ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Alkaloids of the Australian Rutaceæ. G. K. Hughes, F. N. Lahey, J. R. Price and L. J. Webb. (*Nature*, 1948, **162**, 223.) As part of a survey of the occurrence and pharmacology of alkaloids in the Australian flora, three Queensland rain-forest species have been examined. The bark of *Melicope fareana* F. Muell. (bark) contained melicopine ($C_{17}H_{15}O_5N$), pale yellow needles, m.pt. 178·5° to 179·5°C. melicopidine ($C_{17}H_{15}O_5N$), pale yellow prisms, m.pt. 121° to 122°C. melicopicine, yellow prisms, m.p.t. 131° to 132°C. acronycidine ($C_{17}H_{15}O_5N$), colourless needles, m.pt. 136·5° to 137·5°C. While the leaves of *M. fareana* also contained melicopine, melicopidine and melicopicine, acronycidine was absent. Another colourless alkaloid was present but has not yet been obtained pure. The bark of *Acronychia baueri* Schott contained acronycine ($C_{20}H_{19}O_3N$), pale yellow needles, m.pt.174° to 175°C. melicopine, melicopidine and acronycidine, while melicopicine was also isolated from the leaves. The bark of *Evodia xanthoxyloides* F. Muell contained evoxanthine ($C_{18}H_{13}O_4N$), pale yellow needles, m.pt. 217° to 218°C. and melicopidine. It has been established that the five yellow alkaloids are N-methyl acridones. Acronycidine (colourless) is considered to be a quinoline derivative related to skimmianine.

F. H.

ANALYTICAL

Arsenic and Iron, Volumetric Microdetermination of. G. F. Smith and J. S. Fritz. (*Anal. Chem.*, 1948, **20**, 874.) The equivalence point for the potentiometric titration of 0·001N solutions of ferrous iron, oxalic and arsenite ions in 2F perchloric acid solutions by oxidation using 0·001N solutions of perchloratoceric acid in the same acid medium covers the range 0·95 to 1·5 volts, and the ratio of change in potential to oxidant addition is greater at 1·23 volts. Hence, by using a redox indicator, ferrous nitrophenanthrolinium ion (nitroferroin) having a transition potential of 1·23 volts in 2F perchloric acid the more laborious potentiometric titration procedure is avoided. The colour change is red to faint blue. Results are accurate to ±0·5 per cent. In the case of iron, results obtained by the above method and the spectrophotometric determination are of comparable accuracy.

E. N. I.

Capsaicin, Assay of. J. Büchi and F. Hippenmeier. (*Pharm. Acta Helvet.*, 1948, **23**, 327.) In testing capsicum preparations by taste, it is found that the sensitivity of different individuals shows very great variations, the sensitivity of the tongue rapidly decreases, and recovery of the sensitivity requires about 3 hours. A new method of assay is based on a study of the properties of pure capsaicin. Chemically, capsaicin is a vanillylamine acylated with a decylenic acid. The most important groupings are a double bond, an acid amide grouping, a free phenolic hydroxyl, and a methylated phenolic hydroxyl group. Attempts to base a method of assay on the reactions of one of these groupings were unsuccessful for various reasons. A method based on the reducing power of capsaicin is as follows: about 10 mg. of

capsaicin is dissolved, with gentle warming, in 45 ml. of 0·1N sodium hydroxide, and made up to 50 ml. with the alkali; 5 ml. of this solution is treated with 3 ml. of reagent (3 g. of pure phosphomolybdic acid in 100 ml. of water), and, after standing for 1 hour, is examined colorimetrically in a 0·5 cm. cell, using filter S72. A standardisation curve is prepared with pure capsaicin. This method has not yet been extended to the assay of capsicum itself. G. M.

Capsaicin in Drugs, Determination of. J. Büchi and F. Hippenmeier. (*Pharm. Acta Helvet.*, 1948, **23,** 353.) The method is based on the colorimetric reaction for capsaicin previously described by the authors (*Pharm. Acta Helvet.*, 1948, **23,** 327). Details are as follows: 5g. of powdered capsicum is shaken for 30 minutes with 50 ml. of dilute alcohol, and the mixture is filtered into a 250-ml. beaker, the filter being washed with 20 ml. of dilute alcohol. After the addition of 15 ml. of 0·5N sodium hydroxide to the filtrate, the mixture is warmed on the water-bath, with occasional stirring, for about 1 hour until the alcohol is removed and the mixture does not froth on stirring. The solution is cooled and treated with 2N hydrochloric acid, added drop by drop, until it does not turn thymol blue paper blue but still reacts alkaline to litmus paper. This solution, which may be slightly turbid, is then shaken out with 50, 25, and 25 ml. of ether. The combined clear ether extracts are dried with sodium sulphate, and the ether is removed on the water-bath, the residue being dried for 30 minutes in a current of air. The residue is dissolved by gentle heat in 20 ml. of (exactly) 0·1N sodium hydroxide, used in two portions, and the solution is strained and made up to 25 ml. with the alkali. This solution should be clear or at most slightly opalescent. 5-ml. portions are transferred to two 20-ml. measuring flasks, and to a 20-ml. stoppered tube. To each of the flasks, is added 3 ml. of reagent (3 g. of phosphomolybdic acid in water to 100 ml.), while 3 ml. of water is added to the tube. After 1 hour, both flasks are made up to the mark with alcohol (95 per cent.), and shaken vigorously, final adjustment of the volume being made after cooling. The contents of the tube are diluted similarly to 20 ml. The extinction of the liquids in the flasks is then measured against that in the tube, using a 0·5 cm. cell and filter S72. The amount of capsaicin in mg./5ml. of the O·1N sodium hydroxide solution is then equal to the extinction co-efficient $\times \frac{2.5}{1.100}$ The figures obtained from a number of samples of the official Swiss drug (*Capsicum annuum*) range from 0·122 to 0·304 per cent. One sample of cayenne showed 0·415 per cent. In applying the method to the tincture, 50 g. of the latter is shaken out with 50 ml. of light petroleum the extract being washed with 3 quantities, each of 25 ml. of dilute alcohol. The combined alcoholic solutions are combined with the original tincture which has been extracted with light petroleum and, after the addition of 15 ml. of 0·1 N sodium hydroxide, the method is continued as before. For concentrated fluid extract of capsicum (oleoresin of capsicum), 1 g. of the material is dissolved in 40 ml. of light petroleum and this solution is shaken out 3 times with 20 ml. quantities of dilute alcohol. The combined alcoholic solutions are treated as before. G. M.

Extracts, Determination of Moisture in, by Infra-red Heating. M. Bouchardy and A. Mirimanoff. (*Pharm. Acta Helvet.*, 1948, **23,** 321.) The determination of the moisture content of dry extracts by heating in an oven is unsatisfactory, as constant weight is never obtained.

The use of infra-red rays is quicker and more satisfactory. The lamp, placed at a distance of 37 cm. from the material, raises its temperature to about 62°C., and drying is generally complete in 1 to 2 hours, when the extract is spread out in a layer of 1 to 2 mm. thickness and 30 sq. cm. area. Even in this case loss of weight continues after all the moisture has been removed. The presence of residual traces of water may be detected by using the moisture-detecting powder recently described (Baymond, *Pharm. Acta Helvet.*, 1948, **23,** 207; *J. Pharm. Pharmacol.*, 1949, **1,** 44), the extract being placed in a small crucible heated in an oil-bath at 130°C., and covered with a watch glass which has a coating of the water-detecting powder. The dry extracts examined contained in general less than 3 per cent. of moisture. Figures of over 10 per cent., sometimes recorded in the literature, are erroneous. G. M.

Glycerol, Colorimetric Method for the Determination of Small Quantities of. V. H. Mikkelsen. (*Analyst*, 1948, **73,** 447.) The method used depends on the fact that glycerol is oxidised by bromine to 1:3-dihydroxyacetone and probably also to glyceraldehyde; addition of concentrated sulphuric acid yields methyl glyoxal which will react with codeine, thymol, resorcinol and β-naphthol to form coloured compounds. Codeine was chosen for the reaction as it gave a blue colour with a characteristic absorption band with a maximum at 6600Å. By systematic variation of (1) the amount of bromine, (2) the glycerol concentration, and (3) the heating time both for the oxidation and for the codeine reaction, a procedure was obtained which gave reproducible results and proportionality between the extinction coefficient and the glycerol concentration. Details of the final colorimetric method are given, together with details of the method as applied to the determination of glycerol in a morphine injection. In place of saturated bromine water an aqueous solution of potassium bromate and bromide containing an equal volume of 2N hydrochloric acid was used. R. E. S.

Hyoscine and Hyoscyamine, Separation of, and the Alkaloidal Assay of *Duboisia* spp. E. M. Frautner and M. Roberts. (*Analyst*, 1948, **73,** 140.) Hyoscine and hyoscyamine, including atropine, occur together in several solanaceous plants, and are generally determined together as total alkaloids, and calculated in terms of hyoscyamine. Two methods of separation of the two alkaloids are described, the first, an approximately quantitative separation of the alkaloids by chromatographic adsorption on activated silica, and subsequent fractional elution, and the second, the separation and identification of the components of a mixture of alkaloids by fractional precipitation of their picrates. Hyoscine and hyoscyamine are both strongly adsorbed on a silica column from benzene solution, and are separated by elution with absolute alcohol. The hyoscine is removed rapidly but the hyoscyamine only slowly. By adding a trace of dimethylamino-azo-benzene (butter yellow, dimethyl yellow, C.I.19) to the benzene the alkaloids can readily be detected on the column. The dye is only weakly adsorbed by the silica, giving a brilliant red colour, except where the alkaloids are preferentially adsorbed; here a yellow band of unadsorbed dye is left. The dye is quickly removed by elution with ether or absolute alcohol, but if these solvents are then replaced by benzene or, better, light petroleum, containing a little of the dye, the adsorption zones reappear. It can then be seen, which zones have been eluted, separated or spread. The efficiency of the separation of the alkaloids is dependent on the dimensions of the silica column, the authors used a column 12 cm. x 1 cm. The best results are obtained when the ratio of the amount of hyoscine to

that of hyoscyamine lies between the limits 1 : 4 and 6 : 1. The fractions obtained are pure enough to give crystalline picrates, even when only 1 or 2 mg. of alkaloid is present. The picrates are prepared by the addition of a dilute solution of picric acid, followed by crystallisation from chloroform, and are readily identified by their m.pt., hyoscine picrate usually melts at the correct temperature, 187° to 188°C., but the hyoscyamine picrate is frequently less pure and may melt several degrees lower than the correct value, 165° to 166 C. The separation of hyoscine and hyoscyamine picrates is too slow to replace the better and more rapid fractionation which can be achieved with a silica column, it is, however, useful if it is necessary to establish the identity of the main alkaloid present in the hyoscyamine fraction. Using these methods to assay a sample of *Duboisia myoporoides* the authors found that the leaves contained about 2 per cent. of hyoscyamine, 0·7 per cent. of hyoscine, and 0·2 per cent. of other alkaloids. Detached leaves of *Atropa belladonna*, starved to the point of proteolysis, assayed by similar methods, showed that the hyoscyamine content remained unchanged, and that neither hydrolysis nor demethylation takes place to any appreciable extent. L. H. P.

Inositol, Chemical Determination of. P. Fleury and A. Leconles. (*C. R. Acad. Sci., Paris,* 1948, **227,** 691.) The method depends upon measuring the volume of carbon dioxide released when inositol is treated with periodic acid. Though several days are necessary for complete evolution, the majority is released within a short time and this quantity is proportional to the concentration of inositol Interfering substances are first destroyed by treating with magnesia at 100°C. Experiments with known concentrations of inositol added to urine showed that an accuracy of about ±2·5 per cent. was normally obtained, with a maximum error of ±10 per cent. J. W. F.

Iodine in Organic Compounds, Micro-determination of. R. Grangaud. (*Ann. pharm. Franc.,* 1948, **6,** 212.) The method is based on the reaction of iodide, in solution, with silver iodate, forming soluble iodate. The organic compound is decomposed in a tube in a current of oxygen, the gases evolved being passed over heated platinum foil. The iodine is absorbed in 0·75 ml. of 0·2N sodium hydroxide containing 5 drops of 30 per cent. hydrogen peroxide. After the combustion, the hydrogen peroxide is destroyed by heating for 5 minutes on the water-bath, methyl red is added, and the liquid is adjusted to a *p*H of about 5. It is then cooled in ice and treated with about 50 mg. of solid silver iodate, and shaken vigorously for 1 minute. The mixture is filtered by syphoning (Pregl method) through a sintered glass micro-filter covered with a layer of asbestos. The filter is washed with 0·5 ml. of dilute alcohol, then twice with alcohol (95 per cent.). The filtrate is diluted with water to about 30 ml. and treated with a few crystals of potassium iodide and 1 ml. of 10N sulphuric acid. After 1 minute, the liberated iodine is titrated with 0·02N iodine, using starch as indicator. Owing to the formation of traces of nitrous acid, the method must be modified for the assay of nitrogenous substances or for liquids which are sealed into a capillary in presence of a crystal of ammonium nitrate. In this case the hydrogen peroxide is omitted, and after the combustion the liquid in the absorber is treated with 5 drops of sodium bisulphite solution and 0·2 ml. of N sulphuric acid. After heating on the water-bath for 10 minutes to destroy the nitrous acid, the liquid is cooled and treated with 5 drops of hydrogen peroxide and 0·2 ml. of N sodium hydroxide. The assay is then continued as before. G. M.

Iron, Colorimetric Determination of, with *iso*Nitrosodimethyldihydroresorcinol. S. C. Shome. (*Anal. Chem.*, 1948, **20**, 1205.) A spectrophotometric study of the colorimetric determination of iron (ferrous or ferric) has been made using the blue colour given with *iso*nitrosodimethyldihydroresorcinol. The effect of concentration of the reagent was studied together with problems of iron concentration, colour stability, and interference of other ions. The colour formed was found to be stable and both ferrous and ferric iron reacted; iron could thus be determined in the presence of comparatively large amounts of nickel, cobalt, phosphate, arsenate, fluoride, oxalate, citrate, tartrate, borate, and perchlorate in slightly acid medium. Sensitivity measurements indicated that iron can be detected with this reagent to an extent of 1 part in 50,000,000 parts of solution. Analyses of the purified iron-*iso*nitrosodimethyldihydroresorcinol complex showed that an iron atom combines with three molecules of the organic reagent; the complex retained two molecules of water even after drying in a vacuum desiccator over sulphuric acid for a number of days. R. E. S.

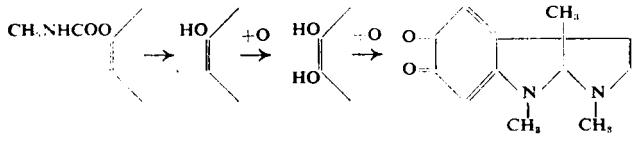
Œstrogens, Synthetic, Colorimetric Determination of. A. Carayon-Gentil and M. J. Cheymol. (*Ann. pharm. Fr.*, 1948, **6**, 129.) Methods are given for the determination of stilbœstrol, hexœstrol and dienœstrol in tablets and oily solutions. *Extraction of tablets:* the finely powdered material, corresponding to 1 to 3 mg. of œstrogen, is shaken mechanically with two quantities of ethyl acetate, 40 ml. in all, each shaking being continued for 1 hour. The mixture is filtered, and the residue washed with water. After evaporation of the solvent, the residue is taken up in methyl alcohol. If the tablets are prepared with esters of the œstrogens, a saponification must follow the extraction. *Oily solutions:* 1 ml. of solution, containing 1 to 2 mg. of œstrogen, is refluxed with 15 ml. of 5N methyl alcoholic solution of potassium hydroxide until saponification is complete. After diluting with 4 volumes of water, unsaponifiable matter is removed by shaking twice with light petroleum, and the aqueous phase is acidified and extracted with ether. The ethereal solution is washed with sodium carbonate solution (1 per cent.) then with water. The ether is removed by evaporation, and the residue is taken up in methyl alcohol so that 1 ml. of the solution corresponds to 50 to 100 μg of œstrogen. For dienœstrol a different method is employed: 1 to 2 ml. of solution, containing 2 to 5 mg. of dienœstrol, is shaken mechanically for 30 minutes with 20 ml. of methyl alcohol, and the extraction is repeated with a further quantity of methyl alcohol. The combined methyl alcoholic solution is filtered and used for the determination. *Determination of dienœstrol.* A coupling method is used. One ml. of the methyl alcoholic solution is mixed with 8 ml. of borate buffer solution, *p*H 12 (20 g. of boric acid and 280 ml. of N sodium hydroxide per l.) heated for 6 minutes at 80°C., then cooled to 20°C. It is then treated with 2ml. of diazotised sulphanilic acid solution and, after 5 minutes, the colour is compared with that obtained from a standard solution of dienœstrol. Dienœstrol from oily solutions may give a final solution which is slightly turbid. It can be cleared by adding 1 or 2 drops of a 10 per cent. solution of calcium chloride. G. M.

Pentaerythrityl Tetranitrate and Glyceryl Trinitrate, Determination of. J. Allert. (*Dansk Tidsskr. Farm.*, 1948, **22**, 188.) By saponification of alkyl nitrates with alkali, nitrites are formed and may be determined colorimetrically with e.g. procaine and α-napthylamine. This method is

however not satisfactory for pentaerythrityl tetranitrate. After *trans*-esterification with sulphuric acid, the nitric acid may be determined with phenoldisulphonic acid, but the brucine method does not give satisfactory results. Details are as follows: 1 ml. of a solution (10 to 100 mg./l.) in glacial acetic acid is treated with 2 ml. of phenoldisulphonic acid and heated for 10 minutes on the water-bath. After the addition of 25 ml. of ammonia solution (25 per cent.), the mixture is made up to 100 ml. The colour is determined photometrically (filter S43) using potassium nitrate as standard. For the determination of pentaerythrityl tetranitrate and glyceryl trinitrate in admixture in tablets the method is as follows: The sample is extracted with 3 ml. of acetone, and rinsed twice with 1 ml. of water. The filtered solution is made up to 100 ml. with water, and the pentaerythrityl tetranitrate which separates out is filtered off. The glyceryl trinitrate content of the solution is determined by the procaine-napthylamine method, and the pentaerythrityl tetranitrate by difference from the total nitrate. G. M.

Persulphates, Iodimetric Titration of. D. J. de Jong. (*Pharm. Weekbl.*, 1948, **83**, 596.) The reaction between persulphate and iodide is slow, but may be accelerated by traces of iron and by warming. The method recommended is as follows: 0·3 g. of the salt is dissolved in 20 ml. of water and treated with 3 g. of potassium iodide and 0·1 g. of ferrous sulphate, followed by 10 ml. of dilute hydrochloric acid and 50 ml. of water. The liberated iodine is titrated with 0·1N sodium thiosulphate until colourless, when starch solution is added, followed by 100 ml. of boiling water. The titration is then continued until the blue colour does not reappear. G. M.

Physostigmine, Photometric Determination of. I. Ehrlés. (*Farm. Revy*, 1948, **47**, 519.) The determination is based on the conversion of physostigmine to rubreserine by way of the following reactions:



The method has been used before, but in the present one somewhat different conditions are employed, with a more closely defined oxidation process. Rubreserine, gives similar, but not identical absorption curves in acid or alkaline solution, with two maxima, as follows:

at *p*H 1·10 { 460 mμ log e = 3·56; 295 „ „ 4·06

in 0·02 M sodium hydroxide { 480 „ „ 3·61; 298 „ „ 4·11

At *p*H values 2 to 6, intermediate curves are obtained.

The pk_A value for rubreserine was found to be 4·03. The methods employed for the determination of physostigmine salicylate is as follows: 1 to 4 mg. is mixed with 1·0 ml. of a 2 per cent. solution of potassium ferricyanide, 1 ml. of M sodium hydroxide, and made up to 50 ml. with water. After 10 to 15 minutes the extinction is read at 480 mμ. A blank test is also carried out on the reagents. The molar extinction coefficient is between 3900 and 4000. G. M.

ESSENTIAL OILS

Cajuput Oil, Characters of. W. Spoon and W. M. Sesseler. (*Pharm. Weekbl.*, 1948, **83**, 593.) A sample of cajuput oil obtained from England showed the following characters; which are compared with the requirements of the Dutch Pharmacopœia:

	Sample	Dutch Pharmacopœia
Density 15°/15°C.	0·9180	0·919 to 0·930
Refractive index 20°C.	1·4698	1·466 to 1·471
Solubility in alcohol (80 per cent.)	in ½ to 10 vol.	in 1 vol.
$[\alpha]_D$	−1·3°	—
Cineol (per cent.)	51·5	—
Distillate between 170° and 190°C.	59	66 per cent.

The sample was clear and colourless, or with at most a faint bluish tinge, and the odour was abnormal. Samples recently imported via Macassar also showed abnormal figures, as follows:

	Boeroe	Moluccas	Moluccas
Density 10°/15°C.	1·9148	0·9138	p·9126
Refractive index 20°C.	1·4691	1·4686	1·4674
$[\alpha]_D$	−4·4°	−1·3°	−1·4°
Solubility in alcohol (80 per cent.)	1-10 vols	1·5-10 vols	1-10 vols
Cineol (per cent.)	59·6	60·4	60·4
Fatty Oils	absent	present	trace
Mineral oils	absent	absent	absen

These abnormal figures are apparently due to the difficulties of the reconstruction period in the producing areas. It would appear that the distillation is not carried as far as previously, so that the oil is deficient in heavy components. The presence of small quantities of coconut oil in two samples is also of interest.

G. M.

FIXED OILS, FATS AND WAXES

Fatty Acids, Saturated Straight-Chain, Separation of. L. L. Ramsey and W. I. Patterson. (*J. Ass. Off. agric. Chem.*, 1948, **31**, 441.) The method given depends on partition chromatography and can be used for the straight-chain saturated fatty acids having 11 to 19 carbon atoms. A column of silicic acid is used with a mixture of furfuryl alcohol and 2-aminopyridine as the immobile solvent and *n*-hexane as the mobile solvent. Percolate fractions are titrated with standard sodium ethylate solution, while the separated acids are determined by titration in alcohol (70 per cent.) with standard sodium hydroxide. Each separated acid is tentatively identified by its threshold volume, confirmation being obtained by a melting-point determination or by the addition of an approximately equal amount of an authentic sample of the suspected acid to the unknown, and testing the homogeneity by chromatographic adsorption of the mixture on a fresh column. The separation of the even numbered acids from each other, and of the odd numbered members from each other, was fairly complete in a single fractionation and recoveries of added acids were essentially quantitative. Details of procedure are given, together with results obtained in investigating the purity of a number of fatty acids obtained from commercial sources.

R. E. S.

Neat's Foot Oil, Component Acids and Glycerides of. T. P. Hilditch and R. K. Shrivastava. (*J. Soc. chem. Ind., Lond.*, 1948, **67**, 139.) The component acids and glycerides of a sample of Irish neat's foot oil were determined by application of ester fractionation and of low temperature crystallisation from solvents. The acids were fractionated by crystallisation from ether followed by fractionation of their methyl esters. They consisted of myristic 0·7, palmitic 16·9, stearic 2·7, arachidic 0·1, tetradecenoic 1·2, hexadecenoic 9·4, oleic 64·4, octadecadienoic 2·3, octadecatrienoic 0·7, and unsaturated C_{20-22} acids 1·6 per cent. (wt.). The component glycerides, studied after partial separation by low-temperature crystallisation from acetone, were found to include about 35 per cent. of palmitodiolein, 23 per cent. of hexadecenodiolein, 8 per cent. of polyethenoid-diolein, 7 per cent. of oleopalmitostearin and probably not much more than 10 per cent. of triolein, with minor amounts of other mixed glycerides. The presence of fairly substantial proportions of hexadecenoic acid in neat's foot oil had not been previously noted. The specific utility of the oil as a lubricant cannot be connected with a high content of triolein, the present work suggesting the possibility that hexadecenodiolein (nearly one-quarter of the oil) and perhaps also the di-oleoglycerides in which the third acyl group is a polyethenoid member of the C_{18}, C_{20} (or C_{22}) series, may contribute specifically to its lubricant properties. R. E. S.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Potato Amylose, Action of β-Amylase on. R. H. Hopkins, B. Jelinek and L. E. Harrison. (*Biochem. J.*, 1948, **43**, 32.) The reaction between the purest amylose and β-amylase obtainable has been investigated with special attention to the reaction kinetics. The mixtures used in the investigations and the method for determining the progress of hydrolysis were as described by Hopkins *et al.* (*Biochem. J.*, 1946, **40**, 507) except that in the presence of antiseptics or butyl alcohol the ferricyanide method of Cole was used. It was found that pure potato amylose was hydrolysed by β-amylase, at a steadily declining rate, to completion. Any "denaturation" of the amylose due to undue exposure to water caused a break in the progress curve and incomplete hydrolysis. Amylose crystallised from potato starch paste at *p*H 4, or from autoclaved paste, hydrolysed faster than from non-autoclaved pastes at *p*H 6 or 9. Relatively shorter molecular chains hydrolysed more slowly. Short-chain molecules (less than 12) were not present in amylose prepared by crystallisation methods according to evidence from iodine colours. The blue value of amylose increased with chain length approaching an asymptotic value although it decreased on hydrolysis. The reaction obeyed the formulation of Michaelis and Menten but the value of K_m varied widely with the preparation. A hypothesis is put forward to explain the observed kinetics. R. E. S.

Potato Starch, Fractionation of. R. H. Hopkins and B. Jelinek. (*Biochem. J.*, 1948, **43**, 28.) A number of methods were investigated with the object of obtaining the purest amylose and amylopectin. The processes of Schoch and of Haworth, Peat and Sagrott were used, and amylose and amylopectin were also prepared from non-autoclaved potato starch paste by successive additions on alternate days of cyclohexanol and thymol; the resulting products from the different methods did not show any great differences. Amylose produced under acid conditions showed a greater

tendency to retrograde in neutral solution. The action of hot water on amylose changed even the portion which does not retrograde, the blue value fell, and β-amylose action was incomplete. Preparations of amylose with 17 times the blue value of the corresponding amylopectin were obtained. Purification of amylopectin was less successful, but fractional ethyl alcohol precipitation removed some amylose. R. E. S.

Potato Starch, Fractionation of. Part V. The Phosphorus of Potato Starch. L. H. Lampitt, C. H. F. Fuller and N. Goldenberg. (*J. Soc. chem. Ind., Lond.*, 1948, **67**, 121.) Fractions of potato starch, ground for differing periods in a ball mill, were examined for the distribution of phosphorus under various conditions. A small amount only of the phosphorus of potato starch was found to be present in dialysable form. Bound phosphorus was not split off from potato starch fractions by precipitation with alcohol and there were no significant differences between the bound phosphorus in the cold-water soluble and hot-water soluble potato starch fractions. The conclusion was reached that the phosphorus of potato starch is present, either largely or wholly, in the form of strongly-bound esterified phosphate groups, whilst in wheat starch, either all or a large proportion of the phosphorus is present in the form of less strongly-bound, adsorbed phosphatides. The work in the present paper coupled with that of other workers suggests that most of the phosphate groups in potato starch are bound to the amylopectin fraction, whereas in wheat starch the phosphatides are adsorbed, either largely or wholly, by the amylose fraction. The net result of this difference is to increase the hydrophilic character of potato starch solutions and pastes, but to decrease that of wheat starch solutions and pastes. R. E. S.

Potato Starch, Fractionation of. Part VI. Retrogradation of Fractions. L. H. Lampitt, C. H. F. Fuller, N. Goldenberg and G. H. Green. (*J. Soc. chem. Ind., Lond.*, 1948, **67**, 179.) The literature concerning the retrogradation (changes in the physico-chemical state that take place in solutions, pastes, or gels on ageing) is briefly summarised and results are reported dealing with the retrograding properties of potato starch fractions as compared with wheat starch fractions. The various cold-water-soluble and hot-water-soluble fractions of starch obtained by grinding potato starch in a ball mill for varying periods were examined and the retrograding properties of the various fractions under varying conditions of temperature and concentration are reported: the properties were qualitatively similar to those of wheat starch fractions published earlier. The differences in retrograding properties of the various fractions depended on the contents of amylose and amylopectin and also on the differences in the nature and distribution of the small quantities of phosphorus present in the two starches. R. E. S.

GUMS AND RESINS

Karaya Mucilage. K. E. Grönkvist. (*Farm. Revy*, 1948, **47**, 623,635.) Karaya gum has been introduced into the new Swedish Pharmacopœia under the name of Gummi sterculiæ. The viscosity of mucilages has been examined, using an Ostwald viscometer. The mucilages were prepared from coarsely powdered gum, and were passed through a homogeniser. When the mucilage was passed repeatedly through the capillary of the viscometer, the apparent viscosity at first decreased, then rose to a constant value. The same pheno-

menon was shown by an alkaline solution, but on ageing the viscosity decreased and became less variable. The maximum viscosity is obtained at *p*H 5 to 7, and decreases sharply on the alkaline side. In all cases of fully hydrated mucilages, the viscosity decreases on keeping, but the decrease is more rapid with a high *p*H value. Heating the dry gum causes the hydration of the mucilage to be very slow, so that such mucilages show a rise in viscosity over a period of some months. The *p*H of the mucilage is not affected by heat, but decreases on storage. G. M.

ORGANIC CHEMISTRY

β-Peltatin, A New Compound from Podophyllin. J. L. Hartwell and W. E. Detty. (*J. Amer. chem. Soc.*, 1948, **70,** 2833.) The fractionation of podophyllin by chromatographic adsorption has yielded 4 per cent. of a new crystalline substance for which the name β-peltatin is proposed. The new compound possesses about the same high necrotising activity for mouse sarcoma 37 as α-peltatin. β-Peltatin crystallises from alcohol in colourless, transparent prisms, m.pt. 231·1 to 238·0°C. (shrinks at 225·5°C.) cor. ; $[\alpha]_D^{20°C.}$ -115° (*c*, 1·009 absolute alcohol). Analysis showed a formula $C_{22}H_{22}O_8$ with three methoxy groups. Molecular weight values (Rast) for derivatives of both α- and β-peltatin agree with the formula $C_{22}H_{22}O_8$ and indicate that the peltatins are thus isomeric with podophyllotoxin. α-Peltatin has one methoxy group less than β-peltatin and podophyllotoxin. Both α- and β-peltatins give an immediate yellow colour with sulphuric acid but with α-peltatin the colour turns reddish-brown, with β-peltatin green, before becoming finally red. R. E. S.

Sodium Citrate, Water of Crystallisation of. T. A. G. Haanappel. (*Pharm. Weekbl.*, 1948, **83,** 687.) The Dutch Pharmacopœia describes crystalline sodium citrate with 5 H_2O. Actually the highest hydrated salt contains $5\frac{1}{2}$ H_2O. The Pharmacopœia also states that the salt effloresces at a relative humidity less than 0·3; investigation showed that this figure should be 0·6. G. M.

Stilbamidine : Instability of. A. J. Henry. (*Brit. J. Pharmacol.*, 1948, **3,** 163.) Hydrolysis of the amidine groups of stilbamidine is a dark reaction. Examination of solution of stilbamidine after storage for three years under various conditions shows that the factors on which the rate of hydrolysis of the amidine groups primarily depends are the *p*H of the solution and the temperature. Storage in the dark at 40°C. of a 1 per cent. solution of the hydrochloride at *p*H 7 will almost certainly produce a good crop of crystals of *trans*-4-amido-4′-amidinostilbene hydrochloride within three months. A *p*H of 5 suppresses hydrolysis almost indefinitely. *Trans*-4-amido-4′-amidinostilbene is more toxic than the parent compound. Its formation in the body—for which conditions of *p*H and temperature would be favourable—from stilbamidine absorbed and retained for long periods may, therefore, in part account for the delayed toxic effects which have been observed. The occurrence of such prolonged storage in the body is supported by recent examination of the urine of kala-azar patients some 18 months after termination of a course of treatment; stilbamidine, or a closely related derivative, is still being excreted at a low level (0·005 to 0·03 mg/100 ml.). It seems highly probable that similar conditions of adsorption, storage and slow release apply for the dimer after administration as appear to apply for stilbamidine. S. L. W.

PLANT ANALYSIS

Alkaloids, Isolation of, From Plants. N. Lörgren. (*Svensk Farm. Tidskr.*, 1949, **53**, 1.) The dried material is first extracted with an organic solvent containing a small quantity of an organic acid which forms insoluble salts with the alkaloids. The alkaloids are then extracted with an organic solvent containing dry ammonia gas. As applied to *Chelidonium majus*, details are as follows. 75 g. of the root is treated with 230 ml. of anhydrous ether containing 2 g. of anhydrous oxalic acid. After shaking for 3 hours, the mixture is filtered and the residue is treated with a further quantity of 150 ml. of ether and 0·5 g. of oxalic acid. After filtering again, the residue is suspended in ether, dry ammonia gas is passed in, and after standing overnight, the extraction is continued in a continuous extraction apparatus for 2 days. The solution is filtered and the ether distilled off. The residue is dissolved in a mixture of 15 ml. of chloroform and 15 ml. of dry ether, then treated with 100 ml. of a saturated solution of anhydrous citric acid in ether. The precipitate is filtered off and dried *in vacuo*. For ergot the defatted drug is extracted exhaustively with ethyl acetate containing ammonia gas. The solution is concentrated at a low temperature, and the alkaloids are precipitated by the addition of a saturated solution of citric acid in ether. The precipitate is allowed to settle, separated by centrifuging, washed with light petroleum, and dried *in vacuo*. It contains all the alkaloids of the drug in very good yield. G. M.

Cascara Sagrada, Chromatographic Isolation of Trihydroxymethylanthraquinones. M. R. Gibson and A. E. Schwarting. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 206.) Chloroformic extracts of extract of cascara sagrada, U.S.P., were run through a 30-mm. tube of celite, 3 parts, and magnesia, 1 part. The red-brown, deep red, orange and pink layers obtained were separated, treated with 10 per cent. hydrochloric acid to dissolve the magnesia and shaken out with chloroform. When the chloroformic solutions were run through 12 mm. tubes of the same adsorbent, the red-brown layer proved to be a mixture of the anthraquinones, whereas the deep red, orange and pink layers were homogeneous, and contained the three anthraquinones (emodin, aloe-emodin and *iso*emodin) each contaminated with an unknown substance, which could be removed by subliming under reduced pressure. The progress of the separation and purification was followed by similar experiments on various mixtures of the pure anthraquinones, and by spectrophotometric measurements. The method is not suitable for quantitative isolation of the anthraquinones unless the nature of impurities appearing in the first resins is known. A pale yellow layer which appeared immediately below the adsorbed anthraquinones in all experiments was not identified but was shown not to interfere appreciably with the spectrophotometric measurements over the range of wave-lengths used. The use of spectrophotometry showed its suitability as a method for the quantitative analysis of mixtures of the anthraquinones. The method described for the preparation of pure *iso*emodin was a modification of that of Green, King and Beal (*J. Amer. pharm. Ass.*, 1938, **27**, 95) and gave bright orange crystals melting at 179°C. G. R. K.

Pyrethrin Content of *Chrysanthemum cinarariæfolium* flowers. M. G. Edwards. (*J. Soc. chem. Ind., Lond.*, 1948, **67**, 379.) The pyrethrins were rapidly extracted from the undried flowers by grinding 400 g. with 120 g. of light petroleum (55° to 60°C.) in a rod mill for 2 hours. A further 500

g. of light petroleum was added, grinding continued for 2 minutes and the marc washed until the washings were colourless. In this way some 95 per cent. of the pyrethrins were extracted: the remainder can be obtained by a 7-days' maceration or in a Soxhlet. Three strains of flowers were investigated—(a) large flowers of low pyrethrin content; (b) "high toxic" flowers; (c) commercial Grade 1 flowers. To obtain a comparison with moisture-free flowers, samples were dried (a) in a draught at room temperature, for 12 to 14 days; (b) in a current of air at 55° C., for 7 to 8 hours; (c) in a commercial drying tunnel at 55° C., for 24 hours. The main extract was concentrated and the pyrethrins determined in aliquot portions by the A.O.A.C. method, modified by the use of hydrochloric acid in place of sulphuric acid to acidify before the light petroleum extraction of the monocarboxylic acid. The free chrysanthemum acids were not extracted and are included in the pyrethrins. Tables show the pyrethrins in the extracts of fresh flowers, and corresponding dried flowers, calculated to moisture-free basis. The results indicate that the undried flowers contain about the same amount of pyrethrin I, and about 10 per cent. more of pyrethrin II than the same flowers dried in the most favourable way, and 3 to 4 per cent. more pyrethrin I and 12 to 13 per cent. more pyrethrin II than flowers dried by one particular commercial method. H. F.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Antipyretic Action and Catalase Activity. J. Williamson and E. A. Rudge. (*Biochem. J.*, 1948, **43**, 15.) Many antipyretics can be used to stabilise hydrogen peroxide solution and several representative antipyretic substances were therefore tested for inhibitory activity on the isolated catalase-hydrogen peroxide system in an attempt to provide evidence for the hypothetical suggestion that such substances lower the temperature of the body by reducing the rate of oxidation, conceivably by interfering with hydrogen peroxide metabolism. Of the substances tested, acetanilide and phenacetin represented typical aniline derivatives, salicylic acid and acetylsalicylic acid represented typical phenol derivatives, phenazone represented pyrazolone derivatives, and quinine represented quinoline derivatives; *p*-hydroxybenzoic acid was also examined. Parallelism with antipyretic activity was not found since inhibition of the *in vitro* catalase-hydrogen peroxide system was observed with some, but not all, of the substances tested. A horse liver catalase extract was used: phenacetin, phenazone and quinine showed no inhibition: acetanilide showed a slight inhibition, the greatest inhibitory action being obtained with salicylic acid, acetylsalicylic acid and *p*-hydroxybenzoic acid. It is suggested that the observed inhibitions with the hydroxybenzoic acid derivatives result from the presence of phenolic -OH groupings. R. E. S.

Bacitracin. Production and Properties of Crude Substance. H. S. Anker, B. A. Johnson, J. Goldberg and F. L. Meleney. (*J. Bact.*, 1948, **55**, 249.) Bacitracin, which was first extracted from a culture of an organism of the *Bacillus subtilis* group isolated from an infected wound, has not yet been obtained in the pure state. An arbitrary unit has been defined as the amount which when diluted 1 in 1024 completely inhibits the growth of a stock strain of group A hæmolytic streptococcus. It can be assayed by a serial dilution method, or by a plate method using

strains of *Corynebacterium*. Suitable media for production purposes are an *l*-glutamic acid synthetic medium or a soya bean digest medium. Extraction cannot be effected by a number of common solvents but *n*-butanol extracts 85 to 90 per cent. of the activity. The butanol is distilled from the extract in the presence of water, giving a 100-fold concentration. Purification is effected by butanol-ether extraction at *p*H 3 to 4, the activity remaining in the aqueous layer. The latter is extracted with ether, distilled under reduced pressure, and freeze-dried after neutralisation. An alternative method of purification using magnesium oxide with subsequent precipitation as the salicylate is also described. Aqueous solutions of crude bacitracin are stable at 0° to 5°C. for 8 to 12 months, but at higher temperatures there is a loss of activity which is complete at 37°C. in two weeks. Alkali causes rapid inactivation; so also does hydrogen peroxide, but thiol compounds cause no measurable change. Thioglycollic acid partially reactivates peroxide-inactivated material. The substance is soluble in alcohols but insoluble in many other organic solvents. Diffusion experiments indicate a molecular weight below 2000. It is precipitated by heavy metals and by several organic acids, sometimes, for example, by salicylic acid, without loss of activity. Chemical examination shows that the substance is not a peptide, and that it lacks guanido and phenolic groups. H. T. B.

Diphtheria Toxoid: Improvement in Preparation. L. B. Holt. (*Brit. J. Exp. Path.*, 1948, **29**, 343.) Diphtheria toxoid produced in the ordinary way is first treated with magnesium hydroxide to remove colour, inorganic phosphates, and some protein. It is then treated with cadmium chloride solution and finally fractionated with ammonium sulphate. H. T. B.

Liver Extract, Purified; Chemical Nature as Determined by Paper Partition Chromatography. G. H. Tishkoff, A. Zaffaroni and H. Tesluk. (*J. biol. Chem.*, 1948, **175**, 857.) Results are given for the investigation of a commercial, highly purified, liver extract containing the antipernicious anæmia factor using two-dimensional paper partition chromatography. The presence of one or more polypeptides of high molecular weight was proved; the polypeptide material was separated and the free amino acids were liberated from it by hydrolysis. The following amino-acids were obtained: leucine, glycine, alanine, aspartic acid, valine, proline (relatively large amounts); arginine, lysine, glutamic acid; serine, phenylalanine, threonine, hydroxyproline (medium amounts); histidine, cysteine, methionine (small amounts). Some free amino acids were found in the original preparation and also riboflavine, but folic acid and xanthopterin were not found. R. E. S.

Penicillin, Radioactive, Studies with. D. Rowley, J. Miller, S. Rowlands and E. Lester Smith. (*Nature*, 1948, **161**, 1009.) In view of the possibility that penicillin may act by depriving sensitive organisms of glutamic acid, which is probably essential to their growth, the authors have attempted to ascertain whether penicillin, by being itself absorbed, blocks the passage of glutamic acid through the cell wall. As the amount of penicillin taken up by organisms is too small to detect by biological methods, it was decided to attempt its detection by using radioactive penicillin of a high specific activity, namely, 0·05 microcurie per I.U., the radioactivity of the penicillin being measured by means of a Geiger-Müller counter having a background of 14 counts per minute. For the absorption experiments, *Staphylococcus aureus* was grown on agar plates

at 37°C. for 9 hours, scraped off, transferred to 25 ml. of broth, so as to give a thick suspension, and incubated for a further 2 hours. A measured amount of radioactive penicillin was then added and an aliquot portion of the suspension removed for radioactive assay, this removal of a standard volume of the suspension being repeated at intervals as a control. After ½, 2, 6 and 24 hours, 5 ml. of the suspension was removed and filtered through a "Gradocol" membrane, and washed through with 2 ml. of water into a graduated receiver. Aliquot portions of these filtrates were taken, dried, weighed and counted. Any uptake of penicillin by the bacteria should be shown by a decrease in penicillin concentration in the filtrate. From the results of an experiment in which 0·07 I.U. per ml. was employed in a suspension containing $3{\cdot}2 \times 10^9$ viable organisms per ml., it was seen that the greatest difference in the penicillin concentrations in the filtrates was no greater than that for the suspensions. Corresponding results were obtained from other experiments, using penicillin concentrations of from 1 down to 0·05 I.U./ml. From these experiments the authors conclude that absorption of penicillin, if any, probably amounts to less than 10 molecules per bacterium. S. L. W.

Pyridine Nucleotides, Extinction Coefficients of the Reduced Band of. B. L. Horecker and A. Kornberg. (*J. biol. Chem.*, 1948, **175,** 385.) Precise values for the extinction coefficients of diphosphopyridine nucleotide and triphosphopyridine nucleotide were determined on partly pure preparations by the use of pure substrates in reactions which are essentially complete. Such determinations have been made with pyruvic acid, acetaldehyde and *iso*citric acid. The systems actually used were pyruvate di- and tri-phosphopyridine nucleotide, *iso*citrate-triphosphopyridine nucleotide, and acetaldehyde-diphosphopyridine nucleotide. A molecular extinction coefficient of $6{\cdot}22 \times 10^6$ for the reduced forms of both di- and tri-phosphopyridine nucleotides at 340mμ. was obtained. R. E. S.

BIOCHEMICAL ANALYSIS

Ascorbic Acid in Food Preparations, Estimation of. S. A. Goldblith and R. S. Harris. (*Anal. Chem.*, 1948, **20,** 649.) It is shown that both the indophenol method and the method based on coupling with 2 : 4-dinitrophenylhydrazine give satisfactory results in estimating the ascorbic acid content of fresh vegetables. The indophenol technique measures biologically active ascorbic acid while the dinitrophenylhydrazine method measures *l*-ascorbic acid, dihydroascorbic acid and 2 : 3-diketogulonic acid. The ascorbic acid in oxalated slurries (with four parts of 0·5 per cent. oxalic acid) was oxidised almost completely to dehydroascorbic acid in 21 days while the values obtained by the dinitrophenylhydrazine method remained constant for 14 days. It is claimed that inasmuch as the indophenol and dinitrophenylhydrazine methods agree when used to measure ascorbic acid in ground-fresh plant materials and disagree more and more during storage after the plant is taken from the ground, these methods should prove useful in checking the freshness of perishable vegetables. The ascorbic acid content of garden-fresh edible plants may thus be measured in a laboratory remote from the harvest area. Both methods may be employed to establish the freshness of vegetable foods. R. E. S.

Barbiturates in Blood and Tissues, An Ultraviolet Spectrophotometric Procedure for the Determination of. L. R. Goldbaum. (*J. Pharmacol.*, 1948, **94,** 68.) A simple, rapid and highly specific procedure is described

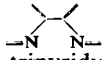
for the determination of barbiturates in blood and tissues based on the characteristic ultraviolet absorption spectra of the malonylurea ring structure. The drug is extracted by an organic solvent, usually chloroform, and then re-extracted with alkali. The ultraviolet absorption spectra of the alkaline solution is determined against a reference blank solution of sodium hydroxide using the photoelectric quartz spectrophotometer. Absorption spectra of some representative barbiturates, e.g., seconal and amytal, showed an intense ultraviolet absorption with a maximum at 225mμ and a minimum of 235mμ. At 255 mμ the concentration bears a linear relationship to the optical density up to at least 20 μg./ml. for these barbiturates. The method is sensitive to 0·4 mg./100 ml. of blood and 1·0 mg./100 g. of tissue, with an error of less than 10 per cent. E. N. I.

Heparin, Assay of. C. N. Mangieri. (*J. Lab. clin. Med.*, 1947, **32**, 901.) Fresh bovine blood containing 50 ml. of 8 per cent. solution of sodium citrate per l. is used. The plasma may be used fresh or after storage at −20°C. with equal accuracy. It is recalcified before assay with a predetermined amount of calcium chloride dissolved in 0·2 ml. of physiological saline solution so that the end-point for clotting lies near the middle of the series of twelve tubes. Two series of tubes are set up, one containing the standard and the other, the unknown heparin. 10 μg./ml. of standard or purified heparin or 20 to 40 μg. of crude heparin is used. The standard is diluted to 1·0 Toronto unit/ml. The first tube of each series contains 0·23 ml. of heparin solution and this is increased by 0·02 ml. up the series. The volume of every tube is then made up to 0·8 ml. with saline solution. To the first tube of each series 1·0 ml. of plasma and 0·2 ml. of the calcium chloride solution are added. The contents are gently mixed and incubated at 37°C. for 3 hours. This is repeated with each 2 corresponding tubes in the series at a time. The end-point is the tube in which clotting is just prevented after 3 hours. Fluorescent light is recommended for detecting thin films of clot. The activity of the heparin under test is obtained from the quantities of heparin in each of the end-point tubes. The author claims that the end-point is definite and that results are reproducible with less than 10 per cent. of error. A. D. O.

Theophylline in Blood and Urine, Determination of. A. J. Plummer. (*J. Pharmacol.*, 1948, **93**, 142.) To 4 ml. of a methyl alcohol solution of theophylline in a 15-ml. centrifuge tube add 5 ml. of a saturated solution of copper acetate, and allow to stand tightly-stoppered for 4 hours to ensure complete precipitation of the theophylline-copper compound; centrifuge for 15 minutes at 1000 r.p.m.; decant the supernatant fluid, drain, and wash the precipitate with 5 ml. of methyl alcohol; again centrifuge, decant, and drain; dissolve the precipitate in 4 ml. of 0·2N sulphuric acid and add 0·5 ml. of potassium iodide solution (1 g. to 1 ml. of water); titrate the liberated iodine with 0·02N sodium thiosulphate, using soluble starch solution as indicator; each mg. of theophylline is equivalent to 0·57 ml. of 0·02N sodium thiosulphate. For blood, deproteinise by adding 25 parts of blood to 40 parts of 13 per cent. trichloracetic acid; allow to stand 20 minutes; filter or centrifuge; render just alkaline to litmus with 2·5N sodium hydroxide; add 10 ml. of a phosphate buffer of *p*H 8·0, the final *p*H must be between 7·3 and 8·2; extract the theophylline by shaking the buffered filtrate with three 20-ml. quantities of a mixture of 3 volumes of chloroform and 1 volume of isopropyl alcohol for 5 minutes each extraction; evaporate the combined extracts just to dryness on a water-bath;

dissolve the residue in methyl alcohol; transfer the solution to a 15-ml. graduated centrifuge tube, keeping the final volume of methyl alcohol between 0·3 and 0·5 ml.; determine as previously described, using 0·005N sodium thiosulphate solution for the final titration; each mg. of theophylline is equivalent to 2·28 ml. of 0·005N sodium thiosulphate. For urine, adjust the *p*H of the urine to between 7·3 and 8·2, and continue with the determination as for blood, commencing with the words "extract the theophylline . . ."; the volume of methyl alcohol used to dissolve the theophylline should be from 1 to 2 ml., and 0·01N sodium thiosulphate should be used for the final titration; each mg. of theophylline is equivalent to 1·14 ml. of 0·01N sodium thiosulphate. The method is sensitive to 0·13 mg. of anhydrous theophylline per 100 ml. of blood or urine. From 15 to 20 ml. samples of blood are satisfactory. A dilute urine may be used directly, but concentrated urine should be diluted with 3 or 4 volumes of water. Water should be rigidly excluded from the solutions when precipitating the theophylline with copper acetate, or the precipitation may not be quantitative. Caffeine, theobromine, uric acid, ethylenediamine and sodium acetate do not interfere with the determination, nor does any normal blood property or constituent. S. L. W.

CHEMOTHERAPY

Anthelmintic Potency in Relation to Chemical Constitution. E. Baldwin. (*Brit. J. Pharmacol.*, 1948, **3**, 91.) A report on the results of tests carried out *in vitro* on over 200 chemical compounds for the detection of anthelmintic potency, using an *Ascaris* preparation of which the muscle is directly exposed to the action of the drug. Significant activity was found among aliphatic-aromatic and aromatic-aromatic ketones, but nothing approaching the activity of santonin was discovered in this group. Considerable activity was observed among lactones, but here again none approached the activity of santonin. These facts seem to support the suggestion that the outstanding anthelmintic efficacy of santonin is due to the simultaneous presence of both ketonic and lactonic groups in its structure, rather than to either alone. Among the lactones, phenols and pyridines tested it was noted that anthelmintic activity increased with the addition of a second (usually a benzene) ring to the parent molecule and that activity was greater when the two rings were independent than when they were fused. The value of phenolic carbamates was confirmed, and an unusually high order of potency demonstrated in 2-hydroxydiphenyl carbamate. None of the thiazoles examined showed much promise of useful potency, but among the pyridines an outstanding order of activity was shown by 4-benzylpyridine, and more especially by 2-2′-dipyridyl and 4:5-phenanthroline. The  linkage in the last two compounds and in the corresponding tripyridyl possesses properties which seem to offer considerable possibilities in the search for new and highly efficacious anthelmintics. No activity was discovered among a number of microbial antibiotics, and there is no reason to think that penicillin, or the sulphonamides can yield new anthelmintic agents of any practical value. The importance of using experimental material of nematode origin as the basis of methods of this kind is strongly emphasised. S. L. W.

Antimalarial Compounds. Studies in the Chemotherapy of Tuberculosis. E. Hoggarth and A. R. Martin. (*Brit. J. Pharmacol.*, 1948, **3**, 156.)

Antituberculous activity in mice has been demonstrated with a new group of compounds, 2-arylamino-4-dialkylamino-6-methyl-pyrimidines, some members of which are active as antimalarial drugs. The compound showing most promise was 2-*p*-chloroanilino-4-δ-diethylamino-α-methylbutylamino-6-methylpyrimidine dihydrochloride (No. 3300). No activity was found with the other antimalarial drugs tested, including quinine, mepacrine and pamaquin. S. L. W.

Diaminomethylpyrimidines and Related Compounds: Studies in the Chemotherapy of Tuberculosis. E. Hoggarth, A. R. Martin, M. F. C. Paige, M. Scott and E. Young. (*Brit. J. Pharmacol.*, 1948, **3**, 160.) More than 100 diaminomethylpyrimidines and related compounds have been examined for antituberculous activity in mice. The aim of the investigation was to discover a compound with greater activity than that possessed by No. 3300 (*Brit. J. Pharmacol.*, 1948, **3**, 156). This aim was not realised, and it would appear that in compound No. 3300 itself and a number of closely related compounds the maximum activity possible in this particular chemical group has been reached. S. L. W.

Sulphonamides: Studies in the Chemotherapy of Tuberculosis. E. Hoggarth, A. R. Martin and E. H. P. Young. (*Brit. J. Pharmacol.*, 1948, **3**, 153.) The activity of a series of 2-sulphanilamido-4 : 6-dialkoxypyrimidines and some closely related compounds against *M. tuberculosis in vitro* has been studied. In the former group, activity *in vitro* increases with increasing size of the alkoxy groups, but activity is limited by the consideration that substituents sufficiently large to confer high activity *in vitro* result in such poor adsorption that activity *in vivo* cannot be expected. The di-*n*-propoxy and di-*iso*propoxy compounds produced a significant increase in the mean survival times of groups of mice infected with *M. tuberculosis* when the drug was given both before and after the mice were infected, but when drug treatment was delayed for 24 hours no therapeutic effect was demonstrated. The higher members of the series were very poorly absorbed and failed to show any therapeutic action. S. L. W.

PHARMACY

DISPENSING

Sodium Citrate, Sodium Chloride and Glucose, Preparation of Sterile Solutions of. P. G. Hoorweg and G. V. D. Reyden. (*Pharm. Weekbl.*, 1948, **83**, 684.) A solution containing 3·3 per cent. of sodium citrate, 5 per cent. of glucose, and 0·9 per cent. of sodium chloride was found to develop a turbidity on storing, especially under tropical conditions. The deposit consisted of silicates, although a specially hard glass was used for the containers. The precautions which were found necessary to prevent this precipitation were found to be the following: use of special hard glass; closures of a suitable plastic, and not of rubber; washing the asbestos filter, used to remove pyrogen, with a very large quantity of distilled water before use. G. M.

Sulphathiazole for Injection, Sterilisation of. P. Morch. (*Arch. Pharm. Chemi*, 1948, **55**, 575.) Sulphathiazole dissolves in alkali with a yellow colour, which is increased on heating. In alkaline solutions the compound is actually present as a resonance form and hydrolysis may occur. The author

describes a method for determining 3 of the products as a measure of the degree of decomposition. Results are summarised thus:

Atmosphere in ampoule	Heat treatment	Decomposed per cent.
Air	1 hour at 100°C.	0·04
Air	20 minutes at 120°C. ..	0·08
Air	1 hour at 120°C.	0·28
Nitrogen ..	20 minutes at 120°C. ..	0·05
Oxygen ..	20 minutes at 120°C. ..	0·12

The discoloration of the solutions was proportional to the amount of decomposition. The preparation is very sensitive to light: after standing in sunlight for three weeks the solution became dark red and had decomposed to the extent of about 3 per cent. The content of sulphathiazole was not appreciably changed by heat treatment. It is concluded that sterilisation of a sulphathiazole solution (*p*H about 9·5) should be carried out at 120°C. for 20 minutes. G. M.

GALENICAL PHARMACY

Morphine and Apomorphine, Stability of Solutions of. A. Ionescu-Matiu, A. Popescu and L. Monciu. (*Ann. pharm. Fr.*, 1948, **6**, 137.) The degree of decomposition of a solution of morphine or apomorphine may be determined by determining the ferricyanide value, as follows. To 1 to 5 ml. of a 1 per cent. solution of morphine 4 ml. of ferricyanide reagent (4 per cent. of potassium ferricyanide with 4 per cent. of potassium hydroxide), and 20 ml. of water are added. After boiling for 5 minutes and cooling, 50 ml. of water is added, followed by 5 ml. of sulphuric acid (20 per cent.). The mixture is then titrated with permanganate until a pink colour persists for 1 minute. By using this reaction, it was shown that a solution for injection of morphine hydrochloride had undergone from 2 to 12 per cent. of decomposition in 1 year, according to the conditions under which it was kept. The decomposition may be prevented by the addition of sulphite according to the following formula: morphine hydrochloride 0·40 g., sodium bisulphite solution (10 per cent.) 1 drop, water to 10 ml. Sterilisation is for 20 minutes at 100°C. A corresponding formula is used for apomorphine. In tinctures and other liquid galenical preparations, the ferricyanide process may be applied to the extracted morphine. The results showed that the degree of decomposition in one year did not exceed 15 per cent. It is recommended that these preparations should be stabilised with sulphite or benzoic acid. G. M.

PHARMACOGNOSY

***Atropa Belladonna*, Frequency Determinations of.** D. D. Boswijk. (*Pharm. Weekbl.*, 1948, **83**, 609.) Frequency determinations of *Atropa Belladonna* have now been extended to 6 further samples from different localities. The results in general agree with those found previously (*Pharm. Weekbl.*, 1948, **83**, 225; *Quart. J. Pharm. Pharmacol.*, 1948, **21**, 534), but a sample from Leyden showed an abnormal frequency for the stomata in the upper epidermis, ranging from 63 to 88. In samples from Groningen, Delft and Leyden, trichomes with unicellular stalks and multicellular glands were prominent; while multicellular stalks and unicellular glands were general in *Atropa Belladonna* var. *lutea*. G. M.

***Cinchona Ledgeriana* Bark. Distribution and Interrelationships of Alkaloids in.** H. F. Birch and L. R. Doughty. (*Biochem. J.*, 1948, **43**, 38.) The distribution of alkaloids throughout the whole bark of 3 trees of *Cinchona Ledgeriana*, each 7 years old, was investigated. Two of the trees had suffered damage at an earlier stage of their growth and differed markedly in shape from the third tree which was of apparently normal growth and straight in form. Samples of bark were analysed for total alkaloids, quinine, cinchonidine, and for amorphous alkaloids. The distribution of alkaloids in the stem bark was a function of bark thickness which is at any point inversely proportional to the distance of that point from the base of the tree. This basic regular decrease in alkaloid content from the base of the tree upwards was interrupted by zones of relatively high alkaloid content due to local thickness of the bark where the main stem forked, usually caused by damage of the stem at an earlier stage in the tree's life, where secondary leaders arose and where large branches joined the stem. Significant increases in alkaloidal content associated with unit increase in bark weight were found for total alkaloids, quinine, and cinchonidine/unit increase in bark weight, and for quinine, cinchonidine, and cinchonine/unit increase in total alkaloids. These increases were large compared with the small and insignificant increases (and decreases in the case of one tree) found for the amorphous alkaloids, an indication of the progressive conversion of amorphous to crystallisable alkaloids during the life of the tree. When the trees were felled the amorphous alkaloid content was that currently available for transformation to the crystallisable alkaloids, while the crystallisable alkaloid content represented the accumulation of the end products of the amorphous alkaloid transformations throughout tree growth. Cambial activity further governed the efficiency or degree of conversion of the amorphous to the crystallisable alkaloids, for in one tree the highest crystallisable to amorphous alkaloid ratios obtained where the tree bent and where the secondary leader arose. In general, the distribution of the alkaloids throughout the bark is governed primarily by the history of the tree as reflected in its form. R. E. S.

***Datura Stramonium*, Growth Effects produced by 2 : 4-Dichlorophenoxyacetic Acid applied to the Stems.** H. W. Youngken, Jr. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 196.) Hydrous wool fat containing 2:4-dichlorophenoxyacetic acid in concentrations varying from 0·0001 to 5 per cent. was applied to the stems of seedlings about 3 in. high and older plants about 8 to 10 in. high. Seedlings treated with 0·0001 and 0·001 per cent. showed no abnormal effects, but higher concentrations produced a systemic effect proportional in degree to the concentration used. The effect was manifested by stunted growth, skin bending and swelling, cellular proliferation and roughening and curling of the leaves. Seedlings did not recover from the effects if the concentration was greater than 1 per cent. Those older plants treated with 1 to 5 per cent. showed, after 45 days, stunted growth, swollen stem bases with warty outgrowths, marked curvature of the stems above the first branching, folding of the leaves, increased pubesence along the leaf veins and petioles, and retardation of flower development, only the lower flowers maturing; other flower buds which formed remained unopened. Two plants treated with 1 to 0·1 per cent. and on which mature fruits formed produced spineless capsules. After three weeks the seeds of these fruits were chocolate brown and either flattened and kidney-shaped, or vermiform to crescent or horn-shaped, and smaller. Older plants treated with 0·1 per cent. or less showed little if any toxic effects. The determination of the total alkaloids of leaves from plants which were 85 days old and had received four

applications of 0·001 per cent. of 2:4-dichlorophenoxyacetic acid showed no significant difference from the control plants. Since, however, stronger concentrations produce somewhat drastic effects on the leaves, the effect of these on alkaloidal formation is being investigated. G. R. K.

PHARMACOLOGY AND THERAPEUTICS

Alcohol, Sensitisation to, by Drug. J. Hald and E. Jacobsen. (*Lancet*, 1948, **255**, 1001.) Doses of 0·5 to 1·5 g. of diethylthiuramdisulphide [bis(diethylthiocarbanyldisulphide)], $(C_2H_5)_2NC(S).S.S.C(S)N(C_2H_5)_2$, although without effect by themselves produce unpleasant symptoms if alcohol is taken subsequently. The symptoms following 10 to 20 g. of alcohol include flushing of the face, dilatation of the scleral vessels, palpitations and possibly slight dyspnœa. Larger doses of alcohol cause nausea and vomiting. This sensitisation seems to be due to a great increase in blood acetaldehyde which occurs when both the drug and alcohol are taken, but which is absent when either is taken separately. Sensitisation begins about 3 hours after the drug is taken and may last about 48 hours depending on the dose. The drug is stated to be non-toxic by itself and is excreted very slowly. W. W. W.

Alcoholism treated by Sensitising Drug. O. Martensen-Larsen. (*Lancet*, 1948, **255**, 1004.) The author has used tetraethylthiuramdisulphide in the treatment of 83 cases of alcoholism. This drug although innocuous by itself causes unpleasant symptoms if alcohol is taken subsequently. 74 of the patients developed distaste for alcoholic drinks as a result of the treatment; 9 refused to continue with it. After physical and psychiatric examination the patient received 1·0 to 1·5 g. of the drug followed by 0·5 g. daily, and informed of the consequences should he drink alcohol, but he is encouraged to try it to show the effect of the treatment. Although heavy drinkers can still take a fair amount of alcohol at the start of the treatment, their tolerance for alcohol soon diminishes and finally all desire for drink seems to be lost. W. W. W.

Analgesics, A Method of Testing in Man. A. J. H. Hewer and C. A. Keele. (*Lancet*, 1948, **255**, 683.) Instead of the usual method of testing analgesics by measuring the threshold intensity of some stimulus required to elicit pain, tests have been made on the power to relieve pain experimentally induced by the contraction of ischæmic muscles. By means of a cuff the distal circulation of the arm was cut off and the hand and forearm muscles were then exercised by rhythmically compressing a bulb. After 50 to 60 contractions had been made there was slight or moderate pain in the forearm muscles. The contractions were then stopped and the ischæmia still maintained. Soon the pain began to increase and became intolerable after 8 to 15 minutes. If the cuff was deflated the pain disappeared in a few seconds. Ten grades of pain could be distinguished and are described in units from 1 to 10. The drugs were given when the pain was of the value of 4 to 5 units, gases by inhalation, the others by intravenous injection, thus eliminating differences due to different rates of absorption. It was found preferable, instead of trying to find the smallest dose that would give complete relief from pain of 4 to 5 units of intensity, to find the smallest dose that would produce a definite effect at this stage. For this purpose a narrowing range of doses, well above and below the threshold, were given, until there was only a small differ-

ence between the effective and ineffective doses. Nitrous oxide from 10 per cent. to 40 per cent. in oxygen, and cyclopropane, 2 and 3 per cent. in oxygen, were used. Results are given for the following drugs: (1) morphine (hydrochloride or sulphate); (2) pethidine hydrochloride; (3) *dl*-2-dimethylamino-4:4-diphenyl-heptan-5-one hydrochloride, amidone; (4) *dl*-1-dimethylamino-3:3-diphenyl-hexan-4-one hydrochloride (Hoechst 10582); (5) *dl*-2-dimethylaminomethyl-3:3 diphenyl-hexan-4-one hydrochloride (isoamidone); (6) *dl*-2:1-morpholino-4:4 diphenyl-heptan-5-one hydrochloride (C.B. 11); (7) procaine hydrochloride; (8) thiopentone sodium; (9) benadryl hydrochloride; (10) N-*p*-methoxybenzyl-N-dimethylaminoethyl-2-aminopyridine maleate (neoantergan, antistin, pyranisamine maleate); (11) N-phenyl-N-benzyl-2-methylimidoazoline (antisan); (12) tetraethylammonium bromide. The results show great variations between the 4 persons studied and clinical differences in response to these drugs can, in part, be ascribed to differences in sensitivity towards them. It was found, for example, that amidone and C.B.11 were both 30 times as potent as pethidine, while in another subject morphine and amidone were both 10 times as potent as pethidine but only 1/10 as powerful as C.B.11. Again, in another subject morphine, amidone and C.B.11 were about equally analgesic and were 20 to 25 times more potent than pethidine. H. F.

Calciferol by Intramuscular Injection. T. Lightbound. (*Lancet*, 1948, **255**, 1010.) Initially, patients with lupus vulgaris, lupus verrucosus, erythema induratum and normal controls were treated orally with 150,000 I.U. daily of calciferol for 6 months. The dose had to be reduced in some patients owing to nausea and vomiting. To overcome this, intramuscular injections of 600,000 I.U. were given thrice weekly for 3 weeks and then twice weekly. The intramuscular route is preferred because results were more rapid, pigmentation was absent, there were no toxic symptoms, there was little or no hypercalcæmia, less chance of reduced packed-cell volume and of raised blood urea. All cases, however, showed diminished kidney function (urea clearance test), and therefore treatment should not exceed 4 months without a rest period. W. W. W.

Chloroquine, Chronic Oral Toxicity of. O. G. Fitzhugh, A. A. Nelson and O. L. Holland. (*J. Pharmacol.*, 1948, **93**, 147.) A 2-year chronic toxicity study with rats fed on diets containing from 100 to 1,000 p.p.m. of chloroquine showed that the toxicity of the drug was very slight or questionable at 100 p.p.m. and became progressively more severe with each increase in dosage. There was a significant retardation of growth at a concentration of 400 p.p.m. A progressive increase in mortality occurred at dosage levels of 200 p.p.m. or more, and 800 and 1,000 p.p.m. caused early death of all animals. The outstanding hæmatological change was a leucocytosis, predominantly neutrophilic, marked in the group on 800 p.p.m., less striking in the group on 400 p.p.m., and scarcely noticeable in the group on 200 p.p.m.: there was increase in the hæmoglobin concentration and erythrocyte counts in the rats on 800 p.p.m. Histopathological changes increased from very slight or absent in rats on 100 p.p.m., to marked in those on 800 and 1,000 p.p.m.: the two prominent lesions at toxic doses were a slow focal necrosis of striated muscle, especially cardiac, and a moderate degree of centrolobular hepatic necrosis and fibrosis. In relation to bodyweight of the rat, the lowest dosage of chloroquine which produced slight toxic effects in some animals corresponds to approximately 4 mg./kg./day for 2 years. This corresponds

approximately to the prophylactic dosage in man; however, taking the length of time into consideration, the amount of chloroquine that will produce toxic effects in rats is above the therapeutic or prophylactic dose for man. The toxicity of chloroquine was found to be slightly less than that of mepacrine; at the low dosage level of 4 mg./kg./day there was no noticeable difference between the toxicities of the two substances. S. L. W.

Dimercaprol in the Treatment of Experimental Lead Poisoning in Rabbits. F. G. Germuth and H. Eagle. (*J. Pharmacol.* 1948, **92,** 397.) Rabbits which had received 5 consecutive daily subcutaneous injections of lead acetate in a dosage of 240 mg./kg. all died in from 3 to 40 days, the survival time averaging 26 days after the last injection. A series of animals receiving this dosage of lead acetate were then treated for 5 days with dosages of dimercaprol varying from 20 to 80 mg./kg. daily. Dimercaprol significantly hastened their death, the average survival time at the smallest dosage of dimercaprol being reduced from 26 days to 15 days, and at the highest dosage from 26 days to from 1 to 12 days, the mortality being greatest within the first four days. This experiment was carried out in the hottest part of the summer; in a second similar experiment carried out in the winter months dimercaprol did not accelerate death but it still failed to exert a protective action. Similar results were found in acute lead poisoning induced by intravenous injections of lead acetate, the animals treated with dimercaprol dying approximately in the same time as the untreated controls. Further experiments to determine the effect of dimercaprol on urinary lead excretion showed that it caused a striking increase. For two hours after a single injection of dimercaprol at 20 mg./kg. the urinary excretion of lead increased 11- to 40-fold in animals with a subcutaneous depot and 3- to 7-fold in animals injected intravenously, this favourable effect lasting for 4 hours after a single injection. The magnitude of the lead-excretion response decreased with each additional injection of dimercaprol, suggesting that only a small proportion of the lead injected could be dissociated by dimercaprol from its combination with the tissues. The reason for the failure of dimercaprol to protect the animals in spite of increased urinary lead excretion may be due in part to the fact that only a small portion of the total body store of lead is mobilised and the amount eliminated is too small to effect the outcome. A second reason for failure may be the fact that the lead mobilised by dimercaprol is shown to form a lead-dimercaprol complex which is almost as toxic as lead acetate itself, which may explain why in some experiments the administration of dimercaprol actually accelerated death, since the lead complex when formed may act on other organs more vulnerable to the toxic effects of lead, or more vital to the host, than were the tissues in which the lead was originally deposited. In spite of these results, the authors consider, in view of the striking effect of dimercaprol on the urinary excretion of lead, its cautious therapeutic trial in man is justified. S. L. W.

Hetrazan, Mode of Action in Filariasis. F. Hawking, P. Sewell and P. Thurston. (*Lancet,* 1948, **255,** 730.) Experiments were carried out on cotton-rats infected with *Litomosoides carinii,* to study the mode of action of a new compound, hetrazan (1-diethylcarbamyl-4-methylpiperazine), which has been introduced for the treatment of human filariasis due to *Wüchereria bancrofti.* Intravenous injection of 6 mg./100 g. was followed by rapid diminution of the microfilariæ, 80 per cent. disappearing in 1 minute and over 90 per cent. in 2 minutes. Microfilariæ surrounding

the adult worms in the pleural cavity remain active despite intensive treatment and form a reservoir from which the supply in the blood is constantly replenished. Hetrazan has little effect on the adult worms. Experiments *in vitro*, and the histological examination of the distribution of the microfilariæ in the different organs suggests that hetrazan has an opsonin-like action on the microfilariæ and renders them susceptible to destruction by the reticuloendothetial system. Microfilariæ *in vitro*, and in the pleural cavity are not in contact with phagocytes and are not so rapidly affected by hetrazan. E. N. I.

Niaara: a Digitalis-like Colombian Arrow-Poison. K. Mezey, C. Uribe-Piedrahita, J. Pataki and J. Huertas-Lozano. (*J. Pharmacol.*, 1948, **93**, 223.) Niaara, an arrow-poison from Colombia, is the latex of the tree "Pacuru-niaara," or "poison tree," *Ogcodeia ternstroemiiflora* Midbr. A white, amorphous principle, niaarin, having chemical and pharmacological properties characteristic of the cardiac glycosides, has been isolated from this latex. The intravenous LD50 in cats is 0·21 mg./kg.; it is poorly absorbed from the gastro-intestinal tract. Seven cases of congestive heart failure in man were successfully treated with niaarin, injected intravenously daily in doses of 0·50 to 0·75 mg. for the first two days, and then 0·25 mg. daily for a further 2 or 3 days. The therapeutic effect was evident within less than 24 hours after administration. On the whole, niaarin compares closely with strophanthin in rapidity and brevity of action. Cumulation is not important when 0·25 mg. is given as the daily maintenance dose. No side effects were noted in these cases, and no curare-like action is produced. Although niaarin is an effective therapeutic agent, the necessity for intravenous administration limits its field of usefulness. On the other hand, the actions of niaarin develop with remarkable rapidity which may occasionally be desired. S. L. W.

Ouabagenin. K. K. Chen, R. C. Anderson and H. M. Worth. (*J. Pharmacol.*, 1948, **93**, 156.) Employing a crystalline form of ouabagenin, the authors determined it to be approximately one-half as active on the heart as ouabain in cats and about one-third as active in frogs. It is more effective than ouabain in causing vomiting of non-anæsthetised cats, weight for weight, and the emetic dose is therefore no measure of the cardiac activity when different compounds are compared. Unlike digitoxigenin, it does not cause convulsions in cats or frogs. S. L. W.

Penicillin Treatment of Nasopharyngeal Diphtheria. E. W. Bixby. (*Amer. J. med. Sci.*, 1948, **215**, 509.) A series of 139 cases of diphtheria in young men, all of whom had been previously immunised according to the U.S. Army Schedule, were selected for study. Although the typical grey membrane was seldom found, each case included in the report had a positive nasal and/or pharyngeal culture for *Corynebacterium diphtheriæ*, and gave a confirmed positive virulence study. Every case received 100,000 units of diptheria antitoxin intramuscularly, and general routine treatment. Of the 88 cases receiving only this treatment 53 (60 per cent.) developed a carrier state after two weeks. To the remaining 51 cases, penicillin was administered intramuscularly immediately on confirmation of the presence of *C. diphtheriæ*, in doses of 20,000 units every 3 hours for 50 doses. Of these, 43 (79 per cent.) had permanently negative cultures after 2 weeks. The average time required in hospital by patients with a 2-week negative culture was 35 days compared with an average of 50 days for those cases with a two-week

positive culture. In contrast to these results, the use of penicillin later in 40 cases of the two-week positive group who had previously received only the routine treatment was not successful, 60 per cent. remaining positive. The incidence of post-diphtheritic complications was not affected by penicillin although the severity of complications was modified. H. T. B.

Sodium Salicylate, Cutaneous Absorption of. G. Valette and R. César. (*Ann. pharm. Franc.*, 1948, **6**, 16.) The object of this investigation was to apply the characteristic elimination of sodium salicylate in the urine, to the study of the factors governing the absorption by the skin of non-liposoluble substances. The addition of eucalyptol markedly enhances the cutaneous penetration of sodium salicylate; the degree of penetration is dependent on the proportion of eucalyptol, but when the proportion exceeds 20 per cent. of the volume of the solution it produces an irritant effect. Sodium salicylate is hydrolysed in solution: when eucalyptol is added to such a solution it dissolves the salicylic acid liberated, with which it gradually becomes charged and penetrates the skin, and the irritation observed is thus attributable not to the eucalyptol but to the salicylic acid dissolved in it. This action of eucalyptol is less marked in alcoholic than in aqueous solutions. The addition of an emulsifying agent to a mixture of eucalyptol and solution of sodium salicylate does not increase the effect produced, though the results are better with oil-in-water than with water-in-oil emulsions. The renal excretion of sodium salicylate after cutaneous application of an aqueous solution containing eucalyptol, follows almost the same rhythm as after administration of the salt orally or subcutaneously, elimination reaching its maximum in 3 hours and continuing for about 48 hours. The alkalisation of solutions of sodium salicylate (to *p*H 8·4) was found to hinder the cutaneous penetration of the salt, while acidification (to *p*H 4·6) was found to increase it. S. L. W.

Sulphetrone, Pharmacology and Chemotherapy of. G. Brownlee, A. F. Green and M. Woodbine. (*Brit. J. Pharmacol.*, 1948, **3**, 15.) Sulphetrone is 4 : 4'-*bis*(γphenyl-*n*-propyl-amino)diphenylsulphone-tetra-sodium sulphonate, an amorphous material containing, when air-dried, from 5 to 7 per cent. of water. It is insoluble in alcohol and other organic solvents, but is exceedingly soluble in cold water to give a syrup; 20 and 40 per cent. w/v solutions are stable when neutral or slightly alkaline, and may be autoclaved. A 10 per cent. w/v solution is isotonic with 0·91 per cent. sodium chloride solution, and hypertonic solutions up to 60 per cent. are readily obtained. In mice and dogs sulphetrone has an acute toxicity many times less than that of sulphanilamide. Very large doses can be given by mouth to mice and dogs without producing symptoms or pathological changes, but similar doses in rabbits produce anæmia. It causes hyperæmia and hyperplasia of the thyroid gland. Sulphetrone is not hydrolysed to diaminodiphenylsulphone in the body. When given orally or parenterally, it raises the alkali reserve of the plasma in the rabbit and the dog, but over a period of time, equilibrium is established. When given by mouth not only is sulphetrone the least toxic of the sulphones, but is also less toxic than any of the sulphonamides. It is only slowly absorbed from the intestinal tract; when given intravenously it is excreted in the urine almost completely in 24 hours, but when given orally only 75 per cent. is excreted in the same time. The drug is conjugated in the experimental animal or in man. It penetrates all tissues with extreme rapidity, with the exception of the brain, and is present in them in about the same concentration as in blood, but it enters the cerebrospinal fluid rather more slowly than do other sulphonamides. It has no action

on smooth muscle, heart, blood pressure or respiration. Antibacterial *in vitro* studies show sulphetrone to approach the efficiency of diaminodiphenlysulphone against avian, bovine and human strains of *Mycobacterium tuberculosis;* blood from guinea-pigs which had previously received parenteral sulphretone inhibits *in vitro* strains of virulent mycobacteria. The authors conclude that sulphetrone may prove effective in the treatment of experimental tuberculosis in laboratory animals, and that its administration to man in large doses for protracted periods is a practical possibility. S. L. W.

Sulphetrone, Treatment of Tuberculosis with. D. G. Madigan. (*Lancet*, 1948, **255**, 174.) In 70 cases of tuberculosis affecting different organs, sulphetrone was given for periods varying from a few days in tuberculous meningitis to eighteen months in more chronic cases. A blood-sulphetrone level of 7·5 to 10 mg./100 ml. should be aimed at by a scheme of gradual dosage. A suitable initial dose for adults is 1·5 g. daily (0·5 g. eight-hourly) for the first week, and 3 g. daily (0·5 g. four-hourly) for the second, the daily dose then being increased by 1 to 2 g. each week until the required blood level is reached, usually with 6 to 10 g. daily. If given intramuscularly combined with streptomycin, for miliary tuberculosis or tuberculous meningitis, 0·05 g. kg. of bodyweight every 4 to 6 hours for the first 24 hours is suitable, increased to 0·1 g./kg. during the second 24 hours; when the meninges are involved, levels above 5 mg. 100 ml. may cause vomiting. It is essential to give iron and brewers' yeast for a fortnight before and throughout sulphetrone treatment to avoid hypochromic and nutritional anæmia; the yeast also prevents the onset of peripheral neuritis, occasionally seen. Even so, a residual hæmolytic anæmia develops and continues throughout treatment leading to a fall in hæmoglobin content to a level as low as 60 per cent.; should it fall below this figure the sulphetrone should be withdrawn. Besides a weekly check of red cells and hæmoglobin, there should be a weekly estimate of blood-sulphetrone, which should not exceed 12·5 mg./100 ml. Danger signals are continuous headache, loss of appetite, nausea and vomiting, gastro-intestinal discomfort, dizziness and mental confusion; these conditions are associated with high blood-sulphetrone levels and measures should be taken to hasten elimination of sulphetrone by giving fluids by all routes. Stasis also should always be guarded against. In general, no beneficial effect was detected from sulphetrone therapy of acute infections, but improvement was observed in chronic lesions. Thus, 12 out of 17 cases of acute pulmonary fibrocaseous disease, and 13 out of 22 chronic cases improved. All of 4 cases of primary pulmonary tuberculosis, and 6 out of 8 strictly exudative lesions, improved. All of 4 in the chronic hæmatogenous group and 3 out of 4 in the productive pulmonary infiltrative group improved. In general, all exudative phases of infiltrative disease were halted and reversed by sulphetrone. The need for long-continued courses is emphasised and routine laboratory control is essential. Sulphetrone is useful as an adjuvant with definite objectives in view. S. L. W.

Thenylene: A New Antihistamine Compound. A. S. Friedlaender and S. Friedlaender. (*Amer. J. med. Sci.*, 1948, **215**, 531.) A new antihistamine compound, N-(α-pyridyl)-N-(α-thenyl)-N′, N′-dimethylethylenediamine hydrochloride, has been synthesised. Under the name of thenylene (of histadyl) it has been examined to ascertain its effectiveness in preventing fatal histamine shock and anaphylaxis in guinea-pigs, and in alleviating allergic symptoms in man. A protective dose of 3 mg. kg. was administered intraperitoneally to male guinea-pigs 15 minutes prior to the intravenous injection of histamine. This dose protected all animals against one lethal

dose of histamine, while 50 per cent. survived approximately 8 lethal doses. Marked protection was given against fatal anaphylaxis in guinea-pigs sensitised by the intraperitoneal injection of 0·1 ml. of normal horse serum, followed 12 days later by a shock dose of 1 ml. of the same serum intravenously. Of the control animals 100 per cent. died, while only 20 per cent. of fatalities occurred amongst a group receiving 1 mg./kg. 15 minutes before the dose of antigen. The clinical action was studied in 117 patients with one or more allergic complaints, in doses of 100 mg. for adults 4 times daily, or as necessary when symptoms were intermittent. Children were given one-quarter to one-half this dosage. Symptomatic relief was obtained in many cases of urticaria, hay fever and perennial allergic rhinitis. Results in asthma were not striking. Mild side effects occurred in 25 per cent. of patients, but rarely affected administration of the drug. Drowsiness was most common, occurring in 13 patients, and vertigo, headache, gastro-intestinal distress and dryness of mucous membranes were also reported. Toxic symptoms were usually relieved by a reduction of dosage. H. T. B.

BACTERIOLOGY AND CLINICAL TESTS

Antibiotics, Induced Resistance of *Staphylococcus aureus* to. J. W. Klimek, C. J. Cavallito and J. H. Bailey. (*J. Bact.*, 1948. **55,** 139.) It is known that many antibacterial substances are inactivated by various thiol compounds, some reacting rapidly with a large number of -SH compounds, some with cysteine or related β-aminoalkane thiols only, and others displaying reactions intermediate between these two. A study of the development of resistance of *Staphylococcus aureus* to several antibiotics was undertaken to determine whether a correlation existed between development of resistance and the known reactivity of the antibiotics with thiol compounds. The antibacterial agents studied were penicillin, streptomycin, pyocyanin, gliotoxin, aspergillic acid, mercuric chloride, and the active principles of *Allium sativum*, *Asarum canadense* and *Arctium minus*. The susceptibility of *S. aureus* to the antibiotics was determined by growing the organism in a series of beef broth cultures containing increasing quantities of the test agents. Results of the experiments are demonstrated in figures, and show the ability of *S. aureus* to develop rapid and marked resistance to penicillin, streptomycin and the active principle of *Asarum canadense*. The organism developed an intermediate degree of resistance to pyocyanin and gliotoxin, very little resistance to mercuric chloride or the active principle of *Arctium minus* and no resistance to aspergillic acid. These results run parallel with the degree of specificity of reactivity with sulphydryl groups. The more selective the antibiotic as to the type of -SH compound with which it will react, the more readily does it induce bacterial resistance. Reversibility of resistance occurs with antibiotics which react non-selectively with thiols, while the resistance induced by antibiotics reacting selectively with thiols is likely to be non-reversible. H. T. B.

Streptomycin. Activity in Presence of Serum and Blood. E. B. Schoenbach and C. A. Chandler. (*Proc. Soc. exp. Biol. N.Y.*, 1947, **66,** 493.) Bactericidal tests were carried out on the growth of *Staphylococcus aureus* in the presence of streptomycin, various factors possibly affecting such tests, i.e. phagocytosis, hæmolysis, immune serum and labile

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