaserine from culture broth filtrates of a *Streptomyces*, are described. Azaserine crystallises as light yellow-green needles from aqueous ethanol. It undergoes decomposition over a wide range (146 to 162° C.) upon melting. Dissolved in *p*H 7·0 phosphate buffer, it shows characteristic absorption in the ultra-violet region with one sharp, well-defined peak of $E_{1\,cm.}^{1\,per\,cent.}$ 1140 at λ 250·5 mμ. The biological activity is destroyed and a hyperchromic shift to λ 252 mμ ($E_{1\,cm.}^{1\,per\,cent.}$ 1230) occurs in 0·1N sodium hydroxide. There is a complete disappearance of ultra-violet absorption in 0·1N hydrochloric acid which is associated with a total loss of biological action and an evolution of nitrogen. The infra-red absorption curve is recorded. A sharp absorption band at 4·66 μ is present and, like the ultra-violet absorption peak at 250·5 mμ, disappears completely upon acidification. The purity of azaserine was established by the Craig countercurrent technique. A. H. B.

PLANT ANALYSIS

Corchorgenin, A New Cardiac-active Aglycone from *Corchorus olitorius* L. J. K. Chakrabarti and N. K. Sen. (*J. Amer. chem. Soc.*, 1954, **76**, 2390. A new cardiac-active aglycone, $C_{23}H_{32}O_6$, m.pt. 227° C., $[\alpha]_D^{21}$ + 90 (ethanol) was isolated by chromatography of an ethanolic extract of the defatted seeds of *C. olitorius L.* grown in west Bengal. The aglycone is extremely bitter, soluble in ethanol, methanol and pyridine; sparingly soluble in water-chloroform and benzene, and insoluble in ether. It contains a hydroxyl group which can be acetylated to yield a monoacetate m.pt. 240 to 242° C. It exhibits a typical digitalis-like cardiac action which can be demonstrated with a concentration of 5×10^{-6}; this activity is practically identical with that of ouabain. In cats, its potency indicates that it is more active than either of the isomeric genins, corchortoxin or strophanthidin of *S. kombe*. It has been provisionally named "corchorgenin." A. H. B.

β-Sitosterol and Corchorolic Acid, Isolation of. G. Soliman and W. Saleh. (*J. chem. Soc.*, 1954, 1506.) The oil obtained by extraction of the seeds of *C. olitorius* with light petroleum was hydrolysed with ethanolic potassium hydroxide and β-sitosterol was obtained from the unsaponifiable matter. The latter was also isolated in a similar manner from the seeds of *C. capsularis*. The yellow substance obtained from the ethanolic extract of the seeds was a mixture of a phenol and an aliphatic hydroxy-acid, corchorolic acid, $C_{26}H_{52}O_3$, m.pt. 96° C. A. H. B.

TOXICOLOGY

Toxicology, Paper Chromatography in. A. S. Curry and H. Powell. (*Nature, Lond.*, 1954, **173**, 1143.) A solvent system is reported for the chromatographic separation of basic organic compounds obtained in the toxicological analysis of viscera. The use of the modified Dragendorff reagent of Munier and Macheboeuf (*Bull. Soc. Chim. Biol.*, 1951, **33**, 846), provided an additional criterion to the R_F value, giving a variety of shades of orange and red; 20 μg. of a substance could be easily detected. The solvent was a mixture of *n*-butanol (50 ml.): water (50 ml.): citric acid (1 g.), the upper layer being used. Before use the paper (Whatman No. 1) was dipped in a 5 per cent. sodium dihydrogen citrate solution, any excess liquid removed by blotting, and then dried at 60° C. for 25 minutes. The R_F values are given for 28 basic substances. R. E. S.

BIOCHEMISTRY

BIOCHEMICAL ANALYSIS

Calcium in Biological Materials, Estimation of. J. G. Llaurado. (*J. clin. Path.*, 1954, **7**, 110.) A method is described in detail for the estimation of calcium, when mixed with other cations in biological materials, using a flame photometer burning coal-gas, an interference filter with a peak at 620 mμ, and an additional didymium glass to remove interference by sodium. The sample is treated with ammonium oxalate, the precipitate removed by centrifugation, dissolved by hydrochloric acid and submitted to flame photometry; a linear relationship between galvanometer deviations and calcium concentrations from 0 to 40 p.p.m. was shown. Tables of results are given and the sodium and potassium concentrations which can be present without affecting calcium

estimation are discussed. An interference effect of oxalic acid on calcium flame excitation is described for the first time, this being overcome by heating at 300° to 400° C., to destroy the oxalic anion. Results are given showing complete recovery of calcium in distilled water and in human serum. R. E. S.

Isoniazid, Estimation of. E. I. Short. (*Lancet*, 1954, **266**, 656.) A rapid and convenient method for the determination of isoniazid blood levels depends upon the reaction of the $-NH_2$ group of the hydrazide with 1 : 2-naphthoquinone-4-sulphonic acid and measurement of the colour (absorption maximum 455 mμ) which develops. Since isoniazid does not penetrate the circulating red cells, samples of serum or plasma may be used instead of whole blood. A "protein-free" filtrate is prepared by adding to 4 ml. of plasma or serum, 5 ml. of distilled water, 4 ml. of a 9 per cent. solution of zinc sulphate and 5 ml. of 0·1 N sodium hydroxide solution, mixing after each addition, heating in a water bath for 3 minutes, cooling and filtering through Whatman no. 42 paper. To 9 ml. of the filtrate is added 1 ml. of a 0·1 per cent. w/v solution of 1 : 2-naphthoquinone-4-sulphonic acid The mixture is allowed to stand for about 1 hour before measuring the light absorption through an Ilford no. 601 or 621 filter. The measurement is made against a blank consisting of normal serum or plasma similarly treated by protein precipitation and reaction with 1 : 2-naphthoquinone-4-sulphonic acid. The result is read from a standard curve prepared with the aid of normal serum or plasma containing added isoniazid. Recoveries are satisfactory for clinical purposes, the average in these experiments being 100·5 per cent. The method is more accurate with the higher levels of isoniazid, the standard error of 6 estimates falling from ± 3·7 per cent. at 0·125 mg./100 ml. to ± 2·7 per cent. at 0·3 mg./100 ml. The method, with some modifications, may be applied to tissue, such as lung, or urine. G. B.

Isoniazid, Estimation of, in Biological Fluids. W. F. J. Cuthbertson, D. M. Ireland, W. Wolff and S. W. A. Kuper. (*Brit. med. J.*, 1954, **1**, 609.) The method depends on the reaction between isoniazid and picryl chloride (2-chloro-1 : 3-5-trinitrobenzene) to form a compound which may be measured absorptiometrically. For the determination in plasma (heparinised) the protein is first precipitated and the protein-free fluid employed. The extraction is carried out by a two-stage process using butanol-ether and 0·1 N hydrochloric acid. The picryl chloride is added to the acid extract, readings made against water in a Hilger absorptiometer, and the results calculated by comparison with a standard curve. This is prepared from readings obtained by applying the method to a series of known freshly prepared solutions of isoniazid in non-hæmolysed plasma. The method is also applicable to urine. The reproducibility of the method is satisfactory; the absorptiometer readings are linearly related to the amounts of isoniazid present within the range 0 to 20 μg. The standard deviation of a single estimation is about ± 10 per cent. *p*-Aminosalicylic acid was found to interfere with the assay, but not streptomycin. The method is not sensitive enough to detect any isoniazid in plasma 12 hours after the last dose of the drug. Isoniazid was estimated by this method in the plasma of 24 patients and in the urine of 9, and the results of the estimations are given. S. L. W.

Methanol in Biological Fluids, Determination of. M. Feldstein and N. C. Klendshoj. (*Analyt. Chem.*, 1954, **26**, 932.) In the method described, methanol is separated from biological material by diffusion in a standard Conway microdiffusion cell and is absorbed by a solution of sulphuric acid; it

is then determined quantitatively by oxidation to formaldehyde and subsequent reaction with chromotropic acid. Known amounts of methanol, added to blood and urine samples, showed recovery results ranging from 80 to 85 per cent. for less than 0·10 mg. of methanol. The incomplete recovery represents the diffusion equilibrium of methanol between the two solvents in the inner and outer compartments of the Conway cell; since, however, the equilibrium is constant under the conditions of the test, the methanol content can be calculated by applying a diffusion correction factor of 100/82·5 or 1·21. Using this factor, recoveries of methanol were shown to be 97 to 103 per cent. R. E. S.

Salicylate in Biological Fluids, Determination of. P. Trinder. (*Biochem. J.*, 1954, **57**, 301.) A rapid method for the determination of salicylate in biological fluids is presented, based on a reagent containing ferric nitrate, mercuric chloride and hydrochloric acid, which precipitates the proteins and simultaneously reacts with salicylic acid to give a purple colour. Details of the procedure, which can be completed in 5 minutes, are given. Recovery results on samples of serum, whole blood and urine, to which known amounts of sodium salicylate had been added ranged from 97 to 100·5 per cent.; the results were not affected by the presence of 100 mg. of phosphate ion, 20 mg. of bilirubin, 25 mg. of phenol, 10,000 I.U. of heparin, 1000 mg. of glucose or 1000 mg. of urea, per 100 ml. of serum. The blank values on normal serum and plasma samples are less than 1·1 mg. of salicylic acid/100 ml. R. E. S.

Urinary 17-Ketosteroids, Determination of. H. Werbin and S. Ong. (*Analyt. Chem.*, 1954, **26**, 762.) A modification of the Zimmermann reaction is described in which the absolute ethanol technique of Callow *et al.* (*Biochem. J.*, 1938, **32**, 1312) was used for the colour formation; the ethanol concentration of the pink solution was adjusted to 37 per cent. before extraction, and an improved apparatus is introduced for the extraction of the steroid chromogens. The modifications practically eliminated substances which interfered with absorption measurements at 400 mμ and gave an average recovery of 96 per cent. of the crystalline steroid when added to urine extracts. The absorption spectra of the chromogenic material from a number of urine extracts are given together with quantitative results for the concentration of 17-ketosteroids in neutral urine extracts. R. E. S.

CHEMOTHERAPY

8-Aralkyltheophyllines and Related Compounds. G. P. Hager, J. C. Krantz Jr., J. B. Harmon and R. M. Burgison. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43** 152.) A number of 8-(substituted aralkyl) theophyllines were prepared in a search for compounds combining the hypotensive activity of 8-benzyltheophylline with greater solubility in water. They were obtained by fusion of 1:3-dimethyl-5:6-diaminouracil with the appropriate carboxylic acid and ring closure of the amide with sodium hydroxide solution or phosphorus oxychloride. The compounds generally showed a hypotensive effect when injected intravenously, but were inactive orally owing to lack of absorption from the gastrointestinal tract. They appeared to act directly on the arterial musculature. Polar solubilising groups of the electron releasing type did not decrease the activity of the compounds when substituted in the aralkyl group, but electron withdrawing groups decreased the activity slightly. The presence of a substituent at position 7 of the theophylline part of the molecule was found to be detrimental to hypotensive activity. G. B.

8-(9-Fluorenyl)theophylline and Related Compounds. G. P. Hager, C. T. Ichniowski and B. Misek. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 156.) 8-(2-Thienyl) theophylline was prepared by fusion of 2-thienylacetic acid with 1:3-dimethyl-5:6-diaminouracil and cyclisation of the amide with sodium hydroxide. 8-(3-Thienyl) theophylline was obtained similarly. 8-(9-Fluorenyl) theophylline was prepared by dehydrogenation of the Schiff base formed by the reaction of 9-formylfluorene with 1:3-dimethyl-5:6-diaminouracil. In experiments on anæsthetised dogs, 8-benzyl, 8-benzhydryl, 8-(2-thienyl), and 8-(3-thienyl) theophylline produced similar effects, the thienyl derivatives being slightly less active than the other compounds. Suitable doses produced a fall in blood pressure without an accompanying effect on the respiration. 8-(9-Fluorenyl) theophylline when similarly tested produced a pronounced transient fall in blood pressure and transient cessation of respiration followed by a longer compensatory rise in blood pressure, with respiratory recovery. 2-(9-Fluorenyl) 2-iminazoline and 2-(9-fluorenyl) 2-(9-fluorenylidenemethyl) 2-iminazolidine were also prepared but gave variable results during the preliminary pharmacological tests. G. B.

Mercurial Diuretics. L. H. Werner and C. R. Scholz. (*J. Amer chem. Soc.*, 1954, **76**, 2453.) Various mercaptans were combined with 3-hydroxy-mercuri-2-hydroxypropylcarbamylnicotinic acid sodium salt to produce compounds for investigation of the structural requirements of the thiol for maximal detoxification. Animal studies indicated that the polyhydroxyalkylthiols were highly effective in reducing the cardiac toxicity of mercurials; of these 1-thiosorbital appeared most promising. A number of mercurated compounds of different structures were prepared and combined with 1-thiosorbitol. A. H. B.

***iso*Nicotinyl Hydrazide, Mechanism of Action of.** D. S. Goldman. (*J. Amer. chem. Soc.*, 1954, **76**, 2841.) The *iso*nicotinyl hydrazide analogue of diphosphopyridine nucleotide (I) in which the nicotinamide moiety of the latter is replaced by *iso*nicotinyl hydrazide, was prepared by incubating diphosphopyridine nucleotide with beef spleen diphosphopyridine nucleotidase, and a large excess of *iso*nicotinyl hydrazide. Extinction coefficients for (I) at several *p*H's are given and the chemical and enzymatic activities are described. From the result obtained, it was considered that the antituberculous action of *iso*nicotinyl hydrazide might be due to the intracellular formation of an inactive pyridine nucleotide analogue with a concomitant reduction in cellular oxidative metabolism. A. H. B.

PHARMACY

NOTES AND FORMULÆ

Antacid Capacity, A Method for Appraisal of. A. M. Corrente. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 242.) The following method was used. Place 100 ml. of water in a beaker, maintain the temperature at 37° C. and stir continuously so that particles are only gently moved about. Add the test material (powder or tablets) and record the *p*H after 10 minutes. Add 10 ml. of 0·1 N hydrochloric acid, recording the *p*H after 45 seconds and 9 minutes, and continue the process, adding 10 ml. of acid every 10 minutes and recording the *p*H until the 45 second and 9 minute readings are the same, usually about *p*H 2. A variation involving the addition of pepsin (0·5 per cent. in 0·1 N hydrochloric acid) alters the pattern of response. The addition is made after the initial increments of the acid alone has reduced the *p*H to 6. Plot

the volume of acid against the two series of *p*H readings. Powders containing sodium bicarbonate with calcium carbonate or magnesium oxide showed a considerable capacity for neutralising acid, but within a range which is too alkaline, bearing in mind that the usual aim in antacid therapy is to maintain the *p*H within the range 3·0 to 3·5. Tablets of dried aluminium oxide showed an effect which varied with temperature and the presence of pepsin. At 37° C., in the presence of pepsin there was a large buffering capacity at low *p*H (2·0 to 3·0). Tablets of magnesium trisilicate with aluminium hydroxide gel were of limited buffering capacity. Some other commercial tablets were examined and the results tabulated. G. B.

Dextran (*New and Nonofficial Remedies, J. Amer. med. Ass.*, **154**, 154, 241.) Dextran is a water-soluble glucose polymer of high molecular weight obtained by the action of *Leuconostoc mesenteroides* on sucrose. The marketed product has an average molecular weight of about 75,000. It is administered intravenously as a 6 per cent. solution in isotonic sodium chloride to expand plasma volume and maintain blood pressure in the emergency treatment of hæmorrhagic and traumatic shock. The usual dose is 500 ml., given over a period of 15 to 30 minutes, and may be repeated when necessary if blood products are not available or indicated. For the treatment of hæmorrhage, the dose should be just sufficient to raise the systolic pressure to 80 to 85 mm. of Hg to avoid the production of further bleeding and dangerous dilution of the circulating blood. Dextran is excreted in the urine to the extent of 30 to 50 per cent., the remainder being metabolised. Almost no adverse reactions have been observed after repeated injections, although it appears to have a tendency to produce antigen-antibody type of reactions in some patients. These reactions are infrequent and mild if the dextran is refined and hydrolysed sufficiently to provide an average molecular size similar to that of serum albumin. Solutions of dextran do not require refrigeration. They should not be regarded as a substitute for whole blood or its preparations or for combating anæmia secondary to hæmorrhage, or severe wounds. G. R. K.

Red Blood Cells, Preservation of. P. L. Mollison. (*Brit. med. Bull.*, 1954, **10**, 27.) It is now generally assumed that the loss of viability of red cells stored at + 4° C. is due to the continuance of metabolic activity. This activity can be arrested by freezing, but the lowest practicable temperature at which blood can be stored without damage is − 2° C. and red cells stored at this temperature survive no better than those stored at + 4° C. Red cells mixed with glycerol can be frozen and thawed without lysis; there is a critical salt concentration which determines cell damage and in the presence of a glycerol concentration of about 23 per cent. w/v this salt concentration is not reached. It is already possible, using saline-glycerol as preservative, to maintain the viability of red cells for at least 6 months at − 79° C. but has not so far proved possible to preserve them for more than about 3 months at − 20° C. One way of improving preservation at − 20° C. is to leave part of the plasma with the red cells and to use a citrate-glycerol solution instead of saline-glycerol; this solution has a final sodium citrate concentration of about 1·5 per cent. w/v and a glycerol concentration of about 30 per cent. w/v. Red cells stored in this mixture for 3 months at − 20° C. undergo very little lysis and often have about a 90 per cent. survival after transfusion; they are agglutinated about as well as fresh red cells. Before the red cells can be transfused the glycerol must be removed by a continuous washing process such as described by Chaplin and Veall (*Lancet*, 1953, **264**, 218); a bottle of blood can be washed free of glycerol in about 2 hours. S. L. W.

PHARMACOLOGY AND THERAPEUTICS

Adrenaline, Noradrenaline and Dihydroergotamine, Action of, on the Human Uterus. W. Garrett. (*Lancet*, 1954, **266**, 1060.) Adrenaline and noradrenaline stimulate strips of uterine myometrium taken from both pregnant and non-pregnant women; noradrenaline being about 3 times more potent than adrenaline. Noradrenaline also causes uterine contraction *in vivo* during late pregnancy and labour whereas adrenaline inhibits uterine rhythm. *In vitro* after dihydroergotamine adrenaline and noradrenaline no longer cause contraction. *In vivo*, during late pregnancy and labour the action of both amines is unaffected. M. M.

Antiepileptics, Analgesic Effects of Clinically Useful. E. A. Swinyard, D. L. Smith and L. S. Goodman. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 212.) Analgesic potencies were determined by measurement of the dose required to double the reaction time (withdrawal of the tail in response to a heat stimulus) in rats. Minimal neurological toxicity was assessed on the appearance of abnormalities in positioning sense, righting, gait and stance, muscle tone, equilibrium or other signs and was determined at the time of peak analgesic effect. The most powerful analgesic was phenobarbitone, followed by phethenylate, methylphenobarbitone, mephenytoin, phenytoin, paramethadione, phenacemide and trimethadione. All the antiepileptic drugs tested were more effective as analgesics than acetylsalicylic acid and less active than morphine. Neurotoxic action was observed to occur before analgesic activity, except for trimethadione and phenacemide. G. B.

Blood Theophylline Concentration Following the Oral Administration of Theophylline Ethylenediamine and Theophylline *iso*Propanolamine. A. E. Vivino. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 234.) Doses of theophylline with ethylenediamine and with *iso*propanolamine were administered to 25 normal subjects aged 20 to 28. A preliminary blood sample was taken for use in a blank determination. Samples were also drawn at intervals up to 10 hours after oral administration of 0·2 g. tablets of the drug, and assayed. Theophylline appeared in the blood within 15 minutes, and the concentration rose rapidly to a maximum (about 200 μg./100 ml. for theophylline with ethylenediamine and 350 μg./100 ml. for theophylline with *iso*propanolamine) at 1 hour. This high level was maintained for 4 hours, after which there was a gradual decrease in the blood theophylline level. Theophylline with *iso*propanolamine gave a more rapid rise in blood concentration and higher and more prolonged blood levels than theophylline with ethylenediamine. G. B.

Polymyxin B Therapy of Meningitis. J. P. Biehl and M. Hamburger. (*Arch. intern. Med.*, 1954, **93**, 367.) Details are given of 6 cases of meningitis following spinal anæsthesia or other procedures affecting the central nervous system. In 5 patients the infecting organism was identified as *Pseudomonas æruginosa*, while in the sixth it was *Proteus rettgeri*. The first two cases received penicillin, streptomycin and oxytetracycline, the second being given chloramphenicol and sulphadiazine in addition, but treatment was unsuccessful and both died. The third patient was given polymyxin B when his condition was critical but it did not prevent death. The fourth case was treated unsuccessfully with various antibiotics and sulphisoxazole but prompt recovery occurred when polymyxin B was given intrathecally. A prompt recovery also took place in the

fifth patient, who acquired a *Pseudomonas* infection while receiving intrathecal streptomycin for meningeal tuberculosis, when polymyxin B was added to the streptomycin injection. In the sixth patient the infecting organism, *Proteus rettgeri*, was sensitive to chloramphenicol as well as polymyxin B and the contribution of the latter to the patient's recovery may have been insignificant.

H. T. B.

Polymyxin; Meningitis Treated with. D. H. Trapnell. (*Lancet*, 1954, **266**, 759.) A case of *Pseudomonas pyocyanea* meningitis following a cerebrospinal fluid fistula in a boy aged 5½ is described. The causal organism was resistant to sulphonamides, penicillin, aureomycin, oxytetracycline and chloramphenicol, but was sensitive to polymyxin B. Treatment with polymyxin was commenced with a dose of 200,000 units intramuscularly 4-hourly and 40,000 units intrathecally 12-hourly for 3 doses and thereafter once daily. After a marked initial improvement, treatment was stopped on the 8th day, but was resumed 3 days later after a relapse. Polymyxin B was restarted in a dose of 200,000 units intramuscularly 4-hourly and 40,000 units intrathecally daily. Within 5 days temperature again became normal, and the systemic dose of polymyxin was decreased to 200,000 units 8-hourly, and four days later was further decreased to 200,000 units 12-hourly for the next 14 days. Apart from 4 days during which the daily intrathecal dose was halved, 40,000 units was given daily until the 37th day, 20,000 units being given for a further 2 days. Toxic effects included severe eosinophilia and xanthrochromia of the cerebrospinal fluid; other toxic effects observed were nausea and malaise, pain at the injection site, pain down the legs and in the back following intrathecal injections, and sacral œdema. These effects disappeared in spite of continued polymyxin therapy. In spite of its toxic effects, polymyxin is the drug of choice for pseudomonas meningitis. The case in question would probably have proved fatal but for this therapy. S. L. W.

Potassium Perchlorate, Treatment of Thyrotoxicosis with. M. E. Morgans and W. R. Trotter. (*Lancet*, 1954, **265**, 749.) Potassium perchlorate inhibits thyroid function by preventing the thyroid from concentrating iodide. The object of this trial was to ascertain whether potassium perchlorate is effective, safe, and suitable for routine use in the medical treatment of thyrotoxicosis. 108 patients were treated; of these, 25 had had no previous treatment, 10 had previously completed a course of methylthiouracil but had subsequently relapsed, 64 were receiving methylthiouracil up to the time when perchlorate was started, and 9 were receiving methimazole. In the majority of the patients, potassium perchlorate in a dosage of 400 mg. daily was effective in controlling thyrotoxicosis. The rate of response appeared to be somewhat slower than with methylthiouracil, and 1 out of the 25 previously untreated cases was not completely controlled. When patients on maintenance doses of methylthiouracil were changed over to potassium perchlorate effective control of the thyrotoxicosis was maintained in all but 2 of 64 cases. The average dose necessary was from two to four times as great as that of methylthouracil. No toxic effects were seen in any of the patients, except for possible signs of gastric irritation in 2, both of whom had a previous history of dyspepsia. It is recommended as worthy of further trial, with the reservations that it is unsuitable for use in combination with iodides for pre-operative preparation, that in a few cases it has proved relatively ineffective, and that the possibility that it is a gastric irritant for some people has not yet been excluded. S. L. W.

Reserpine, Central Effects of, and their Antagonistic Reactions. J. Tripod, H. J. Bein and R. Meier. (*Arch. int. Pharmacodyn.*, 1954, **96**, 406.) Reserpine, a recently isolated alkaloid from the root of *Rauwolfia serpentina* Benth shows a strong, long-lasting and characteristic depression of the central nervous system. This depression was compared with those of phenobarbital, sodium bromide and mephenesin and by the antagonisms which some drugs exert upon these effects. In its direct sedative action on mice, estimated as the shortening of the "fall-time" (the time the mouse can remain on a slowly rotating rod) reserpine equalled phenobarbital in its potency and was much more effective than sodium bromide and mephenesin; its duration of action was much longer than that of phenobarbital. Atropine prolonged sedation by the alkaloid and sodium bromide, but decreased that by phenobarbital. On the other hand, regitine decreased sedation by reserpine and phenobarbital, but prolonged that by sodium bromide. Reserpine antagonised the psychomotor stimulation induced in mice by pervitin (*d*-α-desoxy-ephedrine), caffeine, morphine, scopolamine and cocaine. Phenobarbital and sodium bromide showed synergism with all the stimulants save scopolamine, which was antagonised by both and cocaine, on which sodium bromide had no effect. Reserpine did not prevent convulsions in mice by strychnine, nicotine, picrotoxin and leptazol, but prevented audiogenic seizures. Phenobarbital, sodium bromide and mephenesin were effective anticonvulsants under each of these conditions. A miotic effect of long duration was observed with the alkaloid and morphine-induced miosis was increased, but reserpine also potentiated the mydriatic actions of atropine and scopolamine. Some antipyresis and a fall in body temperature were seen with the drug. Localisation of its site of action in the higher centres is discussed. G. P.

Tetanus. G. P. Wright. (*Brit. med. Bull.*, 1954, **10**, 59.) Experience with troops during the Second World War proved incontestably that prophylactic inoculation with tetanus toxoid can almost wholly eliminate tetanus under circumstances in which there is a serious risk of contracting the disease. The mechanism of active immunity (Boyd, *Lancet*, 1946, **250**, 113) may be regarded as functioning in two consecutive phases; firstly, neutralisation of toxin by pre-formed antitoxin (deriving from the last inoculation with tetanus toxoid) which is circulating in the blood stream; and secondly, neutralisation of toxin by antitoxin newly fabricated as the result of the toxin stimulus to the previously sensitised reticulo-endothelial system. Even if the antitoxin titre of the injured person at the time of infection were well below a protective level, if he possessed a previously experienced antitoxin-forming apparatus the stimulus of the toxin would suffice to evoke liberation of a large amount of neutralising antibody within a period much shorter than the usual incubation time of this disease. Although the disease is relatively uncommon in Great Britain, its distressing clinical course and high case-fatality rate encourage every effort for its prevention, especially when this can be achieved by so simple a prophylactic procedure. Since it is now well established that simultaneous prophylaxis with more than one antigen is not only feasible but possesses material advantages, there seems no valid reason why combined immunisation against tetanus and diphtheria should not be more widely employed as a public health measure. They need entail neither an additional nor a larger injection, for both the age at which it should be given and the time interval that separates the successive inoculations are the same as those at present current for diphtheria toxoid alone. Furthermore, such early immunisation would be effective at the time of life when the mortality from tetanus is at its peak. S. L. W.

BACTERIOLOGY AND CLINICAL TESTS

***Cassia reticulata* Willd, Antibacterial Activity of.** H. W. Youngken and R. A. Walsh. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 139.) An extract was prepared by macerating the coarsely ground dried leaflets with a mixture of 1 volume of ethanol (95 per cent.) with 2 volumes of water, filtering and removing the ethanol by heating in a water bath. When tested by the F.D.A. agar plate method (modified for certain of the organisms), zones of inhibition were observed with *Escherichia coli*, *Alcaligenes fæcalis*, *Sarcina lutea*, *Proteus vulgaris*, *Salmonella typhosa*, *Bacillus megatherium*, *Pseudomonas æruginosa*, *Micrococcus pyogenes* and *Streptococcus pyogenes*. No inhibition of growth was observed for *Alcaligenes ærogenes*, *Serratia marcescens*, *Bacillus subtilis* and *Hæmophilus influenzæ*. Aqueous extracts of the leaves had a feebler antibacterial activity. Both aqueous and ethanolic extracts contained anthraquinones, emodin and chrysophanic acid. Some macroscopical characters of the crude drug are recorded. G. B.

***Mycobacterium johnei*, Cultivation of.** H. W. Smith. (*J. Path. Bact.*, 1953, **66**, 375.) This paper describes modifications of the liquid and solid media of Dubos (Dubos and Middlebrook, *Amer. Rev. Tuberc.*, 1947, **56**, 334) which have proved satisfactory for the cultivation of *Myco. johnei* and for its isolation from natural sources. Not only did bovine strains of *Myco. johnei* often grow more rapidly on these media than on Taylor's egg medium (Taylor, *J. Path. Bact.*, 1950, **62**, 647; 1951, **63**, 333), but the transparent nature of the solid medium was a great advantage, growth being much more easily detected. The fact that penicillin can be added to both the liquid and solid media to help control contaminants is another advantage not possessed by egg media.

S. L. W.

Tetracycline, Bacteriological Properties of. N. A. Diding. (*Svensk farm. Tidskr.*, 1954, **58**, 273.) The antibacterial effects of tetracycline, chlortetracycline and oxytetracycline were compared, by determining the minimal inhibitory concentrations in a serial dilution method. 2 ml. of the antibiotic solution (2400 μg./ml.) and 2 ml. of culture medium (Difco penassay broth) were mixed in the first tube, 2 ml. of the mixture transferred to a second tube containing 2 ml. of medium and so on. Each tube was inoculated with 1 drop of a 24-hour culture of the organism under test diluted 100-fold with sterile water. The tubes were incubated at 37° C. and observed after 24, 48 and 96 hours. The end-point was taken as the last tube showing no visible growth. The antibacterial effect of tetracycline was found to be of the same order as chlortetracycline and oxytetracycline when based on observations made after 24 hours' incubation. Thereafter the inhibitory concentration rose considerably on prolonged incubation, but the end-point was least affected for tetracycline, possibly because of the greater stability of this antibiotic. Tests were made against 9 organisms. Gram-positive organisms were inhibited by much lower concentrations than Gram-negative, except for a penicillin-resistant strain of *Staphylococcus albus*. Resistance was induced in 4 strains of bacteria by serial transfer in media containing increased amounts of the antibiotics. Induced resistance to one of the three substances was accompanied by a rise in resistance to either of the other two. G. B.