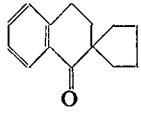
ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

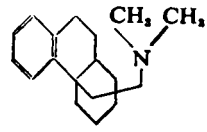
CHEMISTRY

ALKALOIDS

Morphine, Synthetic Approach to Structure of. B. Belleau. (*J. Amer. chem. Soc.*, 1953, **75**, 1159.) The spiroketone, 2:2-tetramethylene-1-tetralone (I) was



(I)



(II)

condensed with methyl bromoacetate to yield a substance which underwent Wagner rearrangement to yield a hydrophenanthroid lactone which, after lithium aluminium hydride reduction followed by dehydration, catalytic hydrogenation, reaction with phosphorus tribromide and subsequent reaction with dimethylamine, was converted to the dihydro desbase of *N*-methyl*iso*morphinane (II). The possible reaction mechanisms involved in the formation of the various intermediates are briefly presented. A. H. B.

ANALYTICAL

Aluminium, Fluorimetric Determination of. E. Goon, J. E. Petley, W. H. McMullen and S. E. Wiberley. (*Analyt. Chem.*, 1953, **25**, 608.) Interference in the colorimetric determination of aluminium in steel using the aluminium salt of 8-hydroxyquinoline in chloroform solution is caused by titanium and vanadium 8-hydroxyquinolines which yield absorption bands in chloroform solution which overlap the band of the aluminium complex; as the aluminium complex fluoresces, an investigation was undertaken to see if the fluorescence could be used for a quantitative method. Details are given of a method by which aluminium can be determined by quantitative fluorimetry using prepared calibration curves. Extraction of the aluminium 8-hydroxyquinolate into chloroform was complete at *p*H values between 6·5 to 10·0 and the fluorescence did not vary critically with time. Of the anions acetate, chloride, citrate, nitrate, perchlorate, sulphate, and tartrate, only the citrate and tartrate caused interference, the recovery of aluminium being only 25 per cent. in the presence of the citrate and 82 per cent. in the presence of tartrate. The presence of iron, titanium and vanadium caused quenching, but for a 1 to 1 weight ratio of titanium, vanadium, or iron to aluminium, respectively, 100, 89, and 83 per cent. recovery of aluminium was obtained; the interference from these elements is thus less serious than in the colorimetric method using 8-hydroxyquinoline.

R. E. S.

Bismuth, Lead and Thallium, Spectrophotometric Determination of. C. Merritt, H. M. Hershenson, and L. B. Rogers. (*Analyt. Chem.*, 1953, **25**, 572.) A survey of the absorption spectra of the bromo- and iodo-complexes of bismuth, lead and thallium has been made together with the absorption spectra of elements in hydrochloric acid including arsenic, copper, indium, iron,

molybdenum, selenium, tin, titanium and tungsten; the survey of cadmium and mercury has been extended to bromide and iodide solutions. The position and intensity of the absorption maxima of bismuth, lead and thallium in hydrochloric acid varied with the acid concentration; 6M hydrochloric acid was chosen as the working medium. Graphs are given of the absorption spectra of the elements studied under varying conditions and consideration is given to the possibility of interference by various cations and anions and their elimination. In the absence of interfering substances, the estimation of lead, bismuth, and thallium can be performed without prior separation. If interfering substances are present, a preliminary separation from cyanide medium with dithizone would permit extraction as a group from all other substances with the exception of tin. Sample mixtures of bismuth, lead, and thallium were analysed by means of multicomponent analysis technique, the optical density indexes being evaluated from measurements of the optical density at each of three wavelengths, using known concentrations and cell thicknesses, with satisfactory results. The accuracy with which thallium can be determined in the presence of bismuth may be somewhat less owing to the uncertainty in evaluating the optical density index for bismuth at 245 mμ. R. E. S.

Digitoxigenin, Fluorimetric Determination of. K. B. Jensen. (*Acta pharm. tox. Kbh.*, 1953, **9**, 66.) A method is described for quantitative determination of digitoxigenin, digitoxin and purpurea glycoside A, based on the fluorescence of digitoxigenin. Digitoxigenin is treated with hydrogen peroxide in a solution of hydrochloric acid and methanol, to which has been added ascorbic acid. The ascorbic acid prevents the rapid splitting into non-fluorescent reaction products that otherwise takes place in presence of excess hydrogen peroxide. Maximum fluorescence is attained after about 40 minutes and the intensity then remains unchanged for nearly an hour. Exposure to irradiation by light of the wavelength producing fluorescence (about 425 mμ) causes a relatively rapid reduction of the intensity. The time of development, intensity and stability of the fluorescence depend on the relation between the amount of hydrogen peroxide, the acid concentration, the amount of ascorbic acid and the temperature. Concentrations of from 2 to 20 μg. of digitoxigenin (or equimolecular amounts of the other two A substances) per 10 ml. of test solution give a linear fluorescence curve. The maximum deviations were about ± 5 per cent. in the amounts of digitoxigenin determined. S. L. W.

Local Anæsthetics, Identification of, by Vacuum Microsublimation. J. Büchi, X. Perlia and A. Strebel. (*Pharm. Acta Helvet.*, 1953, **28**, 109.) The appearance of crystals obtained by microsublimation is not a reliable guide for identification, since, on the one hand, different substances may give similar crystals, and, on the other, the same substance may give different crystalline appearances, even in the same sublimate. The method, however, forms a valuable method of purification or extraction, and identification is then possible from the character, especially crystallographic, of the sublimate, together with microscopic reactions. For this purpose the authors suggest the following physical properties: micromelting point, extinction, interference colours, direction of polarisation, number of axes, optical character, refractive indices on different axes and crystal system. In addition reactions with picric acid, trinitroresorcinol potassium iodide, potassium bromide, potassium permanganate, potassium dichromate, hydrochloric acid, iodine solution and bromine water. These characters and reactions are tabulated for a number of local anæsthetics: benzocaine, propæsine, cycloform, scuroform, monocaine, amylocaine, larocaine, tetracaine, intracaine, surfacaine and lidocaine. G. M.

Thiopentone, Test for Chloride in. E. Kühni and G. Stierli. (*Pharm. Acta Helvet.*, 1953, **28**, 96.) When a solution of thiopentone-sodium is precipitated with dilute nitric acid, small quantities of free thiopentone are still present in the filtrate, and the precipitate given on the addition of silver nitrate appears to be thiopentone-silver. Suitable tests for chloride are with thallium nitrate or with aniline and *o*-toluidine. The latter, which is the more sensitive is recommended for official adoption. It is carried out as follows: 0·5 g. of the substance is dissolved in 10 ml. of water and precipitated with 1·5 ml. of dilute nitric acid. To 1 ml. of the filtrate, in a microtest-tube, is added a crystal of potassium permanganate and 2 drops of sulphuric acid. A test paper, soaked in a solution prepared from 100 ml. of saturated aniline solution, 20 ml. of saturated *o*-toluidine solution, and 30 ml. of glacial acetic acid, is placed on the mouth of the test-tube, which is then heated slowly. A blue coloration on the paper shows the presence of chlorine. G. M.

Vitamin A in Presence of Tocopherols, Determination of. D. T. Ewing, L. H. Sharpe and O. D. Bird. (*Analyt. Chem.*, 1953, **25**, 599.) Chromatographic separation has been investigated for the determination of vitamin A in the presence of tocopherols. Data are presented for mixtures of pure vitamin A and pure α-tocopherol, distilled natural vitamin A esters and pure α-tocopherol, and distilled natural vitamin A esters and Type IV mixed tocopherols. The vitamin A-tocopherol mixture was saponified and the two vitamins separated by chromatography on activated alumina; hexane solutions of the vitamins were used to deposit the vitamins on the column, the optimum solvent for development being a 1 : 2 v/v mixture of ether and hexane. Curves of known mixtures of α-tocopherol and vitamin A alcohol show essentially complete separation of the two components; Type IV mixed tocopherols containing a relatively high proportion of β, γ and δ tocopherols cannot be satisfactorily treated by this method. An average recovery of vitamin A, from the column, was found to be 94·4 per cent. on the basis of 4 experiments; the Morton-Stubbs correction procedure was applied to the eluted vitamin A alcohol. R. E. S.

Zinc, Gravimetric Methods for. J. E. Vance and R. E. Borup. (*Analyt. Chem.*, 1953, **25**, 610.) Radio-isotopic methods were used to determine the amount of zinc remaining in solution following the classical phosphate and sulphide precipitations, 3 precipitations with organic reagents, and several separations of zinc from elements with which it is commonly encountered. The ^{65}Zn isotope was used since it had decay characteristics which make its determination straightforward with a half life of about 250 days and since gamma rays of 1·11 m.e.v. energy are produced. The phosphate procedure can be modified to advantage by the use of a much smaller excess of reagent than previously suggested, adjustment of the *p*H of the wash liquid, and use of a lower ignition temperature. Zinc oxalate precipitation was an excellent process, with zinc oxide as the weighing form. Anthranilic acid and 8-hydroxyquinoline precipitate zinc quantitatively from solution, but the precipitates do not have the predicted compositions and empirical factors are needed. Zinc can be satisfactorily precipitated as sulphide from a cold sulphate-bisulphate buffer in the presence of as much as 4 parts of iron, 2 of nickel, 8 of manganese, and 4 of aluminium. The modified phosphate procedure, the zinc oxalate precipitation, and the sulphide method can all give average results within 0·1 mg. of theory. R. E. S.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

***Lactobacillus bulgaricus* Factor, Chemical Nature of.** G. M. Brown and E. E. Snell. (*J. Amer. chem. Soc.*, 1953, **75**, 1691.) The close relationship of the two growth factors *Lactobacillus bulgaricus* (LBF–1A) and pantothenic acid is indicated in the fact that, in large amounts, the latter replaces the former as a growth factor for *Lactobacillus helveticus* 80. However, LBF–1A is more than 100 times as active as pantothenic acid in promoting growth. Both pantothenic acid and LBF–1A are inactivated by acetylation and most of the activity is restored by mild hydrolysis with N potassium hydroxide in methanol, while ammoniacal methanol regenerated the activity more slowly. These data indicate the presence of one or more free hydroxyl groups. The compound is essentially neutral and is not destroyed by nitrous acid. Acid hydrolysis of LBF–1A yields β-alanine and an unidentified amine along with the disulphide of β-mercaptoethylamine. The treatment of LBF–1A with a liver enzyme liberated large amounts of pantothenic acid. Therefore, in LBF–1A, pantothenic acid must be combined with β-mercaptoethylamine by an amide linkage. The synthesis of the disulphide of *N*-(pantothenyl)-β-mercaptoethylamine gave a compound (named *pantethine*) equal to LBF–1A in growth-promoting activity for *L. helviticus* 80. Treatment of coenzyme A with intestinal phosphatase liberated a compound which is closely related to, or identical with, pantethine or LBF. A. H. B.

***Lactobacillus bulgaricus* Factor, Isolation of, from Natural Sources.** V. J. Peters, G. M. Brown, W. L. Williams and E. E. Snell. (*J. Amer. chem. Soc.*, 1953, **75**, 1688.) The procedures for the isolation of one form of the *Lactobacillus bulgaricus* factor (LBF–1A) from the culture filtrate of *Ashbya gossypii* are described. These involved adsorption and elution from activated charcoal and subsequent successive chromatography on floridin, charcoal, superfiltrol and alumina. Concentrates obtained in this way provided the material used in elucidation of the chemical nature of the growth factor.

A. H. B.

Thyroxine, Diazo Reaction of. G. Barac and H. Morran. (*Bull. Soc. Chim. biol., Paris*, 1953, **35**, 299.) Since, in thyroxine, the positions *ortho* and *para* to the hydroxyl group are occupied by iodine, the coupling of thyroxine with diazo compounds appears to be abnormal. Analysis of the products obtained by coupling with diazotised arsanilic and sulphanilic acids indicate that these are derivatives of phenyl-1-azo-3′(3 : 5 : 5′-triiodothyronine). Coupling occurs in the *ortho* position after the elimination of an atom of iodine. G. M.

BIOCHEMICAL ANALYSIS

Adrenergic Amines of Human Blood. H. Weil-Malherbe and A. D. Bone. (*Lancet*, 1953, **264**, 974.) In the authors' fluorimetric method for the determination of adrenaline plus noradrenaline in plasma, which depends on the fluorescence of condensation products of the adrenergic amines with ethylenediamine, the adrenaline condensation product gives a yellow to orange fluorescence while the fluorescence given by the noradrenaline condensation product is green. By measuring the intensity of the fluorescence, using first a yellow filter (Chance OY4) and then a blue-green filter (Ilford Bright Spectrum

Filter 623), differential determinations of adrenaline and noradrenaline in plasma and red blood cells can be made. Determinations of the amine content of red blood cells are made after hæmolysis of the cells by treating a suspension in water with cetrimide. On applying the method to the plasma and red cells of 22 males and 21 females, the average adrenaline content of the plasma, in μg./l. of whole blood was 1·18 in males and 1·46 in females; in the red cells the figure was 2·81 for males and 2·27 for females. The plasma concentrations of noradrenaline were 5·29 and 5·16 in males and females respectively, and the concentrations in red blood cells were 2·02 and 3·77 in males and females respectively. There are highly significant differences in the intracellular and the extracellular concentrations of each of the amines in the two sexes. There is also a statistically significant inverse correlation between adrenaline and noradrenaline concentrations in both red blood cells and plasma. H. T. B.

Bromide in Body Fluids, Determination of. G. Hunter. (*Biochem. J.*, 1953, **54**, 42.) Details are given for the application of the Van der Meulen reaction —the oxidation of bromide to bromate by hypochlorite—to the determination of bromide in blood, cerebrospinal fluid and urine. The biological material to be examined is first ashed in an open crucible, the ash extracted with water, and phosphate buffer and hypochlorite solution added; after heating at 100° C. for 10 minutes sodium formate solution is added followed by sulphuric acid and potassium iodide, the resulting mixture being titrated with sodium thiosulphate using starch as indicator. The presence of sodium chloride up to 90 mg. has no appreciable effect on bromine values of 10 μg. but the addition of 500 mg. sodium chloride or more lowers the values seriously even at the 50 μg. level; iodine reacts quantitatively as bromide. Determination of bromide by this method is accurate to about $\pm$ 1 per cent. when the bromine present is greater than about 10 mg./100 ml. and when 0·5 to 1·0 ml. is taken. With bromine values from 3 to 10 mg./100 ml. the error is within $\pm$ 5 per cent. A minimum of about 50 μg. of bromine is desirable with the method. R. E. S.

Citric Acid, Microdetermination of. T. G. Taylor. (*Biochem. J.*, 1953, **54**, 48.) The method of Weil-Malherbe and Bone (*Biochem. J.*, 1949, **45**, 377) was employed and found to be very reliable for 0·2 to 1·0 mg. quantities of citric acid, but with smaller amounts the results were low. A detailed study was applied to the estimation of quantities up to 100 μg. The citric acid is oxidised to acetonedicarboxylic acid in the presence of sulphuric acid followed by the conversion of this compound to pentabromoacetone; the pentabromoacetone is then extracted with light petroleum, a portion of which is shaken with sodium sulphide solution. The intensity of the yellow colour produced in the aqueous phase is measured with a suitable photoelectric instrument. Errors present in microdeterminations were eventually traced to an excess of thiosulphate and, in view of the impossibility of avoiding a local excess, it was decided to use ferrous sulphate; a bromide-bromate mixture was substituted for saturated bromine water and the ammonium vanadate incorporated in this solution. Details of procedure are given and of recovery experiments with pure citric acid. R. E. S.

Folic Acid, Determination of. K. Ilver. (*Dansk Tidsskr. Farm.*, 1953, **27**, 81.) For the assay of folic acid by the reduction and coupling method, zinc dust may be used in place of zinc amalgam, while the addition of gelatin is of no advantage. Folic acid shows an absorption peak at 298 mμ in acid

solution, while *p*-aminobenzoylglutamic acid and *p*-aminobenzoic acid show no absorption at this point, so that it would appear possible to determine folic acid directly. Experiments with a number of samples of folic acid showed that the results obtained in this way did not agree with those of the reduction and coupling method. G. M.

Progesterone, Chemical Assay of. D. G. Edgar. (*Biochem. J.*, 1953, **54**, 50.) The paper describes a method of assay of progesterone in biological material based on the extraction and purification of the hormone by partition between organic solvents, final separation by chromatographic partition on filter paper and subsequent estimation by ultra-violet absorption spectroscopy. Oxalated blood was extracted with a 3:1 (v/v) ethanol-ethyl ether mixture, the solvent being evaporated to low bulk, diluted with water and extracted with ethyl acetate. The residue from the ethyl acetate extraction after purification was taken up in water and extracted with light petroleum, the residue, dissolved in benzene, being used for chromatography. The paper chromatography of progesterone was investigated using alumina papers and an ascending technique, the paper dipping into mixtures of various solvents; a reversed phase technique on silicone treated paper using aqueous ethanol-chloroform and aqueous methanol-benzene as solvents was also used, recoveries of the order of 75 per cent. of progesterone added to blood being obtained. Reversed phase chromatography was generally the most satisfactory, an extract of progesterone-treated blood travelling with an R_F of 0·35 alongside the pure substance. With 70 per cent. aqueous methanol compact spots of both progesterone and testosterone were obtained with a complete separation of the former steroid from extracted impurities. Details of the ultra-violet absorption of progesterone are given ($E_{max.}$ 240 mμ). R. E. S.

PHARMACY

DISPENSING

Sodium Sulphonamides, a Buffer System for Ophthalmic Solutions of. H. B. Kostenbauder, F. B. Gable and A. N. Martin. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 210.) Sulphonamide salts are precipitated from 5 per cent. solutions buffered at *p*H 8·6. Precipitation is not due to chemical incompatibility, but solubility is a function of the *p*H of the solution. Using a modified buffer equation it was shown that a minimum *p*H of 8·97 is necessary to keep 5 per cent. of sulphathiazole in solution, and in practice a buffer of *p*H 9 should be used to prevent precipitation at ordinary room temperatures. The inclusion of 0·1 per cent. w/v of exsiccated sodium sulphite retards discoloration and prevents the growth of moulds in the solution. The following buffer solution is recommended for the preparation of ophthalmic solutions containing 5 per cent. of sulphathiazole sodium: boric acid, 0·043 per cent., sodium borate, 0·42 per cent., and sodium sulphite, 0·1 per cent. The calculated minimum *p*H for 5 per cent. solutions of sulphadiazine sodium and sulphamerazine sodium is close to the *p*H of a 5 per cent. solution of each substance in distilled water. A buffer is unnecessary as satisfactory solutions may be prepared by dissolving the substances in water with the addition of 0·1 per cent. of exsiccated sodium sulphite. G. B.

Phenindione (Hedulin). (*New and Nonofficial Remedies, J. Amer. med. Ass.*, 1953, **152**, 142.) Phenindione is 2-phenyl-1: 3-indandione and occurs as a pale yellow, crystalline, almost odourless substance, m.pt. 148° to 151° C., very slightly soluble in water, and soluble in ethanol (1 in 100) and ether (0·9 in 100). When treated with sulphuric acid, a deep blue to violet colour is produced, which is discharged with formation of a white precipitate on the addition of water. When refluxed for 3 hours with aniline in ethanolic solution, a red crystalline substance melting at 222° to 228° C. is obtained. A 0·0005 per cent. solution in 0·1N sodium hydroxide exhibits ultra-violet absorption maxima at about 280 mμ ($E^{1\ \text{per cent.}}_{1\ \text{cm.}}$, about 1328) and 330 mμ, and minima at about 236 and 315 mμ; the ratio of the absorptions at 280 and 330 mμ is 3·20 to 3·40. Phenindione contains not more than 20 p.p.m. of heavy metals and yields not more than 0·25 per cent. of sulphated ash. When dried at 105° C. for 4 hours, it loses not more than 1·0 per cent. in weight. It contains 95·0 to 105·0 per cent. of phenindione and is assayed spectrophotometrically by measuring the absorption at 280 mμ of a 0·0005 per cent. solution in 0·1N sodium hydroxide. Phenindione is used as a systemic anticoagulant. G. R. K.

PHARMACOGNOSY

Morphine, Production of, from Poppy Stalks. S. Biniacki and H. Ludwicki. (*Ann. pharm. franç.*, 1953, **11**, 121.) In the culture of poppies for seed and oil in Eastern Europe, the plant, deprived of the capsules, forms a waste product ("poppy straw") which is often burnt. An examination was made of the possibility of extracting alkaloids from this material. A method for the extraction of morphine has been published by Kabay, and patented in several countries. Experiments showed that this method extracts the morphine completely, but only about 60 per cent. of the non-phenolic alkaloids. To obtain complete extraction it is necessary to increase the time of extraction, or to use a different solvent. The loss of morphine in the early stages is insignificant, but about 25 per cent. of the non-phenolic alkaloids may be destroyed during the operation, owing to the decomposition of narcotine under the action of sulphur dioxide. In the final stages there may be a considerable loss of morphine, especially during its extraction and numerous crystallisations. Of the other matter present in the plant, it is especially the pentosans or the products of their decomposition which go through into the later stages of manufacture. G. M.

PHARMACOLOGY AND THERAPEUTICS

Adrenaline Cream in Fibrositis. J. S. Lawrence and R. J. Sladden. (*Brit. med. J.*, 1953, **1**, 1085.) The value of adrenaline cream in rheumatic disorders was investigated under the two headings, palliation and recovery times, the investigation being carried out at a clinic for the treatment of rheumatism in miners. Palliation was studied in two stages. In the first, on a group of 65 patients, comparison was made between a cream containing 0·1 per cent. of adrenaline and a simple cream. In the second stage on a group of 60 patients, adrenaline cream was compared with dry massage and with no massage. The assessment was made after each patient had been treated thrice weekly for 2 weeks. Each patient in the first group was then treated in the same way with the simple cream while each patient in the second group was then treated by dry massage thrice weekly for 2 weeks and then again assessed after another

fortnight with no massage. Other physiotherapeutic procedures were continued without change during the investigation. The results obtained, as ascertained by a questionnaire, are tabulated. They show that adrenaline cream and dry massage both gave relief in a greater proportion of patients than a simple cream, and no significant difference was noted between the effects of dry massage and massage with adrenaline cream, but the difference between the adrenaline cream and the control groups was highly significant. The investigation into the effect of adrenaline cream on recovery time was carried out on 64 males, alternate patients being treated with adrenaline cream until either no further treatment was required or no improvement was shown for 4 consecutive weeks; the remainder were maintained on massage with a simple cream in the same way. The result in the two groups was identical; 7 patients in each lost all signs and symptoms; 14 and 13 respectively needed no further treatment and 7 in each group were unchanged. The results, however, show that repeated application of adrenaline cream significantly delays recovery and increases incapacity for work. While all in the control group returned to work, 3 in the treated group did not return, and 2 had to cease work after returning to it. H. T. B.

Aminoazotoluene Dermatitis. T. H. Meara and I. Martin-Scott. (*Brit. med. J.*, 1953, **1**, 1142.) 3 cases of contact dermatitis are described due to the presence of aminoazotoluene in the red and green semi-solid inks used in a popular make of ball-pointed pen. 2 of the patients had used such pens while the third was in contact with green semi-solid ink during her work. Each of the three gave a positive reaction in a patch test with 0·01 per cent. of aminoazotoluene in soft paraffin. In 2 cases the time taken to produce sensitivity was 6 to 8 weeks; in the third case it may have been longer. The dermatitis occurred on the hands and arms, and in 2 cases on the eyelids. In each case the trouble cleared up when contact with the red or green ink was avoided. In view of the absence of previous reports of this dermatitis in spite of the sale of large numbers of the pens, and the absence of dermatitis among workers in contact with these two coloured inks, it is suggested that the dye has a very low sensitivity index. H. T. B.

Antibiotics, Assay for the Histamine-like Activity of. L. W. Rowe and R. A. Brown. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 257.) In the tests for histamine-like activity of certain antibiotics in which the fall in blood pressure is observed after intraperitoneal injection into anæsthetised animals, dogs as well as cats were found to be suitable as experimental animals. Dogs anæsthetised with phenobarbitone sodium are only about 1/5 as sensitive to histamine as cats, but this is relatively unimportant since the tests are made in comparison with a histamine standard and the slope of the response of dogs to graded doses is as steep as that of cats. The standard error with either animal is about ± 20 per cent. Results show that for chemically pure substances such as crystalline penicillin or chloramphenicol, a test for histamine-like activity is unnecessary, but it is desirable for streptomycin and viomycin. A solution of chloramphenicol in *NN*-dimethylacetamide cannot be tested satisfactorily since the solvent causes an appreciable depressor action, but the test is not considered to be necessary for this preparation. The use of antihistaminic drugs to antagonise histamine-like activity in antibiotics is not feasible. G. B.

Ergot Alkaloids, Hydrogenated, Hypotensive Effect of. H. Konzett and E. Rothlin. (*Brit. J. Pharmacol.*, 1953, **8**, 201.) The hydrogenated alkaloids of ergot, dihydroergocornine, hydergine (a mixture of equal parts of dihydroergocornine, dihydroergokryptine and dihydroergocristine) and dihydroergotamine caused a fall of blood pressure when injected into the anæsthetised or decerebrated cat, mainly through an action on the vasomotor centres. After section of the spinal cord at the 7th thoracic vertebra they caused a fall in blood pressure, but after section at the 6th cervical or 1st thoracic vertebra there was either a rise or a fall. In the intact cat, after blocking the ganglia with tetraethylammonium, the alkaloids generally caused an increase in blood pressure; while in the spinal cat, even after section of the cord at the level of the 7th thoracic vertebra, or after complete destruction of the cord, they caused a fall. The main site of action is therefore the vasomotor centres and the upper part of the sympathetic outflow, as pathways conducting the impulses, have to be intact for the hydrogenated alkaloids to cause a fall of blood pressure. Without the vasomotor centres, or their efferent fibres, only the vasoconstrictor effect of these alkaloids is apparent. G. F. S.

Gentisic Acid, Toxicities of Esters of. J. F. Nash, F. W. Bope and B. V. Christensen. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 254.) The acute toxicities of 13 new esters of gentisic acid were determined in mice, the esters with amino alcohols being injected intravenously in the form of aqueous solutions. As the esters of gentisic acid with other carboxylic acids were insoluble in water, they were injected intravenously as aqueous suspensions containing not more than 5 per cent. of acacia and 5 per cent. of ethanol, prepared with the aid of tween 80. All compounds tested were more toxic than gentisic acid. The LD50 for esters with other carboxylic acids varied from 201·2 mg./kg. for the 5-phenylacetic compound to 32·5 for the 5-acetylsalicylic derivative. Esters with amino alcohols were more toxic, ranging from 3-diethylaminopropyl gentisate hydrochloride (LD50, 58·5 mg./kg.) to its 5-methoxy analogue, LD50 22·0 mg./kg. G. B.

Iron Preparations, Physico-chemical Properties and Toxicities of. J. A. Nissim. (*Brit. J. Pharmacol.*, 1953, **8**, 197.) The physico-chemical properties of a number of iron preparations have been investigated and their acute toxicities compared in mice. Of the new preparations ferric glucosate, "ferric hydroxide ferrous ascorbate," "ferrous chloride ascorbate," "ferric chloride caramelate," "ferric chloride lactate," glycine and iron and plasma and iron were all more toxic than saccharated iron oxide, although some showed better physical properties. Of the old preparations ferric chloride, because of its protein precipitating properties, was the most toxic iron preparation. Ferrous sulphate was less toxic in its immediate effect but with a more prolonged action it was as toxic as ferric chloride. Colloidal ferric hydroxide had an immediate lethal effect with few delayed deaths, and was as toxic as ferrous sulphate. Ferrous ascorbate was more toxic than "ferric hydroxide ferrous ascorbate." Iron and ammonium citrate showed a delayed effect. Iron preparations differed widely in their toxicity with LD50 ranging from 11 to 300 mg. Fe./kg. G. F. S.

Isoniazid in Treatment of Lupus Vulgaris. R. Russell, N. A. Thorne and R. V. Grange. (*Lancet*, 1953, **264**, 964.) The encouraging reports of the value of isoniazid in the treatment of pulmonary tuberculosis suggested its possible use in cases of lupus vulgaris. The authors report the results of treat-

ment in 15 cases. 5 of the patients were given tablets of isoniazid orally, 6 were treated with local injections, and the remaining 4 had both local and oral treatment. Oral dosage was 150 mg. daily at first, but later it was found that better results were obtained with 300 mg. daily and as toxic side effects were rare a daily dose of 400 mg. was sometimes given. For local injection, the dose varied between 50 and 250 mg. in 2 to 5 ml. of water administered once a week, intradermally into the whole of a lesion if it were small, or into the same part of it if it were larger. Where both methods were used, the oral dosage was 300 mg. daily and the injected dose up to 400 mg. per week. All the patients except one showed improvement, and in most this was progressive up to the time of publication of the report. None of the patients complained of any subjective symptoms. The only side-effect observed was mild urticaria in one patient treated with weekly injections but it was not certain that the urticaria was caused by the isoniazid. From the continuous improvement shown by the patients during up to 30 weeks' treatment it is concluded that drug resistance did not occur. The authors suggest that the dose by mouth should be at least 300 mg. per day but weekly injections are considered far too infrequent for maximal response. Repeated injections, however, cause considerable fibrosis which makes administration increasingly difficult. H. T. B.

Nalorphine in Racemorphan (Dromoran) Poisoning. M. Bornstein, L. Yorburg and B. Johnston. (*J. Amer. med. Ass.*, 1953, **151**, 908.) 2 cases of racemorphan poisoning resulting from the administration, in error, of 50 and 25 mg. of the drug were treated with nalorphine. 1 patient failed to respond to nikethamide and atropine and the other to caffeine. Amphetamine and oxygen treatment improved the condition for a short period, but a relapse to the comatose state occurred. Respirations in one patient were as slow as 4/minute. Slow intravenous injection of 20 mg. of nalorphine was given to this patient, who returned from a comatose state in 10 minutes; 3 hours later a further dose of 10 mg. of the drug gradually improved the condition such that by the following morning respirations were 18/minute and the mind was alert. The other patient was given the nalorphine intramuscularly in doses of 5 mg. and showed improvement after 20 minutes and reverted to the pre-methorphinan administration condition after 6 hours. It is concluded that nalorphine is a potent and rapidly acting antidote to methorphinan. J. R. F.

***p*-Nitrophenyldiethyl phosphate, Reactions of Rabbits to Poisoning by.** J. M. Barnes. (*Brit. J. Pharmacol.*, 1953, **8**, 208.) *p*-Nitrophenyldiethyl phosphate (E 600) is a potent cholinesterase inhibitor acting like tetraethyl pyrophosphate. An intravenous dose of 0·1 mg./kg. in the unanæsthetised, atropinised rabbit caused muscular fasciculations, respiratory arrest and death within 10 to 15 minutes. With a period of artificial respiration the rabbit could be saved and a series of injections could be effected. While periods of unconsciousness did not increase with successive doses the generalised fasciculations were reduced after several doses. Longer periods of artificial respiration became necessary for recovery. In rabbits anæsthetised with urethane, 1 mg./kg. produced muscular fasciculations within 1 minute and movements of the diaphragm became irregular. Cyanosis rapidly appeared and artificial respiration was necessary for recovery. With successive doses at 30- to 60-minutes intervals the diaphragm became refractory but a gradual failure of respiration and circulation occurred. Responses of the tibialis and soleus muscles to stimulation of the sciatic nerve became smaller after each dose and during the later

stages muscular fasciculations disappeared. While the drug kills the animal, due to cholinesterase inhibition, some rapid reversal of the inhibition is postulated during periods of artificial respiration. Sensitivity to acetylcholine increased after each dose and diminished but did not return to normal within a 2-hour period. It is suggested that some of the changes may be a secondary reaction to the inhibition of cholinesterase and to anoxia. G. F. S.

Sodium Aminosalicylate in Treatment of Pulmonary Tuberculosis. R. McL. Todd. (*Brit. med. J.*, 1953, **1**, 1247.) The value of sodium aminosalicylate in the treatment of primary pulmonary tuberculosis was investigated in 69 children divided into 4 age groups. Alternate patients in each group were treated for 12 weeks with 1 g./lb. daily, in divided doses, two hourly in mixture form. [Elsewhere in the paper the daily dose is stated to have been 0·5 g./lb.] The control group were given no specific chemotherapy but received the same general treatment. Most of the patients had suffered from the disease for 3 months or less. Progress was assessed by the following 6 criteria: clinical impression based on appetite, vitality, temperature and pulse rate, serial blood counts, serial sedimentation rates, weight gain, serial chest X-ray films, and incidence of complications. The clinical impression was that progress was equally satisfactory in both groups, and the other criteria selected indicated no benefit from sodium aminosalicylate. 2 children under 3 years old died of tuberculous meningitis, both in the control group, but in the treated group under 3 years old, one developed renal tuberculosis and one developed tuberculous peritonitis. The drug is most useful in acute exudative tuberculosis and since caseation in the regional lymphatic glands is the most striking feature of primary tuberculosis this conclusion was not unexpected. The large dose used was intended to give a blood level of 8 mg./100 ml. The average level 30 minutes after a dose was 12·5 mg./100 ml., while the average level just before a dose was due was 7·5 mg./100 ml. Wide variations were found in the same individual on different occasions. 1 child out of 35 in the treated group developed a generalised rash and œdema; in the others no toxic manifestations of any kind were observed. H. T. B.

Streptomycin Resistance After Previous Treatment with Salts of Aminosalicylic Acid Alone. F. W. S. Turnbull, A. T. Wallace, S. Stewart and J. W. Crofton. (*Brit. med. J.*, 1953, **1**, 1244.) The authors investigated the development of resistance to sodium or calcium aminosalicylate given alone, and the effect of such resistance on subsequent combined treatment with streptomycin and an aminosalicylate. A group of 9 patients with pulmonary tuberculosis who had been treated with an aminosalicylate alone for periods of from 6 weeks to 16 months were treated with 1 g. daily of streptomycin and 20 g. daily of sodium aminosalicylate. The dosage of the aminosalicylate when given alone was unknown; the interval between the two courses of treatment varied from a few days to 39 months. The results of serial culture and sensitivity tests of tubercle bacilli isolated from these patients were compared with similar results on a second series of 5 patients treated similarly with streptomycin and sodium aminosalicylate but who had not previously been treated with aminosalicylate alone. Of the first group, in 8 out of 9 patients bacteria resistant to aminosalicylate could be isolated at the beginning of the investigation; in some the degree of resistance increased during the combined treatment. When these 8 patients were given the combined treatment, streptomycin resistant

bacilli were obtained from 6 patients within 3 months and from all of them after 5 months. By contrast, a slightly resistant organism was isolated after 5 months from only 1 of 6 patients whose organisms were sensitive to sodium aminosalicylate at the start of combined treatment. It is concluded that the use of sodium aminosalicylate to supplement treatment with streptomycin does not prevent or diminish the emergence of resistance to streptomycin if the organisms are resistant to the aminosalicylate at the commencement of combined treatment. H. T. B.

Thyroxine, Biological Action of Substances Related to. J. H. Wilkinson, W. E. Sprott and N. F. Maclagan. (*Biochem. J.*, 1953, **54**, 16.) The thyroxine-inhibitory properties of a series of 4-hydroxy-3:5-diiodobenzoates of glycols has been studied. A series of five ω-hydroxyalkyl 4-hydroxy-3:5-diiodobenzoates (β-hydroxyethyl to ζ-hydroxyhexyl) were prepared together with the five corresponding polymethylene bis-esters and the monoesters from butane-2:3-diol and propane-1:2-diol; in addition glycerol 1-(4-hydroxy-3:5-diiodobenzoate) was examined. In general, increasing chain length of the alkyl group was paralleled by a substantial diminution in thyroxine-inhibitory activity. The hydroxyethyl and the two hydroxypropyl esters caused significant reductions in the thyroxine responses at total doses of 25 mg./kg. and were thus as effective as the *n*-butyl ester, the most potent compound observed. The substitution of a hydroxy group caused a relatively slight effect on the thyroxine-inhibitory activity of a number of alkyl 4-hydroxy-3:5-diiodobenzoates. A second hydroxyl group in the alkyl chain completely abolished the activity of the propyl ester, for the glycerol ester displayed no action at a dosage of 400 mg./kg. There was no correlation between the length of the polymethylene chain and the antithyroxine activity of the polymethylene bis-4-hydroxy-3:5-diiodobenzoates; the dimethylene compound was highly active whilst of the others only the tetramethylene and pentamethylene esters showed any activity. R. E. S.

BACTERIOLOGY AND CLINICAL TESTS

Surface-active Agents, Effect of, upon Bacterial Growth. L. Gershenfeld and G. C. Johnson. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 187.) Rough strains of *Staphylococcus aureus*, *Streptococcus viridans* and *Neisseria catarrhalis* were grown in broth and on nutrient agar containing surface active agents. None of the organisms showed change from the rough growing habit in the presence of 0·001 to 0·0001 per cent. of sodium lauryl sulphate, and higher concentrations were toxic to the bacteria. Lecithin in concentrations from 0·03 to 0·01 per cent. caused no change in the character of the growth, but stimulated the growth of the streptococcus and staphylococcus while *Neisseria catarrhalis* was unaffected. The addition of tween 80, 0·3 to 2 per cent. to the medium caused the staphylococcus to grow in smooth suspension in broth cultures, although the colony structure on agar remained unchanged. This surface-active agent did not affect the growth of the other organisms. It is suggested that as the staphylococcus is affected by tween 80, the concentration of lipids on the cell wall is the cause of rough growth, and adsorption of the surface-active agent gives rise to a hydrophilic surface. This is evidently not the case with *Streptococcus viridans* which is unaffected by tween 80, but roughness may be the result of the interlacing chain structure of this organism. G. B.

Correction.

THE GRAPHICAL EVALUATION OF RESULTS OF SIMPLE AND MULTIPLE SLOPE-RATIO ASSAYS

BY PAMELA M. CLARKE and ZENA D. HOSKING.

This Journal, 1953, **5**, 586.

Page 588, legend to Fig. 1, last line. *For* CC′ *read* BB′.

Pages 592 and 593. *For v read v.*

Page 593, last line. *For* $t_2 = t\sqrt{n(k-1)(2k+1)/k2d_n}$ *read*

$$t_2 = t\sqrt{n(k-1)(2k+1)/k/2d_n}$$

Page 594, first paragraph. *Read:*

For a multiple assay, the corresponding test for "intersections" may be made using a range test described by Cox[7]. When there is a common zero dose the range of the values of H should not be greater than $t_3 r$ where $t_3 = d_v F_{\nu_1,\nu_2}\sqrt{nk(k-1)(2k+1)/2/(vk+1)d_n}$. F is found from variance ratio tables with ν_1 and ν_2 degrees of freedom, where $\nu_1 = \nu_v$ and $\nu_2 = (vk+1)\nu_n$, using the values of ν given in Table V. When there is no common zero dose, $t_3 = d_v F_{\nu_1,\nu_2}\sqrt{n(k-1)(2k+1)/2k/vd_n}$, $\nu_1 = \nu_v$ and $\nu_2 = vk\nu_n$.

Page 594, second paragraph. *For v read v.*

Correction.

A COMPARISON OF PHYSICAL AND CHEMICAL METHODS WITH BIOLOGICAL ASSAY OF VITAMIN A

BY T. K. MURRAY AND J. A. CAMPBELL.

This Journal, 1953, **5**, 596.

Page 597, the last two sentences of the first paragraph should read:—

"Unpublished results of a similar comparison conducted by an informal committee of the U.S.P.[11] indicated that the Morton and Stubbs correction procedure gave a conservative estimate of biological potency. There was, however, no indication of over-correction to the extent reported by Melnick *et al.*"

Page 599, Table I, column 5, "Potency of Concentrates" the figure 15,900 should read 159,000, and in column 6 "Confidence Limits of Concentrates" the figure 16,100 should read 161,000.