ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

***l-nor*-Ecgonine, Complete Extraction of the Bases and Acid Esters from Coca Leaves.** A. W. K. de Jong. (*Rec. Trav. chim. Pays-Bas*, 1948, **67**, 153.) A considerable quantity of a number of bases has been extracted for quantitative determinations, from coca leaves at 55°C. with a mixture of benzene, methyl alcohol and N/1 ammonium hydroxide, but some of these are not ecgonine alkaloids. Since the ecgonine alkaloids are soluble in ether, the benzene and methyl alcohol extraction was replaced by ether extraction in a Soxhlet apparatus, but this method too had its disdavantages. When it was proved that coca leaves contain *l-nor*-ecgonine, which could be converted by methylation into *l*-ecgonine, and thus was of value for preparing cocaine, a return to the benzene and methyl alcohol extraction at ordinary temperature, to prevent decomposition of the methyl ester, was indicated. A smaller quantity of the bases was extracted at ordinary temperatures than at 55°C. and it was necessary to add water to the menstruum, care being taken not to add more than was required to dissolve the salts of the bases. For the extraction now proposed, coca leaves, 20 g., are mixed with finely powdered calcium oxide, which rapidly absorbs all the water contained in the leaf tissue, and dried for 24 hours. They are then shaken with 100 ml. of anhydrous benzene, 5 ml. of absolute methyl alcohol, and a volume of ammonia solution, containing exactly 1·938 g. of water. After another 24 hours, a quantity of finely powdered calcium oxide, corresponding to the amount of water used in the ammonia solution, is added, and after thoroughly mixing, the extractor is closed and the mixture allowed to stand. The calcium oxide absorbs the water and the calcium hydroxide formed sets free the ammonia and the bases, while the acid esters of *l-nor*-ecgonine are converted into their calcium salts; decomposition of the alkaloids is thus prevented. Percolation is started after 24 hours at a slow rate (about 5 drops per minute), and when the solvent mixture has been used, a quantity of a mixture of anhydrous benzene and absolute methyl alcohol, which boils at 58°C., is added to the leaves. The percolation is stopped when 800 ml. of percolate has been collected, and the benzene-methyl alcohol mixture distilled at 58°C. The remaining benzene is filtered from the calcium salts, which are insoluble in benzene. The residue is shaken with 25 ml. of N/5 hydrochloric acid, and 100 ml. of ether and the quantitative determination of the bases (by titration, using methyl red solution as indicator), of the cocaines (using N/1 sodium carbonate), and finally of *l-nor*-ecgonine is carried out on the hydrochloric acid layer. L. H. P

ANALYTICAL

Apparent Density of Dry Powders, a Method for the Determination of. W. B. Ault. (*J. Soc. chem. Ind., Lond.*, 1948, **67**, 313.) 40 g. of precipitated calcium carbonate is rubbed through a 30-mesh sieve and transferred, without jolting or shaking, to a 250 ml. glass measuring cylinder. The powder occupies a volume V_0, depending upon its lightness or bulkiness, but it is difficult to obtain reproducible results for this quantity. The cylinder is fitted with a bung, placed in a box and allowed to drop through

1 inch, 50 times at intervals of 2 seconds. The powder then occupies a volume V_d, which is reproducible to within ± 1 ml. in 150 ml., giving an apparent density (W/V_d) variation of ±0·002 g./ml., using apparatus of specified dimensions. The dropping interval of 2 seconds is the most rapid procedure that can conveniently be carried out. There is no advantage in using a sieve finer than 30-mesh, and there will be a greater tendency for shearing to alter the apparent density. Increasing the height or number of times the cylinder is dropped lessens the difference in V_d for different grades of powder. The test can be modified for use with other powders, such as light magnesium carbonate, diatomaceous earth, chalk and barium carbonate. G. B.

Jaborandi, Assay of. Report No. 5 of the Poisons Sub-Committee of the Analytical Methods Committee of the Society of Public Analysts. (*Analyst*, 1948, **73**, 311.) In the method recommended, powdered jaborandi leaves made alkaline with dilute solution of ammonia, are extracted by percolation with chloroform; this is continued until complete extraction is effected as shown by a test with Mayer's reagent. The alkaloids are extracted from the bulked chloroform solutions using 0·1N sulphuric acid and the resulting acid extract, after making alkaline with ammonia, is finally extracted with successive quantities of chloroform. The chloroform extracts are combined, evaporated, dried, dissolved in a standard excess of 0·05N sulphuric acid and titrated with 0·05N sodium hydroxide. The result obtained expresses the total alkaloid content calculated as pilocarpine. Details of the extraction procedure and a method for separating pilocarpine nitrate from the total alkaloids are given. R. E. S.

Morphine and Apomorphine, Determination of, by a Volumetric-Colorimetric Method. A. Ionesco-Matiu, J. Popa and L. Monciu. (*Ann. pharm. Franc.*, 1948, **6**, 25.) In a 200-ml. conical flask, place a known volume of the morphine solution, 0·5 to 2 ml., and evaporate to dryness on a water-bath; to the residue add 2 ml. of concentrated sulphuric acid, and heat to boiling on a water-bath for 30 minutes; cool, and add with shaking, 100 ml. of distilled water, and neutralise to sodium, using one drop of phenolphthalein solution. Add to the solution 5 drops of a saturated solution of corrosive sublimate and 5 drops of a 10 per cent. solution of sodium acetate; heat to boiling for one minute, when the solution changes from colourless to green, through violet to an intense blue. Cool the solution under a current of water, add 2 ml. of 20 per cent. sulphuric acid and, using a micro-burette, add drop by drop solution of N/10 potassium permanganate, until the solution has changed to a yellowish-brown colour, the permanganate solution maintaining its colour for several seconds; each ml. of N/10 potassium permanganate is equivalent to 0·00495 g. of morphine hydrochloride. The same method may be employed for the determination of apomorphine, commencing with the words " Add to the solution 5 drops . . . . "; each ml. of N/10 potassium permanganate is equivalent to 0·00294 g. of apomorphine hydrochloride. The determination of either morphine or apomorphine may be made even in the presence of other alkaloids. S. L. W.

Plant Extracts, Identification of. G. di Bacco. (*Boll. chim-farm.*, 1948, **87**, 124.) Extracts of medicinal plants can be identified and their strength and purity established by chromatographic tests. Aluminium oxide is used as the medium, in a glass tube 15 mm. in diameter and 180 mm. long, drawn off to a point so that the liquid can flow out at the rate of

30 drops a minute. The lower end of the tube is plugged with cotton wool and about 10 g. of aluminium oxide suspension in light petroleum is poured in; the column of oxide should occupy 90 mm. When the light petroleum has flowed out, leaving a layer 8 or 10 mm. above the oxide, the solution or suspension of the extract to be examined is poured on, allowed to pass through, and the chromatogram developed by washing with two lots of light petroleum. For liquid extracts of rhubarb, cascara and frangula. 1 g. of the extract is shaken from time to time during 30 minutes with light petroleum 10 ml., benzene 10 ml., ether 5 ml. and the clear supernatant liquid is poured on the oxide. The liquid extract of genuine rhubarb of the Italian Pharmacopœia gave a 2 mm. yellow band, an 8 mm. red band. a 10 mm. pink fringe; a liquid extract of genuine rhubarb soluble in syrup gave a 1 mm. yellow band, a 6 mm. red band, a 12 mm. pink fringe; a liquid extract of European rhapontic rhubarb gave a 0·5 mm. yellow band. a 12 mm. pink fringe; a mixture of equal parts of genuine and rhapontic liquid extracts gave a 0·5 mm. yellow band, a 3 mm. red band, a 12 mm. pink fringe. Evidently the red band is due to anthraquinone derivatives. and shows the rhapontic rhubarb to be inferior to the Chinese and the soluble extract inferior to the official. The liquid extract of cascara of the Italian Pharmacopœia gave a 0·5 mm. yellow band, a 1 mm. red band. a 3 mm. bright red band, a 16 mm. pink fringe (the column viewed from above is coloured yellow). The aromatic, bitterless extract of cascara of the Italian Pharmacopœia gave a similar chromatogram with a more accentuated yellow band; liquid extract of frangula gave a 2 mm. bright red band and a 4 mm. pale pink fringe. To verify an extract, the chromatogram should be compared with one obtained from an extract of proved authenticity and the activity may be judged to be proportional to the length of the coloured bands. H. D.

FIXED OILS, FATS AND WAXES

Whale Oil, Component Acids and Glycerides of. T. P. Hilditch and L. Maddison. (*J. Soc. chem. Ind., Lond.*, 1948, **67**, 253.) The component acids of Antarctic whale oil have been previously separated by means of their lithium and lead salts, with subsequent analysis by ester fractionation. They have now been re-examined by the more recent process of crystallisation from solvents at low temperatures. This method is easier and quicker and gives results agreeing with the earlier. The results are given below.

COMPONENT ACIDS OF ANTARCTIC WHALE OIL

	By lithium and lead salt separations	By low-temperature crystallisation	
		(*a*)	(*b*)
	per cent. (wt.)	per cent. (wt.)	per cent. (wt.
Lauric	0·2	Trace	0·3
Myristic	9·3	9·2	9·3
Palmitic	15·6	15·6	15·6
Stearic	2·8	1·9	2·3
Arachidic	0·3	0·6	0·2
Unsaturated C_{14}	2·5	2·5	2·6
,, C_{16}	14·4	13·9	13·8
,, C_{18}	35·2	37·2	36·9
,, C_{20}	13·6	12·0	12·2
,, C_{22}	5·9	7·1	6·8
,, C_{24}	0·2	—	—

The component glycerides have also been segregated by crystallisation from acetone at −60°C. upwards. The following figures are similar to previous results, though differing in some respects: about 16 per cent. of disaturated and 2·5 per cent. of trisaturated glycerides, about 30 per cent. of tri-unsaturated glycerides, and about 50 per cent. of glycerides containing one saturated acid, one unsaturated C_{18} acid, and one of the other homologous unsaturated acids. About 45 per cent. of the oil contains acids of the C_{20} and C_{22} series, and oleic groups are present in over 90 per cent. of the oil. H. F.

INORGANIC

Sulphur Absorbed by Clays, Chemical Activity of. A. Malquori. (*Ann. Chim. appl., Roma*, 1948, **38**, 146.) The chemical activity of the sulphur absorbed by clays when heated with them was tested by boiling a weight of the clay containing 0·5 g. of sulphur with 0·5 g. of calcium hydroxide and 100 ml. of water and boiling for exactly 1 minute, cooling rapidly away from air and determining the sulphur in 10 ml. by oxidation and conversion to barium sulphate. If these sulphurised clays are kept in a moist atmosphere the sulphur rapidly becomes insoluble in calcium hydroxide; the loss in solubility varies for different clays from 8 to 45 per cent. in 14 days. The author connects this behaviour with the hygroscopic water. H. D.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Anti-pernicious Anæmia Factors from Liver, Purification of. E. Lester Smith. (*Nature*, 1948, **161**, 638.) This is a report of the preparation from ox liver of two pigments, both highly active in pernicious anæmia. The crude extract was purified by the methods employed by Emery and Parker (*Biochem. J.*, 1946, **40**, iv) and then by repeated chromatography; subsequently, proteolysed liver extract was used as the starting material and gave better yields of a product with a higher activity. Four tons of material yielded barely 1 g. of the red material from 6 separate lots of liver. The minimum effective single dose for the best 4 preparations from non-proteolysed liver was assessed at about 0·6 mg., proteolysed liver yielding 1 preparation (L.E.445) effective at 0·3 mg. Ten batches of red material all proved clinically active (in 26 cases), and it was found that activity and colour are inseparable. Further chromatographic purification of one of these preparations, following the action of mixed bacteria, gave a product with 8 times the colour intensity of L.E.445, which, if activity remains proportional to colour, should be effective at 0·04 mg. The products are amorphous solids, with the colour of cobalt salts, but showing only general absorption of visible and ultra-violet light, and with molecular weights, as indicated by the diffusion method, of about 3,000 for the pigments from both proteolysed and non-proteolysed liver. On exposure to daylight there is a gradual change in colour from red to orange, accompanied by a marked change in chromatographic behaviour. The products are exceedingly soluble in water, soluble in nearly anhydrous alcohol, acetone, and glacial acetic acid, but insoluble in ether, chloroform and non-polar solvents. The author concludes that the two pigments are differing forms of the classical liver fraction first postulated by Minot and Murphy, and are not an incomplete substitute, such as folic acid or thymine. True pernicious anæmia and its associated neurological disturbances do not require a multiplicity of factors but respond to a single

factor. With an effective dose equivalent to some 20 mg. (and possibly only 2·5 mg.) daily, this is one of the most potent of known physiologically active substances. S. L. W.

Œstrogens, Preparation from Urine by Application of High Temperatures. Felix Sulman. (*Nature*, 1948, **161**, 605.) The usual methods of extracting œstrogens from the urines of pregnant women, pregnant mares, or stallions, with solvents which are not miscible with water, have certain inherent disadvantages. Large extraction vessels and large volumes of urine are required and this involves using a large distillation apparatus and consequently high fuel consumption for evaporation of the solvent; in addition the œstrogens are usually contaminated with organic material. In overcoming these difficulties it was found that the urine could be evaporated till a sticky, gum-like residue remained; this was heated to a temperature not exceeding 245°C. for 5 minutes so as to carbonise the bulk of the organic matter. The œstrogens, such as œstradiol, œstrone, œstriol, hippulin, equilin and equilinin were not destroyed by this procedure. The carbonised mass was extracted with organic solvents and the extract, containing the œstrogens in rather high concentration, was evaporated. The dry residue obtained possessed a high degree of purity, since most organic impurities had been carbonised, and was further purified by the usual methods. L. H. P.

Stilbœstrol Monoglucuronide, Isolation from Human Urine. K. S. Dodgson and R. Tecwyn Williams. (*Nature*, 1948, **161**, 604.) It has been claimed by Wilder Smith (*Nature*, 1947, **160**, 787) that 50 per cent. of stilbœstrol administered to human beings is excreted as a monoglucuronide. The authors were able to isolate the benzylamine salt of stilbœstrol monoglucuronide in a pure crystalline state from the urine of two women, immediately after parturition. These patients had received a total of 100 mg. of stilbœstrol each in 24 hours. The benzylamine salt had m.pt. and mixed m.pt. 223°C. and was lævo-rotatory with $[\alpha]_D^{20°C.} = -55°$ ($c = 0{\cdot}2$ in 50 per cent. aqueous acetone). It appeared identical with a sample previously prepared from pure stilbœstrol monoglucuronide isolated from rabbit urine. The yield of the benzylamine salt corresponded to 35 per cent. of the stilbœstrol which had been administered. In addition to the monoglucuronide, small amounts of free stilbœstrol were also detected in these urines. L. H. P.

***o*-Thymotinic Acid, Preparation and Inhibitory Properties of Derivatives.** J. P. Street, C. E. Georgi and P. J. Jannke. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 180.) *o*-Thymotinic acid (1-methyl-2-carboxy-3-hydroxy-4-*iso*propylbenzene) is structurally related to thymol and salicylic acid. Prepared by treating a solution of thymol in boiling xylene with metallic sodium and dry carbon dioxide at atmospheric pressure, it was obtained in a yield of 71.1 per cent, as colourless, needle-like crystals, m.pt. 126°C., soluble in organic solvents. The preparation of the mono- and di-sodium, silver, magnesium, calcium, barium, mercuric, zinc, cupric, lead, ferrous, ferric, aluminium and bismuth salts, and the mono- and di-hexamine complexes is described. The determination of their phenol coefficients, using *Staphylococcus aureus* at 37·5°C., showed the silver and mercuric salts to have the greatest activity. Fungistatic activity was investigated using the cup-plate technique and five organisms. The mono- and di-hexamine complexes showed the greatest activity against *Epidermophyton floccosum*, *Microsporum canis* and *Tricophyton purpureum;* the magnesium salt also had a considerable effect on the growth of these organisms. The mercuric

salt was the most effective against *Tricophyton mentagrophytes* and also had a considerable effect on *E. floccosum* and *T. purpureum*. *Candida albicans* was the most resistant of the organisms studied, only the bismuth and magnesium salts having any inhibitory effect. The silver, zinc, ferrous, ferric and lead salts had no fungistatic activity against any of the organisms.

G. R. K.

Trichothecin: an Antifungal Metabolic Product of *Trichothecium roseum* Link. G. F. Freeman and R. I. Morrison. (*Nature*, 1948, **162**, 30.) The substance responsible for the antifungal activity in cultures of *Trichothecium roseum* has been isolated in crystalline form in yields of 20 to 30 mg./l. The name "trichothecin" is suggested for the active substance; after extraction with ether or chloroform from the culture filtrate it was purified chromatographically. From light petroleum it crystallised as colourless needles m.pt. 118°, $[\alpha]_D^{18°C.}$ +44°. On the basis of microanalytical results the formula $C_{15}H_{18}O_4$ is suggested. The compound is neutral, only slightly soluble in water, contains one ketonic and one ethylenic group and the results suggest three $CH.CH_3$ groups. The antifungal activity of this compound is shown against Fungi Imperfecti, Zygomycetes and Ascomycetes. Aqueous solutions of trichothecin were stable at *p*H 1 to *p*H 10 for at least 48 hours at 20°C. At *p*H 12 the antifungal activity was rapidly destroyed even at room temperature. R. E. S.

BIOCHEMICAL ANALYSIS

Cup-Plate Method in Microbiological Assay, with special reference to Riboflavine and Aneurine. A. L. Bacharach and W. F. J. Cuthbertson. (*Analyst*, 1948, **73**, 334.) An assay procedure is developed which follows the principles of the cup-plate assay of penicillin. The medium is made deficient in the single substance to be assayed and the organism chosen is one that will not grow in its absence. Solutions containing the missing substance are put into the cup and after incubation, a "zone of exhibition" is shown, the diameter of which may show a relation to the concentrations of the added nutrient. Conditions are described for producing sharply defined zones of growth together with the results of investigations into the relations between inoculum density, concentration of test solution and diameter of growth zone. Details of the procedure for the determination of riboflavine and aneurine are given. The relative insensitivity and potentialities of the method are discussed. R. E. S.

Histamine, an Improved Colorimetric Method for the Estimation of. S. M. Rosenthal and H. Tabor. (*J. Pharmacol.*, 1948, **92**, 425.) Attempts to utilise the diazo reaction given by imidazole compounds for the estimation of histamine have shown it to be unsatisfactory because of lack of specificity, instability of colours and because of numerous substances in biological extracts which inhibit or interfere. To overcome these difficulties and extend the sensitivity of the test, the authors compared a large number of aromatic amines and finally selected the diazonium salt of 4-nitroaniline as the most satisfactory. The coloured azo compounds formed by 4-nitrodiazobenzene in alkaline solution are extracted with an organic solvent, which concentrates and stabilises the colour. At a suitable *p*H, using certain solvents, the azo compounds of most interfering substances either remain in the aqueous phase or pass into the solvent, methyl *iso*butyl ketone, with a yellow or amber

colour, while that of the histamine goes into the solvent with a rose colour. Ammonia reacts like histamine to give a rose colour in the solvent, but on shaking the solvent with a barbitone buffer of *p*H 7·7 the rose colour obtained with histamine or acetylhistamine is intensified, while that of ammonia and other interfering substances is abolished or changed to a pale yellow. A method for overcoming the effects of inhibitory substances, including those in liver extracts and urine, is described; preliminary results on various tissues and with histamine indicate a satisfactory degree of specificity for the method, which has a sensitivity of approximately 0·5 mg. S. L. W.

Penicillin, Determination of, by Alkaline Hydrolysis. Stella J. Patterson and W. B. Emery. (*Analyst*, 1948, **73**, 207.) The alkaline hydrolysis method has been modified for routine assays on a large number of samples of solid penicillin having a high potency. Several indicators, singly and in combination, were tried, to avoid the use of a *p*H meter; cresol red, which gives a sharp colour change at the required *p*H even in the presence of the yellow pigment of commercial penicillin, was finally chosen. For the assay, 0·1 g. to 0·2 g. of penicillin, accurately weighed, was dissolved in 50 ml. of distilled water, previously boiled for 15 minutes to remove carbon dioxide, and cooled in a flask fitted with a soda-lime tube. 1·0 ml. of 0·1 per cent. neutral solution of cresol red in alcohol (70 per cent.) was added and N/10 sodium hydroxide run in slowly from a micro-burette, delivering drops of from 0·02 to 0·03 ml., until a red colour was obtained; a further 10 ml. of N/10 sodium hydroxide was added, the flask was stoppered with a rubber bung and the mixture was left for 3 hours at room temperature. 10 ml. of N/10 hydrochloric acid was then added, and the excess of acid was immediately back-titrated with N/10 sodium hydroxide, till the indicator changed to the original red colour. The difference between this reading, and a blank, carried out in exactly the same manner but omitting the penicillin, multiplied by 59,340 gives the total number of I.U. in the sample, and the I.U./mg. can then be calculated. The factor 59,340 applies only to salts of penicillin G; quantities of penicillin K, or of other penicillins, which are present in commercial samples, will affect the accuracy of the results. This method has been used to investigate the stability of penicillin. There are several limitations to the method ; it cannot be used to assay the official ointment (500 I.U./g.) or lozenge (500 I.U.) and is applicable only for powders containing more than 900 I.U./mg. Also samples which showed no biological activity still indicated considerable potency when assayed chemically, and it is, therefore, necessary to confirm the results by biological methods from time to time. For penicillin in oil and beeswax the penicillin is separated by extraction which dry anæsthetic ether. The authors also describe an alternative procedure, using α-naphthophthalein as indicator. L. H. P.

Penicillin G in Small Broth Samples, Estimation of. J. A. Thorn and M. J. Johnson. (*Anal. Chem.*, 1948, **20**, 614.) The method described is based on the fact that, on a column of Super Filtrol (an acid-treated bentonite), penicillin G is more strongly adsorbed under given conditions than any other known penicillin and may be eluted as a separate fraction. The method is particularly applicable to fermentation broths and is not affected by the number of types of penicillins occurring in the sample to be analysed. The method is not intended for use on purified samples, for which the more accurate physical and chemical methods are available. A broth liquid adjusted to *p*H 4·6 with 50 per cent., phosphoric acid is used, diluted if necessary with 0·05M potassium monobasic phosphate so as to contain between 50 and 200 units of penicillin

per ml. The column is washed through with a phosphate buffer solution adjusted to *p*H 6·1, the elution of the penicillin occurring in the following sequence: first, X and dihydro F; second, F; last, G. Penicillin K appears to be largely if not entirely inactivated upon adsorption. The average recovery of adsorbed penicillin G varied from 75 to 90 per cent., depending on the adsorbent. A factor is thus needed for a particular adsorbent; this is obtained by adsorbing known amounts of penicillin G. The application of aqueous chromatography to resolution of mixtures of the other known penicillins is described as well as the effect of other acids as eluents. R. E. S.

Pregnanediol, Rapid Method for Estimation of. J. Rabinovitch. (*Nature*, 1948, **161**, 605.) Pregnanediol can be detected rapidly by a method using zinc dust to protect the hormone during acid hydrolysis, and to prevent discoloration which would affect the final colour reaction. 1·5 g. of zinc dust is added to 100 ml. of urine and the mixture is heated to boiling-point, when 10 ml. of concentrated hydrochloric acid is added and the mixture boiled for 5 minutes. The flask is immersed in cold water and the zinc allowed to settle. The supernatant liquid is poured on to a sand column, and the residue of zinc is washed successively with 25 ml. of N/1 hydrochloric acid, 25 ml. of N/10 hydrochloric acid and three quantities each of 25 ml. of water; the washings are poured on to the sand column, and the column is dried by sucking hot air through it. The residual zinc is shaken with three quantities, each of 20 ml. of hot alcohol (95 per cent.), and the hot extract is decanted on to the sand column. The alcoholic extract is passed rapidly through the sand and the filtrate is evaporated to dryness. The residue is dissolved in a mixture of 5 ml. of alcohol (95 per cent.) and 20 ml. of N/10 sodium hydroxide, and allowed to stand in the cold for 1 hour. A precipitate, which contains the pregnanediol fraction, is formed, and is separated by filtration through the sintered glass, washed with water and dried. It is extracted with 10 ml. of hot alcohol, the solution may be used for the Gutterman colour reaction. In this the solution is evaporated and the residue dissolved in 5 ml. of concentrated sulphuric acid, a deep yellow or orange colour quickly develops if more than 0·5 mg. of pregnanediol was present in the original 100 ml. of urine. A negative result is shown by a colourless or pale yellow solution. The method may also be used for quantitative estimations by using larger quantities of urine and gravimetric methods. The sand-zinc method gives results of practically the same order as the quantitative method of Astwood and Jones, and is rapid and easy to carry out. L. H. P

Tryptophane, Methionine, Cystine and Tyrosine, A Modified Method for the Microbiological Assay of. E. C. Barton-Wright and N. S. Curtis. (*Analyst*, 1948, **73**, 330.) In this modified method, peptone is treated with hydrogen peroxide to destroy these 4 amino-acids and the resulting product is substituted in the basal medium for the usual series of individual amino acids. Details of the treatment of the peptone with hydrogen peroxide are given and the individual assay media are described. The organism recommended for the tryptophane assay is *Lactobacillus arabinosus* 17/5, the incubation temperature is 30°C. and the range of tryptophane to establish a standard curve is 2 to 10 μg. For the assay of L-methionine, L-cystine and L-tyrosine the organism used was *Leuconostoc mesenteroides* P.60. The range of L-methionine was 15 to 40 μg., that of cystine 5 to 35 μg., and that of tyrosine 10 to 50 μg., to establish a standard curve. The assay of DL-methionine was accomplished using *Lactobacillus fermenti* 36. Protocols of typical standard curves are given. R. E. S.

CHEMOTHERAPY

Curariform Activity of Certain Chondrodendrine Derivatives. D. F. Marsh, C. K. Sleeth and E. B. Tucker. (*J. Pharmacol.*, 1948, **93**, 109.) The authors compared the activity of *d*-N-methyl-chondrodendrine iodide and *d*-*o*-methyl-N-methyl-chondrodendrine iodide with *d*-tubocurarine chloride pentahydrate and *d*-*o*-methyltubocurarine iodide trihydrate in rats, rabbits, cats and man. All these compounds produce skeletal muscular paralysis and differ only in quantitative activity. *d*-N-Methyl-chondrodendrine is about one-half as active as the isomeric *d*-tubocurarine in rats and rabbits but only about one-fourth to one-eighth as active in cats and man. Although the *d*-*o*-methyl-N-methyl-chondrodendrine is about equipotent with *d*-tubocurarine, it is only one-sixth to one-eighth as active as its diasteroisomer, *d*-*o*-methyltubocurarine. S. L. W.

Curariform Activity of *iso*Chondrodendrine Derivatives. D. F. Marsh and M. H. Pelletier. (*J. Pharmacol.*, 1948, **92**, 454.) A comparison with *d*-tubocurarine chloride pentahydrate and *d*-*o*-methyltubocurarine iodide trihydrate in rats, rabbits and cats, showed *d*-N-methyl-*iso*chondrodendrine to be about a twentieth, and *d*-*o*-methyl-N-*iso*chondrodendrine about a fourth as paralysing as *d*-tubocurarine, which in turn is only about one-tenth as active as *d*-*o*-methyltubocurarine. Like the tubocurarine compounds, these *iso*-chondrodendrine derivatives have relatively little effect in intact animals other than lissive action on skeletal muscles. The authors obtained the *d*-*iso*chondrodendrine from *Pareira brava* by a modification of the method of King (*J. chem. Soc.*, 1940, 737) and prepared the derivatives as the iodide salts by the method of Dutcher (*J. Amer. chem. Soc.*, 1946, **68**, 419). S. L. W.

Curarising Properties of R.P.3697. R. Wien. (*Arch. int. Pharmacodyn.*, 1948, **77**, 96.) This compound, the triethyliodide of tri(diethyl-amino-ethoxy):1:2:3 benzene, was studied as a possible substitute for *d*-tubocurarine. Its curarising properties were assayed in comparison with *d*-tubocurarine by the rabbit head-drop method, the frog rectus abdominis preparation, the cat sciatic gastrocnemius preparation, and the rat, rabbit or kitten phrenic nerve-diaphragm preparations. The results of the assays showed that by the rabbit head-drop method it was one-third as active as *d*-tubocurarine, on the frog rectus abdominis preparation it was only one-twentieth as active, and on the rat phrenic nerve-diaphragm only one-eightieth as active; on the rabbit and kitten phrenic nerve-diaphragm, however, it was one-fifth as active. The curarisation effects were easily reversed by neostigmine or eserine. Compared with similar doses of *d*-tubocurarine there was no effect on blood pressure and less effect on respiration in rabbits anæsthetised with ether and thiopentone, in chloralosed cats and in decerebrate preparations. Unlike some other synthetic curarising compounds it compared very favourably with *d*-tubocurarine for its absence of anticholinesterase properties.
S. L. W.

Thio-antimonials, Organic, in Schistosomiasis. L. W. Clemence and M. T. Leffler. (*J. Amer. chem. Soc.*, 1948, **70**, 2,439). Oil-soluble substances of the general formula $(RS)_3Sb$ have been prepared, where R may be *n*-octyl, *n*-decyl, *n*-undecyl, *n*-dodecyl, *n*-tetradecyl, *n*-hexadecyl, *n*-octadecyl, β-phenylethyl, β-(1-naphthylethyl), β-(*p*-di-*iso*butylphenoxy-ethoxy)-ethyl, β-*cyclo*hexylethyl, ω-*cyclo*hexylamyl, ω-(β-tetralyl)-butyl, ω-(β-decalyl)-butyl, or β-(2-pyridyl)-ethyl. These substances are prepared by the

reaction of mercaptan and antimony trichloride in chloroform, and show some promise in preliminary experiments on schistosomiasis. During the investigation the following substances, not previously described, were synthesised and characterised: ω-(β-tetralyl)-butyl and ω-(β-decalyl)-butyl alcohols; β-*cyclo*hexylethyl, ω-*cyclo*nexylamyl, ω-(β-tetralyl)-butyl and ω-(β-decalyl)-butyl *iso*thiouronium bromides and mercaptans. G. B.

PHARMACY

DISPENSING

Folic Acid in Liquid Prescriptions. S. Scheindlin. (*Amer. J. Pharm.*, 1948, **120**, 103.) Folic acid is unstable to oxidation, reduction, acid, alkali, dry heat, acylation esterification, methylation, benzylation, nitrous acid, bromine, hypobromite, hydroxylamine, zinc dust and acetic acid (stable to acetic acid alone) and 1 per cent. hydrogen peroxide, and it is unstable to light but not destroyed by autoclaving in the dark. Folic acid is only very slightly soluble in water, but its sodium salt is water-soluble. An elixir of folic acid containing 5 mg. of folic acid in 4 ml. of a solution containing 10 per cent. of alcohol, artificial red colour, flavouring agents and preservatives. The folic acid was present as the sodium salt and the *p*H was 8·2. A series of mixtures with a wide range of commonly used medicaments was then prepared and stored in clear glass bottles exposed to electric light (but not to direct sunlight) for the first week, and then transferred to a box where they were protected from light. The mixtures were examined for precipitation, colour change, liberation of gas, or change of *p*H after 1 day, 1 week, and 1 month. Varying concentrations of alcohol, glycerin and propylene glycol produced no change in any of the solutions after one month. At and below *p*H 4·1 immediate precipitation of folic acid occurred; at *p*H 5·1 the mixture remained clear for over 24 hours, but at the end of 1 week some precipitation had taken place. No change was observed at any other *p*H. Mixtures of folic acid elixir with the following drugs showed a precipitate which could not be easily suspended or which contained a potent medicament, and the author recommends that such mixtures should not be prescribed:—phenobarbitone, chloral hydrate, tinctures of hyoscyamus, stramonium, nux vomica and digitalis, and quinine dihydrochloride; in addition, sulphadiazine was found to destroy folic acid activity very rapidly. S. L. W.

GALENICAL PHARMACY

Suppository Bases, Examination of Physical Characters of. P. Malangeau. (*Ann. pharm. Franc.*, 1948, **6**, 50.) The essential requirements of a suppository are: (1) that it should melt at a sufficiently low temperature to become liquid in the rectum (without being water-soluble), and (2) that it should offer sufficient mechanical resistance to enable its easy introduction. A simple method of determining the melting-point is as follows: when the melted mass is poured into the mould, a thin, polished metal rod is placed upright in the centre of the suppository cavity and maintained in that position until the suppository has set, so that when the mould is unscrewed the suppository is fixed on the end of the rod. The rod with the suppository attached is then placed in a water-bath containing 2 l. of water, the suppository being placed

at the same level as the bulb of a thermometer and immersed under not less than 3 cm. of water. The temperature of the water is then gradually raised, at the rate of 1° every 2 or 3 minutes, to 30°C., the water being stirred mechanically. The melting-point is taken when the mass of the suppository slides off the metal rod. For the determination of mechanical resistance, the author describes a simple apparatus, by means of which a solid cylinder of the suppository is subjected to varying degrees of vertical pressure at different temperatures. Experiments conducted with this apparatus, using cocoa butter, cocoa butter with the addition of propyleneglycol stearate, hydrogenised oil with stearates of propyleneglycol and triethyleneglycol, and hydrogenised oil with propyleneglycol stearate, showed that, whereas at ordinary temperatures the mechanical resistance of these four bases is fairly comparable, at higher temperatures cocoa butter loses its mechanical resistance much more quickly and is less likely to lend itself to the manufacture of suppositories containing a high percentage of liquid ingredients. S. L. W.

Tablet Disintegration Testing. V. M. Filleborn. (*Amer. J. Pharm.*, 1948, **120**, 233.) Tablets are immersed for a definite time in an artificial saliva bath, and enclosed in a plastic tablet container which is placed in a glass vessel containing artificial gastric juice, agitated by a pump and maintained at 37°C. Fresh artificial gastric juice is admitted by a drip-feed, and the excess allowed to flow out of the vessel. Particles of sterilised sponge may be added to simulate the presence of food. Disintegration is regarded as complete when the tablet is broken into pieces small enough to pass through the 1/16th inch holes of the plastic tablet container. Tests in which the disintegration of radio-opaque tablets, which have been swallowed whole, is observed in human subjects, show that the disintegration times obtained by the "artificial stomach" method are approximately the same as those in the human stomach. When phenobarbitone, ephedrine hydrochloride, mepacrine hydrochloride, sulphathiazole, sulphapyridine, sulphanilamide and sulphadiazine tablets are submitted to the disintegration tests of the Swiss Pharmacopœia and of the 7th Addendum to the British Pharmacopœia, 1932, the observed disintegration times are generally smaller than for the "artificial stomach" method and there appears to be no relationship between the results obtained by the three methods. G. B.

PHARMACOLOGY AND THERAPEUTICS

Amellin Ineffective in Diabetes. H. Whittaker. (*Brit. med. J.*, 1948, **1**, 546.) A mixture of amellin (an extract of *Scoparia dulcis*), calcium gluconate and lactose was given to 2 patients with diabetes in doses of 5 gr. (0·32g), by mouth, thrice daily for 3 months. One patient also received insulin and the progress of his disease was unaltered by the administration of amellin. The patient receiving amellin without insulin became progressively worse and heavy glycosuria and hyperglycæmia were constantly present. After 3 months' treatment with amellin, traces of ketone bodies were found, and the blood-sugar was 348 mg./100 ml. After doses of 16 units of protamine-zinc-insulin daily, the urine became sugar-free and blood sugar 4 hours after breakfast was 206 mg./100 ml. G. R. B.

Amidone (Methadon), Clinical Evaluation of. Elizabeth B. Trioxil. (*J. Amer. med. Ass.*, 1948, **137**, 920.) Amidone (physeptone) was administered by mouth, as capsules, tablets, or elixir, or by hypodermic or intravenous

injection. Onset of action occurred in 2 minutes after intravenous injection, 15 to 20 minutes after hypodermic injection or administration as elixir, and 30 minutes after administration as capsules or tablets; the average duration of action by all routes was 3 to 4 hours, but sometimes the effect lasted for 8 to 12 hours. Usually the hypodermic route was used, but the elixir was found to be equally efficacious and more suitable for prolonged use. When tested on a group of 400 patients showing all degrees of pain from a variety of clinical conditions, amidone gave complete and adequate relief to 81 per cent. in doses varying from 2·5 to 20 mg. Side-effects occurred in 13 per cent. of the patients and included nausea and vomiting, sedation (generally a slight drowsiness) and dizziness. When compared with morphine and pethidine on a group of 90 patients, the analgesic effect of 10 mg. of amidone was found to be equivalent to that of 15 mg. of morphine or 150 mg. of pethidine. No evidence of addiction was encountered in three patients who were treated with the drug for one year. Morphine, pethidine, " pantopon " and dihydromorphinone addicts experienced no withdrawal symptoms when the narcotic was replaced with amidone, or after the end of treatment. No contraindications were met with, but the routine use of elixir in patients with dysmenorrhœa was not encouraged because of the high incidence of nausea and vomiting. For the relief of obstetric pain, amidone is inferior to pethidine. G. R. K.

Arsenicals, an Improved Method for Assay of Toxicity. W. L. M. Perry (*Nature*, 1948, **161**, 975.) In a quantal response assay, each animal can contribute only a positive or a negative reading (in this case, death or survival), and in the event of death there is no indication whether the dose given was the exact minimum individual lethal dose, or whether it was considerably in excess. Thus, the method is wasteful of information, and only when a continuous variate such as survival time cannot be used is recourse to quantal response methods necessary. In the case of the arsenicals there seems to be no such difficulty. Using the survival time as the continuous variate it has been found possible to perform an assay with increased speed, accuracy, and economy in animals; a definite numerical estimate of the toxicity of the drug for that particular animal is obtained, and provided a linear dose-response relationship can be employed it is to be expected that more information will be gained per animal used. A series of experiments with neoarsphenamine, so designed that the methods of quantal responses and measurement of survival times could be compared, was carried out. The dose range in the latter case was chosen to ensure that all the animals treated should die, and that the longest period of survival should not exeed 10 hours. A linear relationship between the dose of drug and mean survival time was established by using logarithmic transformations. Statistical analysis of the results shows the graded response method to be accurate and unbiassed. The limits of error for the graded response assay were shown to be about half as wide as those for the quantal response assay, and the accuracy of the estimation of potency about 4 times as great.

S. L. W.

Atropine Poisoning, Acute. R. B. Welbourn and J. D. Buxton. (*Lancet*, 1948, **255**, 211.) A report of 9 cases of acute atropine poisoning, arising from a dispensing error, 1/6 grain of atropine sulphate being given by subcutaneous injection, instead of the 1/100 grain prescribed pre-operatively. All the patients were young men with septic conditions requiring minor operations, and the poisoning was not suspected until after operation. Only 4 of the patients showed toxic effects, namely, acute delirium and blurred vision,

and all recovered completely. In these 4 cases difficulty was experienced in anæsthetising with soluble thiopentone and nitrous oxide and oxygen. Of the remaining 5 cases, who showed no toxic effects and in whom no difficulty was experienced in producing anæsthesia, 3 were anæsthetised with trichloroethylene which has a more powerful and lasting depressive action on the central nervous system than nitrous oxide and oxygen or soluble thiopentone. S. L. W.

Aureomycin; Experimental and Clinical Investigations. M. S. Bryer, E. B. Schoenbach, C. A. Chandler, E. A. Bliss and P. H. Long. (*J. Amer. med. Ass.*, 1948, **38**, 117.) Aureomycin is supplied as the hydrochloride of an antibiotic from a strain of *Streptomyces aureofaciens*. It consists of yellow crystals, soluble in water giving a solution of *p*H about 4·5, and slightly less soluble in isotonic sodium chloride solution. Alkaline solutions are unstable. *In vitro*, 0·1 to 5·0 μg./ml. inhibits the growth of various Gram positive and Gram negative bacteria, but *Pseudomonas æruginosa* and strains of *Proteus* are unaffected by 20 μg./ml. Fifty times the concentration is required when 50 per cent. of human serum is present. Mice treated orally with 50 mg./kg. of body weight are protected against β-hæmolytic streptococci (C203), but not against *Klebsiella pneumoniæ* A. and *Diplococcus pneumoniæ* I (S.V.I.). In human patients, coli-aerogenes and *Streptococcus fæcalis* infections of the urinary tract are sterilised by 10 to 60 mg./kg. of body weight per day, orally. Favourable initial responses are obtained in patients with Rocky Mountain spotted fever, and with brucellosis, using 3 mg./kg. per day, intramuscularly. G. B.

***Cyclo*heptenylethylbarbituric Acid, Toxicology and Pharmacology of.** W. A. Halbeisen, C. M. Gruber, Jr., and C. M. Gruber. (*J. Pharmacol.*, 1948, **93**, 101.) The intraperitoneal LD50 of *cyclo*heptenylethylbarbituric acid (medomin) is 284 mg./kg. for mice and 220 mg./kg. for rats. When injected intravenously it is 119 mg./kg. for rabbits and 105 mg./kg. for dogs; both rabbits and dogs appeared to develop a tolerance for the drug. Large doses rapidly given intravenously produce a sudden fall in arterial pressure, the extent being directly proportional to the amount given and the speed of administration. An increased heart rate occurs during the fall and persists for some minutes after blood pressure has returned to the control level. Large doses given rapidly intravenously cause marked slowing of, and may permanently stop, respiration in expiration; the respiratory mechanism fails before the heart. When the fall in blood pressure is not extensive there appears to be dilatation of the vessels of the spleen, intestine, kidney and limb; when it is sudden and extensive, a decrease in the volume of these organs is observed, which the authors believe to be passive in character. There is dilatation of the vessels of the skin. The drug appears to have a less depressant effect on the cardiac vagus nerves than other intermediate-acting barbiturates such as amytal sodium. Like other intermediate-acting barbiturates it is destroyed in the body and is excreted as the parent substance only when excessively large doses are given. S. L. W.

Digitalis Assay; Comparison of Intravenous Pigeon and Intravenous Cat Methods. H. A. Braun and L. M. Lusky. (*J. Pharmacol.*, 1948, **93**, 81.) The procedure used in the intravenous pigeon assay was based on the U.S.P. assay process. Healthy adult pigeons of either sex were employed. After keeping the birds in the laboratory for a week on a commercial pigeon feed, the birds were starved for 18 to 28 hours. On the day of assay they were anæsthetised with ether, weighed, and tied to boards. The alar vein was

exposed and cannulated, the cannula consisting of a blunted 22-gauge hypodermic needle, and the test solution injected from a 10-ml. micro-burette calibrated to 0·1 ml. Very light ether anæsthesia was maintained throughout the assay. The solution to be assayed was diluted so that the estimated fatal dose per kg. was diluted to 15 ml. with 0·9 per cent. sodium chloride solution. When the average death time fell outside 60 to 90 minutes a new dilution was prepared. As the pigeon is slightly more resistant to digitalis than the cat, more tincture per 100 ml. is required; an additional 1 ml./100 ml. of tincture usually suffices. The diluted tincture was injected at the rate of 0·1 ml./100 g. of pigeon at 5-minute intervals until cardiac arrest supervened; the end point is very sharp. At least 6 pigeons were used for every preparation to be assayed. The U.S.P. requirement of a standard error of ±5·7 per cent. was adhered to, and 6 to 8 pigeons were usually sufficient to come within this figure. From a comparison of the results obtained with 30 preparations of digitalis it was found that the potency estimates obtained by this method varied in no case by more than 13·6 per cent. from those obtained by the present U.S.P. method; the average deviation between the two methods was not significantly different from zero. The coefficient of variation of the lethal dose of digitalis for the pigeon was 10·4 per cent., compared with 12·9 per cent. for cats. Preliminary investigations show that not only are pigeons more consistent in a given assay than cats but that various batches of pigeons seem to vary less from each other than various batches of cats.

S. L. W.

Dimercaprol (B.A.L.) Treatment of Gold Dermatitis. N. R. W. Simpson. (*Brit. med. J.*, 1948, **1**, 545.) A 5 per cent. preparation in arachis oil with 10 per cent. of benzyl benzoate was administered by deep intramuscular injection in the treatment of two cases of gold dermatitis. 2 ml. was given 4 times on the first day, 2 ml. thrice daily for 3 days, 2 ml. once daily for 9 days and subsequently, 2 ml. every alternate day. In one case, redness, heat, pain and induration occurred at the site of injection. After treatment was commenced, a lapse of 6 days occurred before improvement in the dermatitis was noted.

G. R. B.

Fluorescein as an Indicator of Antihistamine Activity. S. C. Bukantz and G. J. Daurmin. (*Science*, 1948, **107**, 224.) Fluorescein was used as a tracer substance to investigate the changes in capillary permeability due to antihistamine activity in the skin. The first experiment, determining the fluorescence at skin sites of a dog, showed that the antihistamines NH188 (neohetramine) and benadryl were of approximately equal activity in preventing fluorescence. In a second experiment, fixed concentrations of histamine in varying concentration of the antihistamine drugs NH188 (neohetramine) or benadryl were injected intradermally into each of 5 human subjects and 3 ml. of a 5 per cent. solution of fluorescein was injected intravenously soon afterwards. It was found that there was an inverse relationship between the concentration of the antihistamine drug and intensity of fluorescence; also at dimly fluorescent sites the initial fluorescence took longer to develop and was of shorter duration than at highly fluorescent sites. To determine the effects of histamine and of antihistamines on the rate of absorption of fluorescein injected intradermally, fluorescein (1 in 50,000 of saline solution), fluorescein + histamine (1 in 10,000), and fluorescein + histamine (1 in 10,000) + benadryl (1 in 2,000) were injected into three sites on the forearms of 3 normal and 1 allergic human subject. The fluorescein sites remained visible under ultra-violet light for 30 to 45 minutes in the normal cases, while the fluorescein + histamine sites no longer

fluoresced after 4 to 10 minutes; the fluorescein + histamine + benadryl site fluoresced as long as the fluorescein sites. In the allergic subject the fluorescence of the fluorescein and the fluorescein + histamine sites disappeared within 4 minutes, but that of the fluorescein + histamine + benadryl site remained for 25 minutes. This indicated that normally fluorescein is rapidly absorbed under the action of histamine but that benadryl antagonises this action; in allergic subjects a histamine-like substance released locally causes rapid absorption of fluorescein from the skin and this also is neutralised by the presence of the antihistamine drug. The time for appearance, intensity and duration of fluorescence at histamine-injected sites may thus be quantitively modified by the local presence of antihistamine substances. L. H. P.

Gonadotrophin. Crystalline Human Gonadotrophin and its Biological Action. L. Claesson, B. Hogberg, T. Rosenberg and A. Westman. (*Acid endocrinol.*, 1948, **1**, 1.) A crystalline and electrophoretically homogeneous form of chorionic gonadotrophin was isolated from the urine of pregnant women by a method which is fully described. It possesses a constant biological activity of 6,000 to 8,000 I.U./mg. It shows a marked stimulatory action on the growth and maturation of the follicles and on the formation of corpus luteum in intact mice, rats and rabbits, but fails to do so in hypophysectomised rats; in this latter group the crystalline hormone produces only an extensive development of the ovarian interstitial gland. Administered intravenously, it is well tolerated by patients in daily doses as high at 12,000 I.U. injected on 3 consecutive days, causing increased follicular growth in the human ovary and a forced production of œstrogenic hormones. In amenorrhœa due to pituitary hypofunction. large doses intravenously may induce bleedings from the progestational endometrium. Combined with small doses of serum gonadotrophin from pregnant mares it produces intensive development of the follicles. The granulosa and theca cells show no sign of degeneration. Follicular rupture and corpus luteum formation takes place in contrast to the effects induced by the action of crystalline chorionic gonadotrophin administered alone. S. L. W.

Intestinal Carminatives. Method for Assessing Value. S. Alstead and J. Fleming Patterson. (*Lancet*, 1948, **254**, 437.) A simple method for assessing the value of carminatives for expediting the passage of flatus from the bowel is described. A rubber catheter was passed about 2 inches beyond the anal sphincter. The free end was connected with a glass adapter to a piece of rubber tubing, and the tubing attached to a 500 ml. glass measuring cylinder, inverted in water to act as a gas jar. As bubbles of gas displace the water column, the volume of gas is recorded. The oral administration of a carminative mixture, hot turpentine stupes to the abdomen. radiant heat and the injection of carbachol were found by this test to be ineffective. Pituitary extract was found to be the most valuable; physostigmine and prostigmine were only occasionally effective in increasing the output of flatus. G. R. B.

Myanesin, Relaxant in Children. W. H. Armstrong Davison. (*Brit. med. J.*, 1948, **1**, 544.) A dose of myanesin (α:β-dihydroxy-γ-(2-methylphenoxy)-propane) of the order of 2 ml. per stone (6·36 kg.) of body weight was given to 44 children between the ages of 24 days and 4½ years to obtain relaxation for abdominal surgery. Maintenance anæsthesia was with open ether, nitrous oxide, or ethyl chloride. Relaxation after the dose

of myanesin occurred rapidly and was maintained for 10 to 25 minutes. The injection was made into the intravenous drip, if one was set up, or into the longitudinal sinus at the posterior angle of the anterior fontanelle.

G. R. B.

Neohetramine, A New Antihistamine Drug, Pharmacological Characteristics of. N. B. Dreyer and D. Harwood (*Proc. Soc. exp. Biol., N.Y.*, 1947, 66, 515.) The amount of neohetramine required to abolish the contractions of guinea-pig ileum and uterus, and cat uterus produced by a concentration of 0·03 to 0·3 μg. of histamine base in oxygenated Ringer solution was determined. The ratio of the amount of neohetramine to histamine was then calculated. This was 2·7 : 1 for guinea-pig ileum, 1·4 : 1 for guinea-pig uterus and 0·7 : 1 for cat uterus. In dogs and cats the fall in blood pressure caused by 1 to 2 μg. of histamine was offset by 2·5 mg./kg. of neohetramine. However, the effect of larger doses of histamine was not neutralised. Neohetramine, like pyribenzamine, did not affect the inhibition of rat uterus by histamine, but histamine-induced vasoconstriction in a perfused rabbit ear was counteracted by an equivalent concentration of the drug. Doses of 1 to 5 mg./kg. of neohetramine given to atropinised cats and dogs caused an immediate drop in blood pressure without a change in the heart-rate. The animals recovered in a few minutes. Neohetramine showed little or no effect on sympathetic nerve stimulation. On the parasympathetic nerve system, neohetramine exerted some atropine-like action on the chorda tympani, but even large doses failed to abolish chorda secretion. Neohetramine did not lessen the effect of the vagus on the intestine, and in some cases seemed to potentiate it. The total and free acidities of gastric juice obtained by rhythmic stimulation of the left vagus were unaltered by doses of neohetramine up to 5 mg./kg. A. D. O.

Podophyllin, Effect of, on Transplanted Mouse Tumours. M. Belkin. (*J. Pharmacol.*, 1948, **93**, 18.) Podophyllin dispersed in sesame oil was given subcutaneously in doses of 20 mg./kg. to 12 mice carrying 15-day-old implants of sarcoma 180, a similar group of mice given injections of sesame oil alone serving as controls. Injections were made every 3 or 4 days for 2 weeks on the side opposite the tumour. The controls grew typically, but the tumours in mice receiving podophyllin exhibited a prompt decrease in growth rate; at the end of 2 weeks the average volume of the treated tumours was approximately one-seventh that of the controls. In another experiment similarly conducted the effect of podophyllin was tested on a mammary adenocarcinoma. In this case the terminal volumes of the treated mammary tumours was approximately two-thirds that of the controls. For both kinds of tumours used in these experiments the most prominent and consistent histological finding following podophyllin administration was extensive necrosis. Characteristic nuclear alterations were found in both types of tumour. The podophyllin produced varying degrees of malaise, and diarrhœa. Resistance to repeated administration does not develop, judged by the appearance of the tumours after several injections of the drug. S. L. W.

Procaine Penicillin G. W. E. Herrell, D. R. Nichols and F. R. Heilman. (*Proc. Mayo Clin.*, 1948, **22**, 567.) In the search for methods of prolonging the effective concentration of penicillin in the blood the authors examined the properties of a procaine salt of penicillin G (duracillin). Procaine penicillin G is a crystalline, non-pyrogenic substance prepared by combining one molecule of procaine base with one molecule of penicillin. The resulting compound contains 41·5 per cent. of procaine base and has

a potency of 940 units/mg. It is employed in the form of a suspension in sesame oil, each ml. of the suspension containing 300,000 units of penicillin and 125 mg. of procaine. After preliminary animal experiments to ascertain the non-toxicity of the preparation, intramuscular injections of 1 ml. were given to 10 patients. Determination of the concentration of penicillin in the blood, either by the Fleming slide-cell technique or by the Kolmer serial dilution method, disclosed an effective therapeutic concentration for at least 24 hours after the administration of the injection. The injection appears to be safe and non-toxic; no local irritation, soreness or pain followed. Therapeutic results are the same as would be expected from any other form of penicillin therapy. It is important not to massage the site of injection. S. L. W.

Sulphetrone in Tuberculosis. M. G. Clay and A. C. Clay. (*Lancet*, 1948, **255**, 180.) Of 44 cases of tuberculosis treated with sulphetrone improvement was noted in 22, 5 were unchanged, 6 became worse, and 11 died. Of those improved, 9 improved considerably, 7 moderately, and 6 slightly. Improvement was not dramatic, and at best sulphetrone can only be regarded as an adjuvant and not in any was as a specific for tuberculosis. An attempt was made to keep the blood-sulphetrone level at 7·5 to 10 mg./100 ml. At first sulphetrone was given as long as the patient tolerated it, but later it was given in courses of 14 to 15 weeks, with a rest period of 6 weeks between courses. Parenteral sulphetrone was found to possess no advantages over sulphetrone orally. The hypochromic and nutritional anæmias were corrected by administration of ferrous sulphate 3 to 6 gr. twice daily, and yeast, preferably autolysed or boiled, 2 dr. twice daily. Changes in the alkali reserve were compensated by giving 30 gr. of sodium bicarbonate 3 or 4 times a day. Most patients developed cyanosis, but this was not an indication for stopping treatment. In most patients the treatment caused so little upset that they could continue taking it after they got up. Sulphetrone should be used only if there are facilities for estimating blood-sulphetrone levels and for carrying out blood counts.

S. L. W.

Sulphetrone, Treatment of Experimental Tuberculosis with. G. Brownlee and C. R. Kennedy. (*Brit. J. Pharmacol.*, 1948, **3**, 29.) In an experiment in which two groups of 20 guinea-pigs were infected with a heavy inoculum of a virulent bovine strain of tubercle bacilli, the survival time of the group treated with sulphetrone (0·6 g. daily in the diet) was prolonged, being 77 days compared with 45 days in the untreated group. In a second experiment in which the infection was a heavy inoculum of a human virulent strain, the treated group of 24 animals survived considerably longer than the untreated group of 21 animals. Throughout the entire drug-treated group macroscopic evidence at necropsy showed very much less tuberculosis than in the untreated group. This was confirmed by histological examination, and by the observation that acid-fast organisms were very much less in number than in the untreated group. The most significant histological evidence was the repeated finding of healed tuberculous lesions, often calcified, in the spleen, liver, lungs and lymph nodes. With both the bovine and the human strains the results suggest that sulphetrone exerts a retarding effect on the progressive nature of established experimental tuberculosis in guinea pigs, though it is evident that it is incapable of eliminating the causative organism. S. L. W.

***bis*-Trimethyl Ammonium Compounds, Pharmacology of.** G. E. Glock, G. A. Mogey and J. W. Trevan (*Nature*, 1948, **162**, 113.) The authors confirm the findings of previous workers on the curare-like action of a series

of *bis*-quaternary ammonium polymethylenes. Thus, they find that the C_8 compound curarises but is relatively inactive compared with *d*-tubocurarine chloride on the rat diaphragm, and its action was completely reversed by neostigmine; the C_5 compound had no action on the rat diaphragm. The C_3 compound had marked cholinergic action, together with relaxation of decerebrate activity at a dose of 1 mg./kg.; it produces a response of the rabbit's ileum similar to that of acetylcholine; it has slight activity as an anticholinesterase, but has no action on pseudo-cholinesterase. Its cholinergic activity, and its low curarising activity, render it unsuitable as a clinical substitute for *d*-tubocuraine. The C_5 compound has about the same anticholinesterase activity as the C_3. The overlapping of "muscarine," "nicotine" and anticholinesterase activities is a very striking phenomenon which constantly occurs in complex quaternary ammonium compounds. If the chain includes phenyl groups the effect of increasing the distance between the $\overset{+}{N}$ atoms is not to increase the curarising activity but to develop anticholinesterase activity. With regard to the species variation, results with the two closely related compounds, *d*-tubocurarine and its dimethyl ether, show that not only does the ratio of the potency vary between different species, but also, especially in the rabbit, the discrepancy may be larger in the intact animal. S. L. W.

BACTERIOLOGY AND CLINICAL TESTS

Iodonium Compounds, Antibacterial Activity of. L. Gershenfeld and B. Witlin. (*Amer. J. Pharm.*, 1948, **120**, 158.) In iodonium compounds iodine is present as an integral part of the positive ions. They are strong bases and form stable salts. They are decomposed by heat. Hitherto, investigations of these compounds have been primarily for the preparation and synthesis from the standpoint of valency studies, and they have only recently been studied to determine whether they exert insecticidal or bactericidal effects. The authors conducted antibacterial efficiency tests on the following:—diphenyliodonium chloride, *bis-p*-chlorophenyliodonium sulphate, *bis-p*-bromophenyliodonium iodide, *bis-p*-chlorophenyliodonium iodide, *bis-p*-iodophenyliodonium iodide and diphenyliodonium iodide. These compounds, in powder form, showed bacteriostatic activity when tested by the F.D.A. agar plate technique, but only *bis-p*-chlorophenyliodonium sulphate in saturated aqueous solution showed bactericidal efficiency against *Staphylococcus aureus* at 37°C. within 1 minute. The addition of sodium thiosulphate did not affect the bateriostatic or bactericidal efficiencies. Saturated solutions of the compounds in alcohol (95 per cent.) showed greater bactericidal efficiency than alcohol itself against *Staphylococcus aureus*, and saturated solutions in a solvent consisting of acetone 10 per cent. by volume in alcohol (95 per cent.) were also effective against this organism. A saturated solution of *bis-p*-chlorophenyliodonium sulphate in acetone-alcohol solvent showed bactericidal efficiency in 1 minute against *Eberthella typhosa*, *Serratia marcescens*, *Pseudomonas æruginosa*, *Escherischia coli* and *Proteus vulgaris*, and was capable of killing *Bacillus subtilis* (24-hour culture) and *B. subtilis* spores (4-day old culture) within 4 hours at 37°C.

S. L. W.

Iodonium Compounds, Bacteriostatic Efficiency of. L. Gershenfeld and B. Witlin. (*Amer. J. Pharm.*, 1948, **120**, 170). Bacteriostatic efficiency tests were performed on the following iodonium compounds in aqueous solution:—diphenyliodonium chloride, *bis-p*-chlorophenyliodonium sulphate, *bis-p*-bromophenyliodonium iodide, *bis-p*-chlorophenyliodonium iodide and

diphenyliodonium iodide. The test organisms used were *Staphylococcus aureus, Serratia marcescens, Eberthella typhosa, Pesudomonas æruginosa, Escherischia coli, Proteus vulgaris, Bacillus subtilis, Bacillus mesentericus, Bacillus megatherium* and *Streptococcus hæmolyticus*. The minimum bacteriostatic concentration of the compounds varied from 0·001 mg./ml. for *bis-p*-chlorophenyliodonium iodide (*Staph. aureus*) to 0·8 mg./ml. for *bis-p*-chlorophenyliodonium sulphate (*Proteus vulgaris*). In the over-all picture, considering all test organisms, diphenyliodonium chloride appeared the most generally effective. It was bacteriostatic to all organisms tested, and it showed bacteriostatic efficiency at lower concentrations than any of the other compounds tested in the case of 6 of the 11 organisms for which a reasonable comparison was possible. *bis-p*-Bromophenyliodonium iodide appeared second in general effectiveness, being ineffective only against *Eberth. typhosa* and *E. coli*. It was generally effective at the relatively low concentration of 0·009 mg./ml. From a comparison of the results of diphenyliodonium chloride and iodide with those of *bis-p*-chlorophenyliodonium sulphate and iodide, it would appear that it is the anion which has the effect on the activity. Intraperitoneal injections in mice of diphenyliodonium chloride and *bis-p*-chlorophenyliodonium sulphate caused increased excitability, increased respiration, and paralysis of the hind legs. The lethal dose for both of the compounds was 20 mg./kg. of bodyweight.

S. L. W.

Quaternary Ammonium Disinfectants; a Semi-micro Method for Testing. E. G. Klarmann and E. S. Wright. (*Amer. J. Pharm.*, 1948, **120**, 146.) Two factors interfere with the use of the "phenol coefficient" method in the evaluation of quaternary ammonium compounds, namely, (1) the creation of a condition in the "medication" mixture (of diluted cationic disinfectant plus bacteria), which prevents the transfer of a truly representative bacterial sample to the subculture, and (2) the failure to take due account of, and to suppress, the characteristic and marked bacteriostatic action of the cationic compounds in the transfer tube. The authors have developed a semi-micro method to overcome these factors, using Bacto-Oxgall to suppress bacteriostasis. Depending upon whether *Eberthella typhosa* or *Staphylococcus aureus* is to serve as the test organism, 1 or 5 per cent. respectively of Bacto-Oxgall is used. The composition and preparation for use with *E. typhosa* is as follows:—10 g. of Armour's peptone, 5 g. of Armour's beef extract, 10 g. of Bacto-Oxgall in 1000 ml. of distilled water; boil, and adjust to *p*H 7·4, and autoclave for 30 minutes. Add 5 g. of "Super-Cel" (10 g. with 5 per cent. of Bacto-Oxgall), and filter while hot. Add 5 g. of dextrose per litre, transfer to tubes each containing 20 ml., and autoclave for 30 minutes at 15 lb. pressure. The details of the semi-micro technique are as follows:—pipette 0·05 ml. of a 24-hour F.D.A. broth culture of the test organism on to the bottom of sterile 25 x 150 mm. test-tubes, taking care that the pipette does not touch the walls of the test-tube. Place the tubes in a water-bath at 20°C; add 0·5 ml. of diluted disinfectant, which has also been kept in a water-bath at 20°C, to each tube and mix thoroughly with the culture; 10 minutes later pour 20 ml. of Bacto-Oxgall broth into the tube, using aseptic precautions; incubate all tubes for 48 hours at 37°C. The results obtained by this method suggest that the quaternary ammonium compounds are not entitled to the phenol coefficient figures obtained with the original F.D.A. method; conversely, the latter method does not appear to be directly applicable to the testing of these compounds.

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