ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

***Banisteriopsis inebrians* Morton, Alkaloid of.** F. D. O'Connell and E. V. Lynn. (*J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 753.) About 0·145 per cent. of harmine, $C_{13}H_{12}ON_2$, was extracted from the stems of *Banisteriopsis inebrians* by the following method. 700 g. of coarsely ground stems was moistened with a mixture of strong solution of ammonia, ethanol and ether, allowed to stand for 12 hours and extracted with chloroform in a Soxhlet apparatus. The solution was shaken with N sulphuric acid, the acid solution boiled and the alkaloid precipitated with ammonia. The alkaloid after purification was obtained in the form of white shiny crystals, melting point 260° C. The identity of the substance was confirmed by comparison with a sample of harmine obtained from the seeds of *Peganum harmala* L. Both were colourless substances, showing a blue fluorescence in ultra-violet radiation and gave identical spectrograms, with an absorption minimum at 272 mμ and a maximum at 300 mμ, and a mixed melting point of 260° C. was observed. No other alkaloids appeared to be present in the stems of *B. inebrians*, but the alkaloid appeared to be present also in the leaves. G. B.

***Chondrodendron limaciifolium*, Alkaloids of.** J. A. Barltrop and J. A. D. Jeffreys. (*J. chem. Soc.*, 1954, 159.) *Chondrodendron limaciifolium* is a constituent of Tecuna and Chazuta curares, but, although containing highly toxic materials, the plant does not possess curare-action alkaloids. The dried wood yielded about 5 per cent. of basic material upon extraction with methanol or with tartaric acid solution as solvent. The crude alkaloids were separated into a quaternary and a non-quaternary fraction by neutralising an acid solution with sodium bicarbonate, filtering, and extracting the filtrate with chloroform; the aqueous phase contained the quaternary bases. These non-quaternary bases were then divided into a chloroform-soluble and an insoluble portion, and from the chloroform solutions *iso*chondrodendrine and two new non-quaternary bases were isolated. Reactions of ferric chloride with the alkaloids are discussed and related to the alkaloids' structures. A. H. B.

Ergot Alkaloids, Extraction of, by Tetrahydrofuran and its Mixtures. J. M. Campo and L. G. Gramling. (*J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 747.) A quantity of Spanish ergot was powdered, defatted with light petroleum, and the total alkaloidal content was determined by a modification of the colorimetric method of the U.S. National Formulary IX. 10-g. samples of the defatted powdered ergot were macerated for 48 hours with tetrahydrofuran, its azeotropes with water and with chloroform, and a mixture of equal volumes of water and tetrahydrofuran. Soxhlet extraction was also employed, except for the tetrahydrofuran–water mixture. The alkaloidal extracts were evaporated to dryness *in vacuo* without heating, dissolved in a mixture of 9 volumes of chloroform and 1 volume of ammoniacal methanol, and the assay continued by the method of the U.S. National Formulary. Tetrahydrofuran–water mixture extracted about 50 per cent., tetrahydrofuran alone about 60 per cent.,

the water azeotrope about 78 per cent. and the chloroform azeotrope about 90 per cent. of the total alkaloids. The quantity of colouring matter in the extract increased with the water content of the solvent. The tetrahydrofuran–chloroform azeotrope extracts were very light in colour and did not form emulsions so readily as the others. The marcs remaining after extraction by the various solvents contained very little alkaloid, indicating that the losses are due to destruction of the alkaloids rather than incomplete or selective extraction. G. B.

ANALYTICAL

Atropine Sulphate in Eye Drops, Determination of. A. A. Semenicheva. (*Aptechnoe Delo*, 1953, **2**, No. 4, 15.) The method is based on the conversion of atropine sulphate to the picrate, followed by the reduction of the picrate with sodium sulphide; the picramic acid formed is then measured colorimetrically. 1 ml. of 0·5 per cent. solution of atropine sulphate is treated with 2 ml. of water and 1 ml. of a 1 per cent. sodium picrate. The atropine picrate formed is extracted, first with 10 ml. of chloroform and then with 5-ml. quantities, until the extracts are no longer yellow; the chloroform extracts are filtered through a layer of anhydrous sodium sulphate, and the filter is washed with 3 ml. of chloroform, added drop by drop after the first extract has passed through. To the combined chloroform extracts 10 ml. of water is added, and the chloroform is distilled off on the water bath. The aqueous solution is then made up to 50 ml. 2 ml. of this solution is transferred to a test-tube; 2 ml. of 2 per cent. solution of sodium sulphide is added, and the solution is made up to 10 ml. After heating for 10 minutes on a boiling water bath, the solution is cooled, and the colour is compared (visually or photoelectrically) with solutions prepared by reducing known amounts of picric acid (1 to 1·4 ml. of 0·01 per cent. solution) with sodium sulphide in the same way; 1 g. of picric acid is equivalent to 1·517 g. of atropine sulphate. The method is only suitable for neutral solutions. E. H.

Hexamine, Potentiometric Estimation of. Ya. M. Perel'man. (*Aptechnoe Delo*, 1953, **2**, No. 6, 17.) 10 to 40 ml. of hexamine solution (0·025 to 0·07M) is placed in a titration vessel containing a platinum electrode; the cell is connected by means of an agar bridge to a quinhydrone and calomel electrode system. Quinhydrone is added to the liquid and stirred in with a glass rod, and then 0·1N hydrochloric acid in 1 ml. quantities until the end-point is approached, when 0·1 to 2 ml. quantities are added; half a minute after each addition the E.M.F. is measured with a precision of 1 mV. The method is suitable for mixtures containing vegetable extracts and other coloured substances and for concentrations of hexamine down to 0·025M. The relative error is 1·6 per cent.; the method can also be used for concentrations as low as 0·005M, but the error is then 3·5 per cent. In some cases, especially when the titration was carried out slowly, anomalies, marked by an irregular increase in the potential and the appearance of submaxima, were observed. The glass electrode was also used, but it had no advantages over quinhydrone; the *p*H values of hexamine solutions obtained with the glass electrode were higher than those obtained with the quinhydrone electrode, which agreed with the true values. E. H

Iodine Value, Determination of. M. L. Kuchment. (*Aptechnoe Delo*, 1953, **2**, No. 6, 35.) A sample of the oil or fat (1 to 2 g.) is weighed into a porcelain dish, and 0·5 to 1·0 g. of an emulsifier such as gum acacia is added together with a few drops of water. The mixture is emulsified, gradually diluted with 15 to 20 ml. of water, and then transferred to a 100-ml. flask and made up to the mark.

10 ml. of the emulsion is pipetted into a ground-glass stoppered flask and 25 ml. of iodine solution (32 g. of potassium iodide and 24 g. of iodine made up to 11. in water) is added. After the addition of 12 ml. of 0·1M mercuric chloride, the mixture is well shaken and allowed to stand for 10 minutes (or 20 minutes for oils with iodine values higher than 130). 10 ml. of 10 per cent. potassium iodide is then added, and the free iodine is titrated with thiosulphate in the usual way. A blank determination is also carried out. The method is a modification of that of Fialkov, in which the mercury salt is not used; it was found that in the absence of mercury low results are obtained. The method showed good agreement with the official (Russian Pharmacopœia) Hübl method on caster oil, almond oil, sesame oil, fish oil, and cocoa butter. E. H.

Phenacetin, Colorimetric Determination of. B. N. Afanas'ev. (*Aptechnoe Delo*, 1953, **2**, No. 4, 21.) A weighed sample containing 0·1 to 0·3 g. of phenacetin is dissolved in 200 ml. of 90 per cent. ethanol and the solution is filtered, if necessary. 25 ml. of 5 per cent. aqueous solution of chloramine is added, and the mixture is heating to boiling, and, after cooling, made up to 250 ml. The colour is compared in a Duboscq colorimeter with that of a standard prepared in the same way from 2 g. of pure phenacetin. The method is suitable for mixtures of phenacetin with aspirin, caffeine, methyl caffeine or sodium salicylate, but amidopyrine interferes. The results of replicate analyses on various preparations of phenacetin are given. E. H.

Pseudosapogenins, Qualitative Colour Test for. E. S. Rothman, M. E. Wall and H. G. Cooper. (*J. Amer. chem. Soc.*, 1953, **75**, 6325.) The key step in the transformation of steroidal sapogenins to pregnane derivatives is conversion to the so-called "pseudosapogenin" acetates. It was found that the application of the Tortelli-Jaffé colour reaction (*Chem. Z.*, 1915, **39**, 14) to pseudosapogenins gave a blue colour which differs from the typical given colour produced by ditertiary bridgehead ethylenic bonds or by olefines isomerisable to this type. In the most sensitive method of using the test, 1 mg. of pseudosapogenin dissolved in 1 ml. of chloroform was diluted with 5 ml. of glacial acetic acid and mixed with 1 ml. of 0·1 per cent. bromine in chloroform. The mixture was underlayered with 0·1 ml. of 1 per cent. bromine in chloroform and, after 30 minutes, was diluted to 10 ml. with acetic acid and mixed. Attempts to adapt the reaction for quantitative measurements were unsuccessful. The test is particularly useful as an indication of completeness of reaction in pseudosapogenin transformations, e.g., hydrogenation, oxidation, and the like.

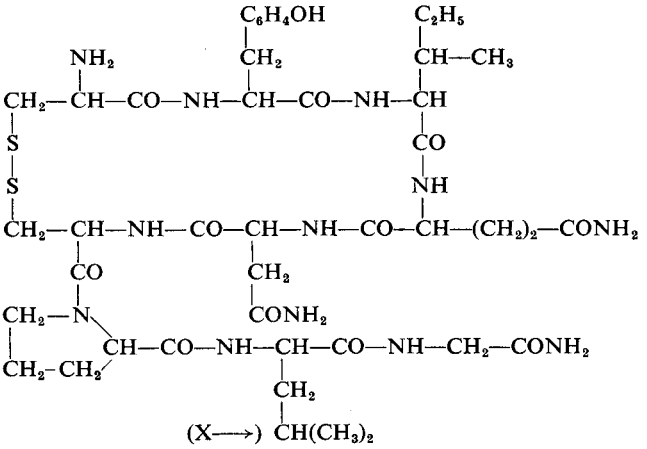
A. H. B.

ORGANIC CHEMISTRY

***p*-Aminosalicylic Acid and its Sodium Salt, Decarboxylation of.** Yu. N. Sheinker and I. V. Persiyanova. (*Zhur. Priklad. Khim.*, 1953, **26**, 860.) The effect of moisture and *p*-aminophenol on the decarboxylation of *p*-aminosalicylic acid was studied at temperatures between 59° and 100° C. The dry acid decomposed in two stages, a slow induction period being followed by rapid decomposition. At 59° C. there was no decomposition after 200 hours, but with increase of temperature the rate increased and the length of the induction period was sharply reduced. The rapid stage followed the Arrhenius equation in the interval 70° to 100° C.; the activation energy was 20,500 k.cal./mol. The presence of water (5, 10, 20 and 44 per cent.) caused a progressive increase in the rate of decarboxylation; there was no difference in the rates of decomposition of the pure and technical grades of the acid in the presence of 44 per cent. of

water. Addition of *m*-aminophenol (3 to 5 per cent.) also increased the rate, and the added phenol had a greater effect than that produced during the decarboxylation. The rate was not affected by varying the initial pressure of the carbon dioxide from 20 to 760 mm. The sodium salt of the acid was stable within the range of temperature studied. E. H.

Oxytocin, Synthesis of an Octapeptide Amide with the Hormonal Activity of. V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, P. G. Katsoyannis and S. Gordon. (*J. Amer. chem. Soc.*, 1953, **75**, 4879.) From evidence from hydrolysis and degradative studies and a knowledge of the molecular weight, the formula (I) was postulated for oxytocin, the principal uterine-contracting and milk-ejecting hormone of the posterior pituitary gland.



(I)

The amino-acid residues have the L-configuration. The total synthesis of this compound is described, and the synthetic material possessed the expected oxytocic activity on the isolated rat uterus. Furthermore, the synthetic product was fully effective in stimulating labour in the human and possessed milk-ejecting activity. No differences were detected in distribution coefficients, electrophoretic mobilities and infra-red patterns between the synthetic material and natural oxytocin. The synthesis reported therefore appears to give a product which is identical with natural oxytocin and this constitutes the first synthesis of a polypeptide hormone. A. H. B.

Vasopressin, Enzymatic Cleavage of Glycinamide from, and a Proposed Structure. V. du Vigneaud, H. C. Lawler and E. A. Popenoe. (*J. Amer. chem. Soc.*, 1953, **75**, 4880.) The structure proposed for arginine-vasopressin is as (I) (see preceding abstract) but with the $CH(CH_3)_2$ group at X replaced by $CH_2CH_2NH—C(=NH)—NH_2$.

The evidence for this structure is based upon (1) partial hydrolysis of performic acid-oxidised arginine-vasopressin and the determination of the structure of the resultant peptides; (2) the application of the Edman degradation to performic oxidised vasopressin and by other degradative reactions; (3) enzymatic cleavage

of arginine-vasopressin with trypsin for 6 hours at 38° C. and *p*H 7 with subsequent identification of the products by paper chromatography and countercurrent distribution. It is suggested that lysine-vasopressin has the same composition as arginine-vasopressin but with lysine replacing the arginine group. A. H. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Intermedin, Chromatographic Behaviour of. R. R. Kohn. (*Nature, Lond.*, 1953, **172**, 1185.) Potentiated intermedin from powdered hog posterior pituitary glands has been submitted to ascending chromatography on No. 1 Whatman paper with butanol-water-acetic acid. The chromatogram was dried, and 1 cm. wide vertical strips from each side were sprayed with ammoniacal silver nitrate and ninhydrin. No reducing carbohydrates were detected. The relative amounts of ninhydrin positive material were measured as a function of chromatogram height photoelectrically. The chromatogram was cut into a series of horizontal strips, the absorbed material eluted, and the eluates assayed for intermedin activity on melanophores in the excised web of the foot of *Rana pipiens*. The measure of eluate activity was based on that dilution which caused the melanophores to attain a stellate stage of pigment dispersion in 1 hour. Activity was found to exist in two concentration peaks; one associated with a small ninhydrin-peak with an R_F value of 0·28, the other with a ninhydrin-positive area with an R_F value of 0·04. J. B. S.

Mycobactin, a Growth Factor for *Mycobacterium johnei*. J. Francis, H. M. Macturk, J. Madinaveitia and G. A. Snow. (*Biochem. J.*, 1953, **55**, 596.) Mycobactin has been isolated from cultures of *Mycobacterium phlei* in a beef-infusion medium containing 4 per cent. peptone and 10 per cent. glycerol. The mycobactin content of cultures was assayed by assessing the amount of growth of cultures of *Myco. johnei* on a solid egg medium to which mycobactin was added, after incubation for 4 to 6 weeks. Mycobactin was extracted with acetone and then precipitated by the addition of light petroleum in which it is insoluble. Purification was effected by adsorption on alumina and elution with hot ethanol, when a crystalline aluminium complex was obtained. A copper complex is also formed, and this can be decomposed to give metal free mycobactin, which is colourless. The yield of mycobactin corresponds to 0·86 per cent. of the dry weight of *Myco. phlei*. Comparable growth promotion tests on *Myco. johnei* with miscellaneous plant and vegetable products in no case showed more than slight growth. Mycobactin appears to be homogeneous, but occasional discrepancies in the optical rotation have been observed. The diacetyl derivative is, likewise, amorphous, but has a reproducible melting point. Both compounds give reproducible ultra-violet absorption spectra. X-ray powder photographs indicate a multi-ring structure with the possibility of the molecule having 7 to 8 rings. The solid exhibits a pale green fluorescence and the solution in methanol a blue one. It is stable up to 150° C. and not oxidised in air; it is soluble in sodium hydroxide, but not in sodium bicarbonate solution. Alkaline solutions are unstable. Mycobactin is insoluble in dilute mineral acids but soluble in concentrated sulphuric acid without decomposition; the latter solution is inactivated on heating. A molecular formula $C_{47}H_{75}O_{10}N_5$ has been assigned to the molecule. A hydrochloride and picrate are reported and cited, in conjunction with electrometric titrations, as evidence of the presence of a weakly basic group. The diacetate gives an intense violet colour with

ferric chloride, indicative of a third hydroxyl (phenolic) in mycobactin, which is not readily acetylated. Optimum growth of *Myco. johnei* is promoted by the presence of 40 to 80 μg. mycobactin/ml. J. B. S.

Oxytocin, Synthetic, and Purified Vasopressin, High Streptogenin Potency of. D. W. Woolley and R. B. Merrifield. (*J. Amer. chem. Soc.*, 1954, **76**, 316.) The bacterial and animal growth factor streptogenin, which is a peptide-like substance liberated during partial hydrolysis of certain proteins, has previously not been isolated in pure condition. The application of ion exchange techniques and countercurrent distribution led to the isolation of a number of peptides. The most active peptide yielded cystine, glutamic acid, glycine, serine, valine and the leucines upon hydrolysis. The similarity in amine and composition of these peptides to oxytocin (du Vigneaud *et al.*, *J. Amer. chem. Soc.*, 1953, **75**, 4879) was observed, and therefore synthetic oxytocin and related peptides were assayed for streptogenin activity. Oxytocin was 300 times as active as a lower extract standard (Sprince and Woolley, *J. Amer. chem. Soc.*, 1945, **67**, 1734). The cystine residue of oxytocin was essential for its streptogenin potency. *SS*-Dibenzyloxytocin was practically inactive, as was also performic-acid-oxidised oxytocin. Several of the smaller synthetic peptides representing sequences in oxytocin or vasopressin were inert. Some variation in the amino-acid composition is compatible with high streptogen potency because purified arginine-vasopressin, free of oxytocin, had a streptogenin activity of 150. However, some specificity of structure seems to be necessary for streptogenin activity. A. H. B.

Sodium-retaining Substance from Beef Adrenal Extract, Isolation of. V. R. Mattox, H. L. Mason and A. Albert. (*Proc. Mayo Clin.*, 1953, **28**, 569.) A crystalline substance with sodium-retaining properties has been isolated by chromatographic techniques from the co-called "amorphous fraction" of beef adrenal extract. The separation was followed by means of a bioassay on adrenalectomised rats, based on the ability of the fraction to cause sodium retention and potassium excretion, the assay being standardised against deoxycortone acetate. The amorphous fraction was obtained by repeated distribution between benzene and water of the extract from beef adrenals. The water-soluble fraction was crystallised until no further crystalline material could be separated. The remaining "amorphous" fraction was biologically active, and when submitted to paper chromatography showed evidence of upwards of a dozen compounds including cortisone. Identifiable substances were insufficient quantitatively to account for the whole of the observed biological activity. Preliminary fractionations were effected by partition of the whole adrenal extract between toluene and propylene glycol on a column of silica-gel. The sodium-retaining substance and cortisone were afterwards separated as acetate by partition in formamide-benzene and formamide-benzene-*cyclo*hexane systems on silica-gel columns. Further purification was effected by partition on paper with formamide as the stationary phase and benzene-*cyclo*hexane as the mobile phase. The diacetate obtained in this way could not be crystallised, and had approximately the same biological activity as deoxycortone acetate. Enzymatic hydrolysis of the diacetate gave a crystalline material, mp. 163° to 164° C., with a biological activity about 100 times that of deoxycortone acetate. A monoacetate was also isolated, about 25 times as active as deoxycortone acetate. Infra-red and ultra-violet absorption spectrographic examination, together with certain chemical reactions, suggest that the unacetylated sodium-retaining substance is an isomer of cortisone. J. B. S.

BIOCHEMICAL ANALYSIS

Calcium in Biological Material, Determination of. P. S. Chen and T. Y. Toribara. (*Analyt. Chem.*, 1953, **25**, 1642.) Variables encountered in the determination of calcium in biological samples by the flame photometric method have been studied in detail. Highest readings were obtained at 556 mμ, but the background was also high and more reproducible results were obtained at 620 mμ. Phosphate suppressed the calcium emission, and protein partially prevented the action of phosphate. Details of procedure are given. Calcium could be determined direct on diluted serum; the potassium content of the serum gave small errors (about +2 per cent.) while the sodum concentration was constant enough to allow for easy compensation. A preliminary precipitation of calcium as oxalate was recommended for urinary calcium, the precipitation also removing sodium, potassium, and phosphate ions. R. E. S.

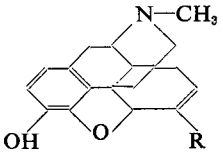
Steroids, Effect of Ultra-violet Light on, during Paper Chromatography. K. Savard, H. W. Wotiz, P. Marcus and H. M. Lemon. (*J. Amer. chem. Soc.*, 1953, **75**, 6327.) Two of the difficulties encountered in the application of paper chromatography to the separation and purification of steroids are (1) the frequent inability to measure steroids quantitatively following chromatography, (2) the inability to obtain, from paper chromatograms, samples of steroids completely free from contaminants which appear as highly polar materials which do not migrate from the starting line in subsequent chromatograms. The observations reported indicate that ultra-violet light (present in artificial light and sunlight) can affect chemical changes in steroid samples during the drying of a paper chromatogram. In synthetic ^{14}C-testosterone, it appears that chemical change occurs prior to the development of the chromatogram. By taking the precautionary measure of drying the chromatograms as rapidly as possible in the absence of light at temperatures not exceeding 50° C., adequate chemical recoveries of $\alpha:\beta$-unsaturated ketosteroids and urinary 17-keto-steroids were possible. Where heat-labile corticosteroids are concerned, it was found best to restrict the drying period to less than one hour at room temperature, shielded from direct sunlight or fluorescent light; the residual solvent (propylene glycol, formamide) was later removed from the eluted steroid preparation *in vacuo* over phosphorus pentoxide, or by partition between ether or methylene dichloride and water. A. H. B.

Streptomycins, Paper Electrophoresis of. M. C. Foster and G. C. Ashton. (*Nature, Lond.*, 1953, **172**, 958.) Streptomycin, mannosidostreptomycin and allied substances have been separated by paper electrophoresis. The apparatus consists of (*a*) a pair of electrode vessels in each of which is situated a pure graphite electrode with a platinum lead; (*b*) a pair of planar glass sheets to act as support and cover for the filter paper, arranged horizontally on levelling screws. Using a power pack providing 0 to 100 m.amp. at 300 volts; a cooling system was found to be unnecessary. A number of spray reagents has been examined. A mixture of equal volumes of 0·1 per cent. aqueous diacetyl, 20 per cent. aqueous potassium hydroxide and 2·5 per cent. α-naphthol in ethanol, develops a magenta colour by reaction with the guanidino groups of streptomycin. After spraying with a solution of naphthoresorcinol in ethanol, acidified with orthophosphoric acid and heating at 100° C. for 1 to 2 minutes, streptomycin shows spots which are intensely fluorescent in ultra-violet light. At high concentrations mannosidostreptomycin shows pink and streptomycin grey,

when viewed in daylight. A spray reagent, suggested by Partridge, gives pink spots, but is less sensitive. Average values of the mobility, μ, of streptomycin mannosidostreptomycin, streptothricin, streptidine and streptamine are recorded. J. B. S.

CHEMOTHERAPY

Δ^6-Desoxymorphines, 6-Substituted. H. D. Brown, I. M. Rasmussen, G. B. Payne and K. Pfister, 3rd. (*J. Amer. chem. Soc.*, 1953, **75**, 6238.) The preparation of a number of Δ^6-desoxymorphines of type I, where R= CH_3, C_2H_5, C_4H_9 and C_6H_5 is described.



(I)

The new analgesics were readily produced by acylation (at OH–3) of the corresponding dihydromorphines followed by dehydration with thionyl chloride and the hydrolysis of the protecting groups. Of the compounds investigated, 6-methyl-Δ^6-dexoxymorphine (I, R = CH_3) was the most potent and gave complete analgesia of rather short duration in all rats at doses as low as 63 μg./kg. Acylation did not greatly alter the activity but shortened the duration. The compounds (I, R = C_2H_5 and R = C_4H_9) were only slightly less active than the methyl compound while the phenyl homologue (I, R = C_6H_5) was significantly less active. Of the intermediates, 3-acetyl-6-methyldihydromorphine was potent but quite toxic. A. H. B.

Quaternary Ammonium Chlorides, Antimicrobial Activity of. R. A. Reck and H. J. Harwood. (*Industr. Engng Chem.*, 1953, **45**, 1022.) The object of this investigation was to establish which quaternary ammonium salt, on the basis of antibacterial activity, physical properties and commercial availability of raw materials, is most suitable for commercial development. Factors considered were degree of unsaturation and alkyl-chain mixtures. Trimethylalkyl quaternary ammonium salts containing the mixed alkyl groups derived from coconut oil, tallow and soybean oil were evaluated, as well as the benzyl-type salt derived from coconut oil. The trimethylalkyl quaternary salts prepared from commercial palmitic and stearic acids were also employed in the investigation. A serial dilution broth screening assay was employed to determine the antibacterial activity of the salts for a broad spectrum of organisms. It was found that the bactericidal activity of the higher aliphatic quaternary ammonium salts containing varying proportions of the saturated and unsaturated C_{18} chain does not vary markedly with the degree of saturation. There is, however, a significant tendency toward higher activity in the mixtures of lower degree of unsaturation. In general, the hexadecyl and the tallow derivatives were found to be superior. The trimethylalkylammonium chloride derived from tallow fatty acids was regarded as the most suitable salt for commercial development. S. L. W.

PHARMACOLOGY AND THERAPEUTICS

Adrenal Ascorbic Acid, Distribution between Particulate and Non-particulate Components of Adrenal and Liver Cells. P. Hagen. (*Biochem. J.*, 1954, **56**, 44.) A study has been made of the distribution of ascorbic acid in cytoplasmic fractions obtained by high-speed centrifugation of tissue homogenates

of ox adrenal medulla in sucrose solution. In all tissues examined, 87 to 100 per cent. of the ascorbic acid was found in the supernatant of the high-speed centrifugate. Little if any ascorbic acid was found in the cytoplasmic particles. Although these results do not provide sufficient evidence to decide whether in the liver cell ascorbic acid is present in the mitochondria or in the cytoplasmic sap, it is significant that other low-molecular weight components of the adrenal medulla are retained by the particles during this procedure. J. B. S.

Adrenaline-Aureomycin in Isolated Frog and Turtle Hearts, Cardiac Arrhythmias Induced by. P. A. Harvey and W. Yang. (*Science*, 1953, **118**, 752.) Isolated frog and turtle hearts perfused by the Straub method were sensitive to the induction of cardiac arrhythmias by adrenaline (1×10^{-6}) when they had been treated previously with Aureomycin hydrochloride (1×10^{-4} to 1×10^{-5}). The adrenaline-induced irregularities did not appear until 20 to 80 minutes after treatment with the aureomycin and could be avoided by pretreatment with potassium chloride just before addition of the adrenaline. G. P.

Adrenolytic and Parasympatholytic Drugs, Blockade of Ovluation in the Hen by. M. X. Zarrow and J. W. Bastian. (*Proc. Soc. exp. Biol. N.Y.*, 1953, **84**, 457.) There is evidence which suggests the presence of a neuro-humoral hypothalamico-adenohypophyseal link in the release of the ovulatory hormone (L.H.) in rat and rabbit (*Recent Prog. Horm. Res.*, 1952, **8**, 139). The authors propose a similar neural link in the ovulatory process of the hen. Normal ovulation in adult White Leghorn hens was inhibited 71 per cent. by atropine and 79 per cent. by SKF–501 (an adrenergic blocking agent). Progesterone-induced ovulation (the progesterone causing a 90 per cent. ovulatory response) was reduced to 20 per cent. after atropine (representing an 80 per cent. inhibition) and to 56 per cent. after SKF–501 (a 44 per cent. inhibition). These results agree with those obtained previously for the rat and the rabbit and suggest both adrenergic and cholinergic links in the neural pathway. G. P.

Aureomycin; Enhancement of Serum Levels. H. J. Eisner, F. E. Stirn, A. C. Dornbush and J. J. Oleson. (*J. Pharmacol.*, 1953, **108**, 442.) The serum levels of aureomycin in rats and guinea-pigs were determined after oral doses ranging from 6 to 800 mg./kg. In general it was found that an increase in dose did not give a proportional increase in the serum concentration of aureomycin. With the simultaneous administration by mouth of certain adjuvants the serum levels in rats were increased up to tenfold. The most effective were citric acid, trisodium citrate, malic acid, tartaric acid, malonic acid, pyruvic acid, monosodium phosphate, tricarballylic acid and lactic acid. The enhancement effect was apparent within 1 hour and lasted for at least 8 hours after the dose was administered, and the effect was noted with doses of aureomycin varying from 8 to 200 mg./kg. S. L. W.

Calomel in Teething Powders, Poisoning by. H. G. Farquhar. (*Lancet*, 1953, **265**, 1186.) A 5 months old boy, having recovered from pink disease produced by ingestion of 52 mg. of calomel in teething powders twice weekly for a month, died 13 months later after another 102 mg. given in 2 doses over 3 weeks. Urine analysis showed a mercury content of 90 μg. in 100 ml. and necropsy revealed gross œdema of all tissues and severe renal damage. A 6 months old boy who had received 13 mg. of calomel every night for 3 months

was admitted to hospital with the symptoms of pink disease and died 6 days later. Urine analysis showed a mercury content of 404 μg. in 100 ml. Analysis by Laug and Nelson's method showed the mercury content of liver to be 3300 μg./145 g. of tissue and of kidney 18,500 μg./45 g. of tissue. In the absence of any conclusive evidence of beneficial effects in infants and in view of its possible harmful effects the sale of teething powders containing calomel should be condemned. J. R. F.

Cortisone in Rheumatoid Disease. H. F. West and G. R. Newns. (*Lancet*, 1953, **265**, 1123.) Doses of cortisone in excess of 75 mg. daily can rarely be maintained for many months and the investigation was planned to provide a definite answer to the question whether 50 to 75 mg. a day orally, continued for a long time, favourably influences the course of rheumatoid disease. 31 patients were started on cortisone treatment 1 to 3 years ago and 27 are still receiving the compound; the average duration of treatment is 19 months. At the commencement, 16 patients were in stage II and 11 in stage III. At first, cortisone was given intramuscularly, but for the last 2½ years it has been given solely by mouth. Progress was assessed by determination of the erythrocyte sedimentation rate, hæmoglobin, packed-cell volume, eosinophil count, plasma-protein, plasma-fibrinogen and plasma-cholesterol, physical examination of joints, grip, weight and blood pressure, and by the effect of rest on stiffness, the use of analgesics, ability to work, and radiographic evidence of an arrest in the disease. A similar group of 27 served as controls. The conclusion is that the treatment does not have a favourable effect on the disease. Five instances of gastric hæmorrhage occurred in 3 patients in spite of precautions to avoid it. Increase in heart size was found in many of the patients. Some weight increase occurred, with a disproportionate increase about the hips in women. The white-cell count was raised. No abnormalities as regards infection, mental behaviour or the endocrine system were observed. In 2 patients death was undoubtedly hastened by the treatment. While it is known that cortisone suppresses the endogenous output of hydrocortisone-like steroids and, after prolonged suppression, adrenocortical deficiency may follow suspension of treatment, no withdrawal symptoms were noted in this series. Although long-continued cortisone therapy is not to be recommended the results do not exclude corticosteroids from a role in the pathogenesis of rheumatoid disease. H. T. B.

Coumarin Anticoagulant, New Intravenous. J. M. Wolff, N. W. Barker, R. W. Gifford, and F. D. Mann. (*Proc. Mayo Clin.*, 1953, **28**, 489.) 3-(α-phenyl-β-acetylethyl)-4-hydroxycoumarin (warfarin), which produces hypoprothrombinæmia, like the other coumarin compounds, has been injected intravenously into 29 patients. Hypoprothrombinæmia reached the therapeutic range within 24 hours and was maximal at 48 hours. The effects were more rapid and consistent than with any of the other coumarin derivatives given orally. While the need for intravenous administration is a drawback, the drug provides a satisfactory introduction for subsequent treatment with dicoumarol and is also valuable to administer to patients who are vomiting or cannot tolerate oral medication. G. F. S.

Dextran Sulphate, Clinical Trial of. C. R. Ricketts, K. W. Walton, B. D. van Leuven, A. Birbeck, A. Brown, A. C. Kennedy and C. C. Burt. (*Lancet*, 1953, **265**, 1004.) The anticoagulant and toxic actions of dextran sulphate are dependent respectively on the sulphur content and the

dimensions of the molecules. Material having the optimum molecular size and sulphur content is less active than heparin on a weight basis, but its biological action is similar and, after a preliminary trial, a large batch was distributed for independent assessment to 3 centres with experience of anticoagulant therapy with heparin. The solution used contained the equivalent of 900 to 1000 heparin units/ml., with sodium chloride 0·72 per cent. and sodium bicarbonate 0·24 per cent. Dosage and frequency of administration varied widely, but in most cases 5000 units 6-hourly, or 500 to 750 units hourly in intravenous drip, was adequate. 5000 units of heparin injected intravenously prolongs the clotting-time for 2 to 3 hours; equivalent dosage of dextran sulphate produced an effect for 5 to 7 hours. On stopping treatment, the clotting-time returned to normal in 4 to 5 hours. No toxic side effects were noticed, but the occurrence of a few hæmorrhagic complications showed that dextran sulphate is no safer than other anticoagulants. In an emergency rapid counter-action of the effects of dextran sulphate could be obtained by blood transfusion. Control of therapy merely requires determination of the clotting-time before each injection. Advantages over heparin are that it is less likely to cause sensitisation reactions since it is not derived from animal tissues, and that it can be manufactured relatively cheaply to specified chemical and physical characteristics. H. T. B.

10-(γ-Dimethylaminopropyl)-2-chlorophenothiazine Hydrochloride. A New Anti-emetic Drug. D. G. Friend and J. F. Cummins. (*J. Amer. med. Ass.*, 1953, **153**, 480.) 10-(γ-Dimethylaminopropyl)-2-chlorophenothiazine hydrochloride, already found to block the emetic effect of apomorphine in animals (*Pr. méd.*, 1952, **60**, 206) has been shown to be effective in eliminating nausea associated with carcinomatosis, labyrinthitis, lymphomatosis and uremia. It also prevented or controlled nausea and vomiting in alcohol-intoxicated patients treated with disulfiram (antabuse) and that produced by aureomycin, folic acid antagonist, codeine, meperidine, methadone, morphine, nitrogen mustard, protoveratrine, oxytetracycline (terramycin) and urethane therapy. In suppressing vomiting the initial intramuscular dose (25 to 50 mg.) was usually effective within 1 hour and remaining doses could be given orally 3 or 4 times a day. Side effects observed were dryness of the mouth, occasionally mild sedation (the drug generally showed no evidence of depression of the central nervous system) and in 3 of the 70 patients treated transient faintness, palpitation and flushing of the face were evident. G. P.

Fumagillin in Amœbiasis. R. Elsdon-Dew, A. J. Wilmot and T. G. Armstrong. (*Lancet*, 1953, **265**, 1180.) 55 African males with acute ulcerative amœbiasis were treated with fumagillin for 10 days. 7 received a 20 mg. dose twice daily and 48 were given a 50 mg. dose 4 times daily. The 40 mg./day dosage was found to be ineffective in controlling acute amœbic dysentery, while the 200 mg./day dosage produced results comparable to those obtained with emetine, 1 g./day for 10 days, but inferior to those obtained with aureomycin at a dose of 2 g./day for 15 days. A mild facial dermatitis in 14 patients receiving the 200 mg. dose was the only toxic symptom observed. J. R. F.

Hibernation Anaesthesia in Major Surgery. A. Smith and J. G. Fairer. (*Brit. med. J.*, 1953, **2**, 1247.) Use has been made, in 36 persons requiring major surgery, of a mixture of chlorpromazine, pethidine and promethazine. Each patient was given 50 mg. of promethazine-8-chlorotheophyllinate by mouth two

hours preoperatively, followed one hour later by a subcutaneous injection of 100 mg. of pethidine and 0·43 mg. of hyoscine hydrobromide. In the half-hour before the operation a mixture of 50 mg. promethazine, 50 mg. chlorpromazine and 100 mg. pethidine was given intravenously in divided doses. Pulse rate during injection rose at first, but later settled to a level just above resting rate. This rate remained unaltered throughout, regardless of severity of surgical procedure or duration of the operation. Respiration became shallow and sometimes increased in rate, while blood pressure fell and was maintained in the region of 100 mm. Hg. At the conclusion of the injection the patient was somnolent, but could still be roused. 2 to 3 ml. of 5 per cent. thiopentone were given to produce unconsciousness. Post-operatively there was no nausea, vomiting or headache and patients required less nursing care than usual.

G. P.

Isoniazid and Streptomycin in Tuberculous Meningitis. J. Torres-Gost. (*Lancet*, 1953, **265**, 693.) Results are reported of treating 100 cases of tuberculous meningitis with a combination of intramuscular streptomycin, oral isoniazid and intrathecal isoniazid. Dosage for children from 2 to 10 years was as follows: intramuscular streptomycin, 0·5 g. daily in one or two doses; oral isoniazid, 200 mg. daily; and intrathecal isoniazid, 20 to 30 mg. daily according to the severity of the illness. Other age groups received appropriately smaller or larger doses. Treatment was continued for 3 months, the frequency of the intrathecal injections being reduced after the first month. There were 6 deaths in the series. Of the remaining 94, the mild cases recovered rapidly, meningeal signs disappearing in a week although improvement in the c.s.f. was slower. In the moderately severe cases, many of whom were at first in deep stupor, progress was slower but still satisfactory. The return to contact with the outside world was usually complete in 2 to 3 weeks but might require 2 months or more. In the severe cases the response was less satisfactory. The persistence of signs and symptoms was often due to extrameningeal tuberculosis. Only 7 patients had significant sequelæ, one having weakness of the hand muscles and the other 6 paresis of the tibialis anterior, causing difficulty in walking. There were 10 relapses, all mild and responding promptly to further treatment. More than 12,000 intrathecal injections of isoniazid have been given in the author's clinic with no difficulties or signs of intolerance. H. T. B.

Isoniazid in Tuberculous Meningitis. T. Anderson, M. R. Kerr and J. B. Landsman. (*Lancet*, 1953, **265**, 691.) 7 unselected consecutive patients with tuberculous meningitis were treated with isoniazid, 10 mg./kg. per day by mouth for 16 weeks. The first four received streptomycin in addition for a week, both intramuscularly and intrathecally. The fifth patient, previously treated with streptomycin, was admitted in relapse and was treated with isoniazid only. The last 2 patients were given supplementary treatment with streptomycin for 4 weeks. As controls, patients treated with streptomycin alone during the immediately preceding period were selected so as to compare as closely as possible in age and severity of illness with the group given isoniazid. All the patients have survived, although the longest period is only 12 months. The c.s.f. was examined weekly for cells, sugar, protein and chlorides, and the duration of the initial fever was noted. The isoniazid-treated patients returned more rapidly to normal and also showed more rapid clinical improvement as judged by appearance, appetite and gain in weight, than the control group. Improvement was least rapid in those treated with streptomycin for 4 weeks, probably owing to the irritant effect of the antibiotic on the meninges. It is

suggested that both the total amount of streptomycin and the duration of its administration can be reduced when isoniazid is given, with benefit to the patient's comfort and an increase in the rapidity of his recovery. H. T. B.

Methimazole in Treatment of Thyrotoxicosis. L. A. G. Davidson. (*Brit. med. J.*, 1953, **2**, 1300.) This report deals with 44 patients treated with methimazole since January 1950. Of these, 34 were untreated thyrotoxicosis patients or people who had relapsed after previous drug treatment with methylthiouracil. In the remainder methimazole was used in maintenance dosage after control with methylthiouracil. The 34 cases of thyrotoxicosis comprised 28 women and 6 men, whose ages varied from 22 to 77 years. The symptoms had been present on the average for 11 months and the average increase in the B.M.R. was +44·6 per cent. The patients received one 5 mg. tablet 3 times a day. As soon as a patient was considered to be euthyroid a maintenance dose of 5 mg. or less daily was begun. A complete remission of symptoms was produced in every patient except one. One, after control, was subjected to surgery. Obvious clinical improvement was noted within a week in every case. The average time until complete control of symptoms was achieved was 56 days; the average B.M.R. after control was +4·7 per cent. 18 patients were able to stop treatment after an average duration of 29 weeks, 6 days; 8 of these relapsed. Toxic effects, mainly skin reactions, occurred in 4·5 per cent. of the patients; there were no serious toxic effects and no evidence of granulocytopenia. Methimazole appears to be the best available preparation for the drug treatment of thyrotoxicosis; it is more effective than other antithyroid drugs, both in rate of response and lack of failures. It is less toxic than methylthiouracil. S. L. W.

Triethylene Thiophosphoramide in the Treatment of Leukæmia. H. Shay, C. Zarafonetis, N. Smith, I. Woldow and D. C. H. Sun. (*Arch. intern. Med.*, 1953, **92**, 628.) This is a preliminary report covering clinical trials of 6 months' duration on 49 patients, 39 with malignant disease originating in the hæmopoietic system and 10 with other inoperable malignant disease. Triethylene thiophosphoramide is dissolved in sterile isotonic sodium chloride solution in a concentration of 10 mg./ml., and is administered intramuscularly or intravenously, in doses of 2, 5, or 10 mg., the size of any one dose depending on the hæmatological findings during the course of treatment. Intramuscularly it is administered undiluted; intravenously the amount of drug to be given is added to 10 ml. of isotonic sodium chloride solution and injected over a period of 2 minutes. Injections were given daily, or at longer intervals, depending on the hæmatological response. The most favourable results were obtained in cases of subacute or chronic leukæmia; it does not appear to be of any value in acute leukæmias. In the dosages used it is remarkably free of any immediate toxic effect such as nausea, vomiting, or local pain after injection; no local venous thrombosis has occurred after intravenous injection. There appears to be a reasonable margin of safety between the "effective" therapeutic dose and the dose which will produce undue bone marrow depression. Encouraging results were obtained in two patients with recurrent and metastatic breast carcinoma, following radical removal of the primary breast lesion; in one there was complete, and in the other partial, disappearance of the metastatic auxiliary mass, with gradual sloughing and healing of the recurrent malignant lesions in the operative scar. The drug is also effective by mouth, in a dose of 10 mg. daily. S. L. W.

L-Triiodothyronine and L-Thyroxine in Human Myxœdema. R. W. Rawson, J. E. Rall, O. H. Pearson, J. Robbins, H. F. Poppell and C. D. West.

(*Amer. J. med. Sci.*, 1953, **226**, 405.) Thyroxine has been regarded previously as the thyroid hormone and the only iodinated compound in the circulation. Recently triiodothyronine has been identified in man and shown to be 3 to 5 times as active as L-thyroxine in myxœdema. This paper compares the total metabolic effects of L-triiodothyronine and also diiodothyronine with those of L-thyroxine in a myxœdematous patient. The results showed diiodothyronine in a dose of 1 mg. to have no effect on the B.M.R. or on metabolism. The same dose of triiodothyronine was followed by a prompt but short-lived increase in the basal metabolic rate and in the excretion of nitrogen, phosphorus and creatine. Thyroxine produced a slow and prolonged effect on the same indices. Disregarding the time of action there were no qualitative and on an equimolecular basis no quantitative differences between it and triiodothyronine. With thyroxine and triiodothyronine labelled with ^{131}I radioactivity disappeared more slowly from the liver area with thyroxine. G. F. S.

BACTERIOLOGY AND CLINICAL TESTS

Penicillin, Paradoxical Effect of. R. F. Parker. (*J. Bact.*, 1953, **66**, 60.) Actively growing cells of staphylococci are killed by penicillin when exposed continuously to it under conditions favouring continued growth. When a culture of staphylococcus is exposed to about 10 times the "minimal inhibiting concentration" increase of the number of viable cells stops abruptly; continued exposure to penicillin results in an initial rapid decrease in viable cell count followed by a lower rate of decrease. If exposure to penicillin is only for 15 minutes no appreciable death of cells occurs during or after the exposure, and after a time growth is resumed. If the penicillin concentration is increased to 50 times the "minimal inhibiting concentration" there is usually an appreciable reduction in the number of viable cells during the 15-minute exposure and the subsequent short period of time required to remove the antibiotic. Then, if conditions are appropriate, the number of viable cells may continue to decrease for more than an hour after complete removal of penicillin, with ultimate reduction in the count by 90 to 99 per cent. Death of the cells may be prevented if they are incubated for a time at 25° C. or if the *p*H of the nutrient medium is reduced from 8·1 to 6·9. S. L. W.

***Bacillus anthracis*, Toxin Production by.** D. G. Evans and J. G. Shoesmith. (*Lancet*, 1954, **266**, 136.) There has been no evidence in the literature that *Bacillus anthracis* can produce a toxin, but the recent demonstration that culture filtrates of the closely related organism *B. subtilis* contain a thermolabile toxin suggested an investigation of culture filtrates of *B. anthracis*. A 7 days old culture of *B. anthracis* in a casein-hydrolysate medium was centrifuged and filtered, the filtrate being saturated at 5° C. with ammonium sulphate. The precipitate was collected, freed from ammonium sulphate by dialysis, and freeze-dried. A saline solution containing 0·5 mg./0·2 ml. of the dried material, when injected intradermally into rabbits, gave within 20 minutes an œdematous raised area about 25 mm. in diameter. After an hour the colour had deepened to dark purple and a vesicle filled with serous fluid had formed. After 1 to 1½ hours the vesicle broke down and after 10 to 20 hours the lesion resolved itself into dark-brown necrotic plaques. The dermonecrotic activity of the toxin was completely destroyed by heating solutions at 80° C. for 15 minutes. The possible significance of this toxin in the pathogenesis of anthrax is under investigation. H. T. B.