ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Ascaridol, in Chenopodium Oil, Determination of. H. Lepetit. (*Trav. Lab. Mat. Med.*, 1943-5, **32**, Part 2.) The method of the French Codex for the determination of ascaridol in oil of chenopodium gives very consistent results, but the results are inaccurate, as the factor used was determined on pure ascaridol, whereas actually the factor varies according to the concentration of ascaridol. The method recommended is as follows. Dissolve a weighed quantity of the oil, between 2·50 and 2·60 g., in sufficient acetic acid to make a total volume of 50 ml. Place a flask, containing 3 ml. of 83 per cent. solution of potassium iodide with 5 ml. of concentrated hydrochloric acid and 10 ml. of acetic acid, in an ice bath. When this has cooled (after about 6 to 8 minutes), add, with shaking, 5 ml. of the chenopodium solution, close the flask, and allow to stand in ice for 5 min. Add 5 ml. of carbon tetrachloride, and titrate the iodine liberated with 0·1N sodium thiosulphate until the aqueous layer is decolorised. Carry out a blank experiment without the oil, adding finally 20 ml. of water before titration. The cooling conditions for this test should be the same as for that with the oil. If N and n are the volumes of thiosulphate solution used for the test and the blank respectively, and a is the weight of sample taken, then the percentage of ascaridol in the oil is given by the formula $0{\cdot}605\ (N-n)/a$. The limits suggested for the official oil are from 60 to 80 per cent. of ascaridol. G. M.

Aspirin, Phenacetin and Caffeine, Infra-Red Determination of. W. H. Washburn and E. O. Krueger. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 623.) A method suitable for the determination of aspirin, phenacetin and caffeine in commercial tablets, etc., depends upon the presence of absorption peaks in the spectra of these substances at 9·27μ, 8·99μ, and 10·26μ respectively. In each case, the absorption due to the remaining 2 substances is a minimum. A quantity of powder, chosen so as to bring the absorption at each of the 3 wave-lengths within the working-range of the spectrometer, is suspended in water and extracted with chloroform. The optical density is determined at each of the 3 given wave-lengths. Reference is made to working curves showing the absorption of aspirin, phenacetin and caffeine at each of these wave-lengths and the proportion of each ingredient is determined approximately, by ignoring the absorption due to the other 2 ingredients whose absorption is not maximal. These approximate figures are used to make a more accurate computation in which allowance is made for absorption due to all 3 ingredients at each wave-length, the exact answer being arrived at by successive approximation. The determination is reproducible within ± 2 per cent., and can be completed within 4 hours. G. B.

Calcium and Magnesium, Separation by Oxalate Method. T. Holth. (*Anal. Chem.*, 1949, **21**, 1221.) For the determination of calcium as oxalate in solutions containing calcium ions the concentration of the solution should be about 0·01M and the precipitates should stand for 4 hours at room temperature before filtration. To obtain quantitative precipitation of calcium oxalate the solution must not contain even relatively small amounts of a strong acid (calcium oxalate cannot be precipitated quantitatively from

a solution containing free oxalic acid) although to obtain precipitates with a particle size convenient for filtration the precipitation should be carried out in neutral or in an acetic acid medium. After precipitation the solution is filtered and the precipitate is washed with hot ammonium oxalate solution 3·0 g./l.), dried at 105°C. for half an hour, and then heated in an electric furnace at 480° to 500°C. to constant weight (40+20 minutes). When calcium is precipitated as oxalate from solutions containing calcium chloride and magnesium chloride, some magnesium oxalate is always occluded. The precipitate should therefore be dissolved in hydrochloric acid and reprecipitated with ammonia; a large excess of ammonium oxalate prevents the precipitation of magnesium from supersaturated magnesium oxalate solutions. In samples containing less than 25 per cent. of magnesium the precipitation can be carried out by neutralising a hydrochloric acid solution of the calcium ion and an excess of oxalate ion with ammonia; ammonium chloride should not be added. For samples containing from 25 to 90 per cent. of magnesium an amount of ammonium oxalate (ten times the amount of magnesium present but not less than 3 g.) is dissolved in 200 ml. of water, the solution is cooled to room temperature, and enough acetic acid is added to make the solution 0·05M. The solution under test containing 100 to 150 mg. of calcium plus magnesium is added, the beaker is then placed on a steam bath for 15 minutes, with stirring, and is then allowed to stand for 4 hours at room temperature after which the filtration is carried out as described previously. R. E. S.

Cinchona and Nux Vomica, Assay of, by Microsublimation. J. A. Zapotocky and L. E. Harris. (*J. Amer. pharm. Ass.*, 1949, **38**, 557.) Heating plant material containing alkaloidal salts produces only minute amounts of crystalline sublimate, and treatment with an alkaline agent such as sodium carbonate is first necessary. A number of alkaloidal bases and the corresponding salts were wetted with sodium carbonate solution to form a paste, dried, powdered, and approximately 25 mg. subjected to microsublimation in a tube (17 cm. long, 7 mm. in diameter) at 5 mm. pressure. The sublimates obtained from the alkaloids were compared microscopically with the sublimates obtained from the respective alkaloids and alkaloidal salts treated with sodium carbonate and in each instance the crystals were found to be identical in shape. Using this procedure the sublimation temperatures of a number of alkaloids were determined and also the temperatures at which alkaloids sublimed from cinchona and nux vomica. In the quantitative determination of the total alkaloids of cinchona, a known weight of dried sample, in No. 60 powder was mixed with anhydrous sodium carbonate in the ratio of 3 to 1. Sufficient distilled water was added to wet the mixture which was then triturated in a mortar and dried in a warm desiccator. The dried mixture was again powdered and approximately 50 mg. was placed in the closed end of a sublimation tube. A piece of cotton wool was inserted over the sample and sublimation was carried out at 5 mm. pressure and through a temperature range of 140° to 175°C. Heat was applied until crystals no longer appeared. The percentage of total alkaloids obtained by this method was 3·25 per cent. against an average of 3·88 by the N.F.VIII assay. If the N.F.VIII assay residue of total alkaloids (including insoluble portions and colouring matter) was purified by treatment with dilute sulphuric acid and activated charcoal followed by extraction with chloroform, an average result of 3·28 per cent. was obtained which was close to the result of 3·25 per cent. obtained by microsublimation. A similar microsublimation assay of nux vomica gave results of 1·26 and 1·22 per cent. alkaloids whereas the N.F.VIII

assay gave results of 1·33, 1·28 and 1·26. It is considered that the micro-sublimation procedure gave results which were more nearly a true determination of the alkaloids present. R. E. S.

Codeine and Diamorphine, Determination of, in Admixture. C. Stainier and J. Bosley. (*J. Pharm. Belg.*, 1947, **2**, 218.) The method is devised specially for the determination of codeine and diamorphine in pilules, containing also terpin hydrate. A quantity of powdered material corresponding to 10 pilules, is treated with 8 ml. of water and 2 ml. of 20 per cent. solution of sodium hydroxide. The mixture is heated, under a reflux condenser for 30 minutes on the water-bath, and transferred to a separating funnel, the solution being rinsed in with 12 ml. of water. It is then extracted in succession with 30, 30, 20, 10 and 10 ml. of chloroform. The chloroformic solution is dehydrated with sodium sulphate, filtered and evaporated to dryness. The residue of codeine is taken up in a mixture of 15 ml. of neutral alcohol and 15 ml. of water, and the solution is titrated with 0·1N hydrochloric acid, using methyl red as indicator. The titration is checked by adding 5 ml. of the acid in excess, and titrating back with alkali. One ml. of 0·1N acid is equivalent to 0·0317 g. of codeine (1 H_2O), or 0·0433 g. of codeine phosphate (2 H_2O). The alkaline solution which has been extracted with chloroform is treated with 2 g. of ammonium chloride, and extracted with 40, 30, 20, 10 and 10 ml. of a mixture of 3 volumes of chloroform and 1 volume of isopropyl alcohol. After dehydration with sodium sulphate, the solution is evaporated to dryness and the residue is dissolved in N hydrochloric acid so that 1 ml. of the solution contains about 1 mg. of diamorphine. To 1 ml. 0·2 ml. of 10 per cent. solution of sodium nitrite, 0·2 ml. of 25 per cent. hydrochloric acid, and water to 5 ml. are added. After 20 min., 1 ml. of 30 per cent. sodium hydroxide solution is added and the colour is determined photometrically in a 10 mm. cell, using filter S47. The amount of diamorphine is then determined from a standardisation curve prepared with morphine hydrochloride, the factor 1·127 being used to convert morphine hydrochloride into diamorphine hydrochloride. G. M.

Ethyl Alcohol in Ethyl Ether, Determination of. J. Lamond. (*Analyst*, 1949, **74**, 560.) The method recommended is as follows. Extract 50 ml. of the ether in a separating funnel with three 15 ml. quantities of water and transfer the combined extract to a 50 ml. Nessler cylinder. Into a second cylinder measure 40 ml. of distilled water and 5 ml. of the reagent (667 g. of ceric ammonium nitrate, $(NH_4)_2Ce(NO_3)_6$, dissolved in the minimum quantity of water, made up to 1 litre and filtered). Add 5 ml. of the reagent to the cylinder containing the aqueous extract and rapidly titrate the contents of the second cylinder with a 3 per cent. w/v solution of alcohol in water. When the colour of the blank almost matches that in the original tube, adjust the volume of liquid in the two tubes to the same level before completing the titration. When the colour is matched the alcohol content of the ether, g./100 ml., equals (ml. of 3 per cent. alcohol required × 0·06). Extraction of alcohol was complete under the conditions of the method and estimation of quantities between 0·10 and 0·20 per cent. was satisfactory. The end-point could be judged to within 0·1 ml., corresponding to an error of 3 per cent. on 0·10 per cent. of alcohol; 0·01 per cent. of alcohol could be detected. Alcohol-free ether could be prepared by washing with a 10 per cent. solution of sodium chloride. The colour is given by all the lower alcohols and interference is caused by aldehydes and amines. R. E. S.

Gallic Acid and Pyrogallol, Paper Partition Chromatography of. S. Rydel and M. Macheboeuf. (*Bull. Soc. Chim. biol.*, 1949, **31,** 1265.) It has been shown that ions resulting from the dissociation of alkaloids cause difficulties when the method of paper partition chromatography is applied to their solutions, but that this difficulty can be overcome by adding sufficient ammonia to repress the ionisation. It is now shown that with acids also, the ions are absorbed on the paper and produce tracks which confuse the chromatograms. The trouble may be avoided by using solvent phases which contain an acid which represses the ionisation. The tests were carried out on gallic acid and pyrogallol, and the spots were developed with ferric chloride or ammoniacal silver nitrate. In *n*-butyl alcohol saturated with water, pyrogallol, which has no acid function, gave well-defined spots, while with gallic acid there were elongated tracks. Both compounds gave satisfactory results in an acid solvent prepared by shaking 4 volumes of butyl alcohol with 5 of water and 1 of acetic acid, and removing the aqueous layer. In alkaline medium (collidine) the phenolic function is ionised and unsatisfactory results are obtained with both compounds. G. M.

Hydrastis, Use of Selective Adsorption in the Analysis of. J. Mendez. E. R. Kirch and R. F. Voigt. (*J. Amer. pharm. Ass.*, 1949, **38,** 538.) The proposed assay is based on the separation of hydrastine from berberine by adsorption on florisil (synthetic magnesium silicate) columns, with selective elution with ammoniacal alcohol followed by spectrophotometric estimation using the colour produced in hydrastine solutions by oxidation with potassium dichromate and sulphuric acid. An extraction of the crude drug is carried out according to the N.F.VIII method to the point where a measured quantity of ethereal extract is taken for analysis. An aliquot of this extract is warmed on a water-bath with 10 ml. of 0·5N sulphuric acid. The resulting acid solution of the alkaloids is quantitatively transferred to a glass wool filter and passed through the prepared adsorption column. The column is washed with 300 ml. of distilled water and then with 50 ml. of ether followed by elution with 100 ml. of ammoniacal alcohol, the ether fraction and the eluate being combined and evaporated to dryness. The residue is dissolved in 0·1N sulphuric acid, aliquot portions of this solution are taken in a 25 ml. flask and 4 ml. of 1 per cent. potassium dichromate solution is added, followed by 5 ml. of concentrated sulphuric acid. This mixture is thoroughly shaken and allowed to stand for 5 minutes in order to develop the maximum colour. Distilled water is added to the final volume, and the light absorption is measured at 600 mμ against a reagent blank. Any berberine present in the eluate aliquot is estimated directly by means of spectrophotometric readings at 430 mμ. Standard curves for hydrastine and berberine are given. It is claimed that the procedure simplifies the purification of the hydrastine; results obtained are lower than those from an N.F.VIII assay on the same sample of hydrastis, the difference representing the other ether-soluble extractives included in the official assay. The average amount of hydrastine in the sample of hydrastis was found to be 2·51 per cent. as compared to 2·67 per cent. when determined by the official N.F. method. R. E. S.

Pentavalent Antimony in the Presence of Pentavalent Arsenic, Polarographic Determination of. I. M. Kolthoff and R. L. Probst. (*Anal. Chem.*, 1949, **21,** 753.) Polarographic measurements on the reduction of pentavalent antimony showed two waves at hydrochloric acid concentrations equal to or greater than 6N, the first wave corresponding to a reduction of Sb^{V} to Sb^{III} and the second to that of Sb^{III} to Sb amalgam. With

concentrations lower than 4N only one wave was observed, the height decreasing with decreasing acidity. No reduction wave was observed in an alkaline medium. The reduction of Sb^{V} to Sb^{O} was complete in a medium of N hydrochloric acid and 4·0 M potassium bromide; one wave only was observed, the anodic bromide wave occurring at a more negative potential than that of the first reduction wave of Sb^{V} to Sb^{III}. The half-wave potential in the bromide-containing medium did not correspond to the true half-wave potential of Sb^{V} to Sb^{O} because this potential is also shifted to a more negative value by the anodic bromide current when the bromide concentration was greater than 2·0 M. The diffusion current constant in the presence of 4·0 M bromide was found to be 7·48 while in 6N hydrochloric acid it was 7·50, indicating that the reduction was complete in 4·0 M bromide. Reduction waves of As^{V} were not observed in solutions containing less than 4N hydrochloric acid or in solutions of 4N potassium bromide and N/1 hydrochloric acid. Waves of small height were found in 6 to 8N hydrochloric acid and this medium is not suitable for the determination of Sb^{V} in the presence of large amounts of As^{V}. The polarographic behaviour of trivalent arsenic and antimony was examined and the characteristics of the waves were discussed; both gave typical anodic waves in 0·1 to 0·5N potassium hydroxide. R. E. S.

Procaine, Photometric Determination of. C. Lapiére. (*Anal. chim. Acta*, 1947, **1**, 337.) The solution of procaine, containing 0·25 to 1·25 mg. of procaine in 0·5 per cent. hydrochloric acid, is cooled in ice and treated with 0·1 ml. of 10 per cent. sodium nitrite solution. After 2 minutes 2 ml. of a 1 per cent. solution of βnaphthol in 10 per cent. sodium hydroxide solution is added. After 30 minutes, 2 ml. of alcohol is added and the volume is made up to 10 ml. with water. The colour is determined, using filter S47. When applied to tablets, the procaine is extracted with peroxide-free ether in presence of ammonia; from ointments, it is extracted by 0·5 per cent. hydrochloric acid from a light petroleum solution. G. M.

Quinine, Spectrophotometric Determination of. C. V. St. John. (*Bull. nat. Form. Comm.*, 1949, **17**, 208.) The proportion of quinine in aqueous solutions of quinine salts may be determined by measurement of the optical density of a solution at 346 mμ. The quantity of quinine is calculated by reference to a calibration curve prepared by using solutions of a pure quinine salt. Using a Beckman quartz spectrophotometer with a band-width of 1 to 5 mμ and a 1 cm. quartz cell, suitable concentrations are 0·005 to 0·3 mg. of anhydrous quinine dihydrochloride per ml. The solutions should be acidified with a little hydrochloric acid, and the reference cell should contain distilled water. Provided that the *p*H is kept below 2·5, the absorption spectrum is unaffected by the concentration of acid present, and identical curves are obtained with sulphate, hydrochloride and dihydrochloride. Interfering substances can be detected by comparing the absorption spectrum with that of a solution of a pure quinine salt. If the logarithm of the optical density is plotted against wave-length in each case, the curves can be superimposed unless interfering substances are present, in which case special correcting systems have to be developed. Provided the cells are carefully cleaned with chromic-sulphuric acid mixture and care is exercised in making the test dilutions accurately, the results obtained spectrophotometrically agree within 1 per cent. with those obtained by the method of chloroform extraction and weighing. G. B.

Strychnine, Colorimetric Determination of. S. Rolland-Leclercq. (*J. Pharm. Belg.*, 1947, **2**, 283.) The method is based on the colour given by

tetrahydrostrychnine with oxidising agents. The solution, containing 0·1 to 0·5 mg. of strychnine, is placed in a large test-tube, the volume being made up to 5 ml. with water. It is then treated with 5 ml. of concentrated hydrochloric acid and 3 g. of amalgamated zinc, and heated on the water-bath for 20 minutes. After cooling, the mixture is made up to 10 ml. and treated with 2 drops of 0·1 per cent. solution of sodium nitrite. After 5 minutes the red colour is determined, using a 10 mm. cell and 520 or 440 filter. The colour given by similar quantities of brucine under these conditions is yellow and much weaker; when using the 520 filter it is negligible. On the other hand, the presence of brucine interferes with the determination of strychnine and produces an irregular increase of the colour due to the strychnine. Thus, in applying the method to mixtures of the two alkaloids, it is necessary first to destroy the brucine by treatment with nitric acid. G. M.

INORGANIC CHEMISTRY

Sodium Citrate, Action of, on Sparingly Soluble Compounds of Calcium and Barium. O. Gengou, P. E. Grégoire, G. Lagrange and J. Thomas. (*Arch. int. Pharmacod.*, 1950, **81**, 61.) Sparingly soluble compounds, such as barium carbonate and sulphate, and calcium oxalate, are more crystalline when produced from less concentrated solutions. Most of these can be dispersed in water by sodium citrate, but generally the more crystalline forms are much less easily dispersed. Sodium citrate is also able to prevent the precipitation of compounds of this type, or, in lower concentration, it modifies fundamentally the microscopic appearance of such precipitates. Independently of the dispersing action, trisodium citrate dissolves a certain quantity of these salts, the amount depending on their nature, the concentration of citrate, and the temperature. Dispersion and dissolution appear to be different phenomena, in which one part of the citrate is used to dissolve the precipitate, while the remainder assists the dissolution of the residue. The resulting solutions have the character of true solutions, following an interchange of ions betwen the trisodium citrate and the barium or calcium compound. In some cases the compound formed crystallises.

G. M.

ORGANIC CHEMISTRY

Oxophenarsine Hydrochloride (Mapharsen), Decomposition Products of. C. K. Banks. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 503.) Arsenicals with the 3-amino-4-hydroxyphenyl structure being known to decompose under unfavourable conditions, investigations were made into the types of decomposition undergone by oxophenarsine as the hydrochloride and as the free base in solid form, as well as solutions of these compounds at various *p*H levels. The oxidative and hydrolytic decompositions under a variety of conditions were studied and the products resulting were determined quantitatively and examined for possible toxicity. Various solid forms of the drug were also investigated. Results showed solid oxophenarsine hydrochloride to be extremely stable when anhydrous, showing no appreciable decomposition in 5 years, but with increasing water content stability decreased. Oxygen did not react with the hydrochloride but was liable to act on hydrolytic products giving coloured oxidation products. Solid hydrated oxophenarsine hydrochloride and oxophenarsine were decomposed to *o*-aminophenol and inorganic arsenic compounds in the absence of oxygen. With oxygen, part of the *o*-aminophenol was converted to iminoquinone intermediate which reacts with more of the aminophenol to produce non-arsenated

*iso*phenoxazones. Solutions of oxophenarsine without oxygen decomposed slowly in the cold, more rapidly with heat, to *o*-aminophenol and inorganic arsenite. In the presence of oxygen, *o*-aminophenol was oxidised and below *p*H 7 condensed with unoxidised o-aminophenol giving non-arsenated *iso*phenoxazones. Above *p*H 7 the *o*-aminophenol was oxidised to give, as final products, arseno*iso*phenoxazones. The decomposition products of oxophenarsine hydrochloride were found to be less toxic than the drug itself, when injected intravenously into rats. G. R. K.

Oxophenarsine Hydrochloride (Mapharsen), Decomposition Rate of. C. K. Banks. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 509.) The rate of decomposition of solid oxophenarsine hydrochloride was found to depend on the moisture content and on temperature, the extent to which it occurred being limited by, and in proportion to, the amount of water available. When anhydrous, the substance decomposed only slightly over a 5-year period at temperatures ranging from 20° to 70°C. Oxophenarsine hydrochloride containing moisture to the extent allowed by the U.S.P. had a decomposition rate approaching a limit of the equivalent of arsenical decomposed for each equivalent of water present. Since the amount of water permissible is small, the maximum decomposition is within the tolerance for the product and is too small to be detected by pharmacological methods. At higher temperatures than the product would encounter the amount of arsenical decomposed for a given moisture content is doubled. Commercial ampoules were stable for 4 years. Solutions of the hydrochloride decomposed by a unimolecular mechanism in a first-order reaction. At a *p*H of about 3·25 the rate was at a minimum. Solutions of *p*H 6 were stable enough for clinical purposes but not for use over a long period of time. When maintained at room temperature or below, solutions made from multiple dose packages were adequately stable for 3 days. Diffused light did no harm but sunlight was deleterious. Such solutions should be kept under a vapour proof stopper to prevent evaporation and subsequent overdosage. G. R. K.

TOXICOLOGY

Morphine, Codeine and Diamorphine from Viscera, Isolation and Determination of. A. Stolman and C. P. Stewart. (*Analyst.*, 1949, **74**, 536.) The use of florisil as an adsorbent in the isolation of morphine, codeine, and diamorphine is described. In experiments on pure alkaloids 1 mg. of the alkaloid was dissolved in 100 ml. of distilled water and the solution was run through a column of untreated florisil. The adsorbed alkaloid was eluted by refluxing with methyl alcohol for 1 hour. Quantitative determination of the eluted alkaloid showed an apparent recovery of 105 to 120 per cent. with morphine or codeine, and 94 per cent. with diamorphine. In order to avoid large quantities of solvent, elution of the alkaloids was carried out from a glass tube containing the florisil in a modified Soxhlet type apparatus. Solid sodium carbonate was placed on top of the adsorption column before elution and oxalic acid was added to the solvent flask to prevent decomposition of the alkaloids in an alkaline solution. Recovery of alkaloids was then substantially complete from *p*H 6·0 to 8·0. Above *p*H 8·0 the morphine was not completely adsorbed; below *p*H 6·0 adsorption of morphine and diamorphine was incomplete, but codeine was still adsorbed at *p*H 4·0, though not at *p*H 2·0. A description is given of initial trial work which indicated that the method could be adapted for the isolation of the alkaloids from dilute alcoholic (Stas-Otto), acidified aqueous (Dragendorff) or from trichloracetic acid (Stewart) extracts of biological tissues. For tissues 100 g.

of liver were macerated in a Waring blender with 200 ml. of alcohol (95 per cent.) acidified with tartaric acid; the mass was mixed with 300 ml. of alcohol and then filtered. Each volume of filtrate was mixed with 4 volumes of 5 per cent. w/v aqueous trichloracetic acid solution and filtered, the filtrate being used for adsorption experiments after the adjustment of the *p*H and the addition of specified amounts of alkaloid. Trichloracetic acid extracts were made by macerating 100 g. of liver with 200 ml. of 10 per cent. w/v trichloracetic acid solution; each volume of filtrate was mixed with an equal volume of water to reduce the concentration of trichloracetic acid to 5 per cent.; each 100 ml. of the 5 per cent. trichloracetic acid solution was mixed with 25 ml. of alcohol (95 per cent.) and then used for adsorption. For blood analyses, 1 volume of blood was mixed with 1 volume of a 10 per cent. w/v aqueous solution of trichloracetic acid and one-half volume of alcohol (95 per cent.); the coagulated proteins were removed by filtration and the filtrate then used for adsorption experiments after the adjustment of the *p*H. Urine was mixed with an equal volume of water and half of its volume of alcohol (95 per cent.), the solution being filtered if necessary and the *p*H adjusted. In the recovery of alkaloids added to tissue extracts it was found that the optimum *p*H found previously for pure aqueous alkaloidal solution gave low results and adjustment of the *p*H to 8·0 to 9·0 was necessary. With this method morphine, codeine, or diamorphine, in amounts ranging from 0·5 to 5·0 mg., contained in 100 to 250 ml. of alcohol or trichloracetic acid extract of tissue, blood or urine, could be quantitatively adsorbed and quantitatively eluted by methyl alcohol. The eluted alkaloids were readily identifiable by the purity of their colour reactions. The determination of the alkaloids was effected by modifications of standard colorimetric methods. R. E. S.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Alvein. K. Gilliver, A. M. Holmes and E. P. Abraham. (*Brit. J. exp. Path.*, 1949, **30,** 209.) Alvein is a new antibiotic formed by *Bacillus alvei* in certain liquid media. It inhibits the growth of a number of Gram-positive and Gram-negative bacteria. Production was obtained by deep fermentation in a medium containing glucose, corn steep liquor and inorganic salts. Chemically the preparation behaved as if it consisted mainly of a strongly basic polypeptide. It is hæmolytic. H. T. B.

Ergothioneine: Preparation from Blood and Ergot. G. Hunter, G. D. Molnar and N. J. Wight. (*Canad. J. Res.* (*E*), 1949, **27,** 226.) The yields from pig blood were very much lower than those reported earlier, 30 gallons giving less than 2 g. Although this variation may be due to dietary factors, the lack of a reliable method of determining ergothioneine in blood precludes an adequate explanation. Ergothioneine was prepared from ergot in 0·26 per cent. yield by the following method. 500 g. of ergot in fine powder was extracted by boiling successively with 2000 ml. of water containing 1 ml. of glacial acetic acid, and two quantities, each of 1000 ml. of water, straining and pressing between each boiling. The filtrate was treated with a slight excess of a saturated aqueous solution of uranium acetate, allowed to stand and the supernatant liquid removed. The residue was washed twice with water and the combined liquid and washings made acid (0·5N) with sulphuric acid and heated to 60°C. A slight excess of an aqueous suspension of cuprous oxide was added, the mixture shaken, allowed to

stand, and the supernatant liquid removed. The copper precipitate was washed three times at 60°C. with its own volume of 0·5N sulphuric acid containing a little copper. The washed copper precipitate was suspended in hot water, treated with hydrogen sulphide and filtered. The residue was washed and the combined filtrate and washings freed from hydrogen sulphide and treated with warm barium hydroxide solution until the solution was neutral on litmus paper. The solution was evaporated *in vacuo* to 15 ml., boiled with charcoal, filtered, and the filtrate evaporated *in vacuo* to 5 ml. when crystals appeared. After further purification, 1·30 g. of anhydrous ergothioneine was obtained. The twice recrystallised product melts with expansion and decomposition at 280°C. It was found that ergothioneine from blood was frequently contaminated with hypoxanthine. Since free ergothioneine is soluble in water and free hypoxanthine is insoluble they may be readily separated. If, however, an acid radical is present, both are soluble and the purine also forms crystals similar to those of ergothioneine. Although copper oxide precipitates purines as well as ergothioneine, it is an eminently suitable precipitant for obtaining the alkaloid from ergot because the latter contains little or no purine, and nitroprusside reacting SH- groups are absent. It has, however, a tendency to gel formation. G. R. K.

Local Anæsthetics and the Potassium Ion. O. Peczenik and G. B. West. (*Nature*, 1949, **164**, 354.) The action of 5 local anæsthetics (procaine-amylocaine, cocaine, amethocaine and cinchocaine) on the isolated rat phrenici nerve diaphragm was studied, with particular reference to the potassium on., All the preparations were left for 2 hours before use in a bath of 70 ml. of Tyrode solution containing 0·2 per cent. of glucose. From the results of 3-minute contacts it was found first that large doses of the anæsthetics produced neuro-muscular block *per se*, and secondly that whereas the addition of potassium chloride potentiated the inhibition produced by amylocaine and amethocaine it had little or no action on procaine, suggesting a difference of action for this compound. In a further experiment in which the potassium chloride was added to the bath for 3 minutes in the recovery phase, after washing out the anæsthetic, it was found that in the case of amethocaine or cinchocaine this always produced block ; without cocaine or amylocaine it had either no action or produced block, while in the case of procaine it was found that the addition of potassium chloride sometimes aided but never prevented recovery. On the other hand the increase of the refractory period of the rabbit auricles produced by cocaine is potentiated by potassium chloride. S. L. W.

BIOCHEMICAL ANALYSIS

Amidopyrin in Body Fluids, Colorimetric Determination of. R. Pulver. (*Arch. int. Pharmacod.*, 1950, **81**, 47.) The reagent is prepared by treating 2 ml. of a 1 per cent. solution of *p*-nitraniline in N hydrochloric acid with 2 ml. of 1 per cent. sodium nitrite solution. After 2 minutes 2 ml. of 1 per cent. sulphamic acid solution is added, and, after a further 20 minutes, 14 ml. of water. The reagent may be kept for 12 hours. This solution gives, with amidopyrin, an intense yellow colour, which does not appear to be due to coupling. For the examination of body fluids, 2 ml. of plasma, serum or liquor is treated with 1 ml. of acetate buffer solution (56·5 ml. of glacial acetic acid and 118 g. of sodium acetate in water to 1 l.) diluted with 10 ml. of water, and heated for 5 minutes on the water bath. The solution is then filtered into a separating funnel, the filter being washed twice with 2ml. of hot water. After cooling, 1 ml. of 2N sodium hydroxide is added, and the liquid is shaken out with two 20 ml. quantities of ether. The ethereal extract

is evaporated to dryness, and the residue is treated with 2 ml. of 10 per cent. sodium acetate solution, and, after cooling, 0·5 ml. of reagent. After 10 minutes, the mixture is transferred to a separating funnel, being washed in with 2 ml. of 2N hydrochloric acid and then with 2 ml. of water. The colour is shaken out into 10 ml. of chloroform, the chloroformic solution being dried with sodium sulphate prior to photometric determination in a 2 cm. cell using filter S43. G. M.

***p*-Aminosalicylic Acid in Biological Media, Determination of.** M. Pesez. (*Bull. Soc. Chim. biol.*, 1949, **31**, 1369.) The author has shown previously that diazotised *p*-aminosalicylic acid, unlike the corresponding compound of *m*-aminophenol, is very unstable, and passes into *p*-hydroxysalicylic acid. *p*-Aminosalicylic acid itself is easily converted into *m*-aminophenol by warming in acid solution. Thus the method of diazotisation and coupling with *p*-hydroxysalicylic acid (derived from *p*-aminosalicylic acid and nitrite) may be used for the determination of both *p*-aminosalicylic acid and of *m*-aminophenol. The sample containing 10 to 50 μg. of *p*-aminosalicylic acid, is diluted to 2 ml. with water, treated with 1 ml. of 10 per cent. solution of trichloracetic acid, and heated for 30 min. in a water-bath. A similar sample is kept in ice during the same period, then treated with 1 ml. of trichloracetic acid. Each tube, cooled in ice, is then treated with 3 ml. of water, 0·5 ml. of 0·1 per cent. solution of sodium *p*-aminosalicylate ($2H_2O$) and 0·5 ml. of 1 per cent. sodium nitrite solution. After exactly 3 minutes at 0°C. 2 ml. of 10 per cent. solution of anhydrous sodium carbonate is added, then, after 15 minutes at ordinary temperature, the volume is made up to 10 ml. with water. The difference in colour between the two tubes is determined photometrically using filter 43. In the case of blood, serum or plasma, 2 ml. of water and 2 ml. of trichloracetic acid solution are added to 1 ml. and, after shaking, the mixture is diluted with 5 ml. of water, filtered and centrifuged. The test is then carried out on 2 ml. of the filtrate. Urine is diluted to 100 volumes with water, and 1 or 2 ml. taken for the test. G. M.

Ergothioneine: Determination in Simple Solution and in Blood. G Hunter. *Canad. J. Res.E.*), 1949, **27**, 230.) *Simple Solution.* If x ml. is the volume of test solution to be used, 2x ml. of water, 1 ml. of diazo reagent, 2 ml. of alkaline buffer solution and x ml. of test solution are put, in that order, into a properly matched Evelyn photo-electric colorimeter tube, cooled in ice-water and mixed. In the presence of ergothioneine a lemon-yellow colour develops. After 45 seconds the tube is removed from the ice-water, 5 ml. of 10N sodium hydroxide is added and the contents mixed. After 3 to 4 minutes the final purplish pink colour has fully developed and the tube is read in the colorimeter A blank test with 2 ml. of water is used to set the colorimeter at 100 per cent. transmittance. The Evelyn filter No. 520, transmitting in the range 495 to 550 mμ, was found to be suitable. The diazo reagent is prepared as follows. To 1·5 ml. of a solution containing 9 g. of sulphanilic acid and 90 ml. of 37 per cent. hydrochloric acid per l., cooled in ice-water, add 1·5 ml. of 5 per cent. sodium nitrite solution, allow to stand for 5 minutes, add a further 6 ml. of nitrite solution and after a further 5 minutes adjust the volume to 50 ml. with ice-cold water. The alkaline buffer consists of an aqueous solution containing 1 per cent. of anhydrous sodium carbonate and 10 per cent. of anhydrous sodium acetate. *Blood.* To 0·45 ml. of whole blood in a centrifuge tube, add 4·5 ml. of 0·0045 N acetic acid containing 160 mg. of sodium oxalate per l., mix, immerse in water which is just boiling, and stir. Centrifuge, separate the supernatant liquid and to it add 0·05 ml. of lead

subacetate solution. Mix, centrifuge, separate the supernatant liquid and add 1 drop of a 10 per cent. aqueous solution of sodium dihydrogen phosphate. Mix, centrifuge and again separate. The supernatant liquid is the test solution and is assayed by the method described above. The strength of the acetic acid-oxalate solution used depends upon whether whole blood, corpuscles or plasma is being assayed and upon the extent of the dilution of the specimen. Ergothioneine added to whole blood was recovered quantitatively by this method. All plasmas were found to give a significant yellow colour, which was attributed to histidine or tyrosine or both and not to ergothioneine. G. R. K.

Penicillins, Total Determination of, by the Iodimetric Method. Report of the Analysts' Sub-committee of the Ministry of Health Conference on the Differential Assay of Penicillin. (*Analyst*, 1949, **74**, 550.) To express the total penicillins in a crystalline penicillin G sample the basic iodimetric method of Alicino (*Ind. Engng. Chem., Anal, Ed.*, 1946, **18**, 619) as modified by Mundell, Fischbach, and Eble (*J. Amer. pharm. Ass., Sci. Ed.*, 1946, **35**, 373) was used. The method was examined by collaborative work and the following modifications were tried (a) buffered reactants to reduce the effect of non-penicillin impurities to a minimum; (b) different strengths of alkali for inactivation of the penicillin; (c) longer or shorter periods of inactivation and iodination; (d) temperatures of inactivation and iodination ranging from room temperature to 30° C.; (e) higher concentrations of potassium iodide in the standard iodinating reagent. The final process recommended was as follows. Weigh accurately about 60 mg. of the sample, dissolve in distilled water and dilute to 50 ml. Transfer a 10 ml. aliquot to a stoppered flask, add 5 ml. of N sodium hydroxide and allow to stand for 30 minutes in a water-bath at 30° C. Acidify with 5 ml. of 1·1 N hydrochloric acid, add 30 ml. of 0·02 N iodine solution, close the flask with a wet stopper and place it in a water-bath at 30° C. for 15 minutes. Titrate the excess of iodine with 0·01 N sodium thiosulphate solution, adding 1 ml. of starch solution near the end-point. A blank determination is completed by transferring a 10 ml. aliquot of the penicillin solution to a stoppered flask, adding 30 ml. of 0·02 N iodine solution and titrating immediately with 0·01 N sodium thiosulphate. The difference between the two titrations (each should be duplicated) represents the amount of iodine that has reacted with the penicillin, each ml. of 0·01 N iodine being equivalent to 0·382 mg. of sodium benzylpenicillin. R. E. S.

Streptomycin B (Mannosido-streptomycin), Colorimetric Determination of. W. B. Emery and A. D. Walker (*Analyst*, 1949, **74**, 455.) The method developed deals with the assay of streptomycin B in the presence of streptomycin itself; it depends on the determination of streptomycin B from the mannose residue in the molecule using a 0·2 per cent w/v solution of anthrone in 95 per cent. v/v sulphuric acid as used for the quantitative determination of carbohydrates by Morris (*Science*, 1948, **107**, 254). Under the conditions described the glucosamine and streptose moieties in the molecules of both streptomycins did not react with the reagent. A calibration curve was prepared using various solutions of pure mannose (10 to 100 μg. per ml.) which were added to the reagent, allowed to stand for 20 minutes and examined photoelectrically using Chance glass O.R.2 red filters (peak transmission 640 mμ) and 2 cm cells. Experimental details are given for the measurement of the solution under test and at the same time the total streptomycin (in units /ml.) present in the test solution is determined by chemical assay based on the maltol-ferric ion reaction. The molecular proportion of

streptomycin B (i.e., the molecular percentage of the total streptomycin present) in the sample may be calculated from the expression:

$$\text{Molecular proportion of streptomycin B} = \frac{323\,z}{x} \text{ per cent.}$$

where a solution of streptomycin at x chemical units per ml. contains z μg. of mannose per ml. The method described has been applied to a number of samples of streptomycin hydrochloride and streptomycin calcium chloride complex containing from 3 to 95 per cent. of streptomycin B. A table is given of results obtained together with the biological/chemical assay ratios, and the proportions of streptomycin B calculated from these ratios; ratios of 0·2 and 1·0 for pure streptomycin B and streptomycin A respectively were assumed. The results obtained by the method given agreed with those calculated from chemical and biological assays assuming the relative biological activities. R. E. S.

PHARMACY

GALENICAL PHARMACY

Bentonite, Dried, as a Disintegrating Agent in Compressed Tablets of Thyroid. C. B. Granberg and B. E. Benton. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 648.) Tablets of thyroid, 65 mg. were prepared according to 12 different formulæ in order to compare the properties of dried starch and dried bentonite as disintegrating agents. The tablets were examined for appearance, for hardness expressed as kg. of pressure required to fracture the dry tablet in a special hardness tester, and for disintegration, expressed as the time taken for a weight of 20 g. to force a blade to cut through a tablet immersed in acid pepsin solution. A working plan of the apparatus for disintegration tests is given. The use of bentonite instead of starch as disintegrant gives tablets of greater hardness and longer disintegrating time, but tablets having a suitably short disintegrating time are obtained when some bentonite is used as a filler. A suitable formula is:—lactose, 35 mg. and bentonite, 10 mg. as filler; bentonite, 12 mg., as disintegrant; magnesium stearate, 1 mg. as lubricant; 15 per cent. starch paste, q.s. as binder. The presence of bentonite does not interfere with the U.S.P. XIII assay for iodine in thyroid. G. B.

NOTES AND FORMULÆ

Choline Chloride. (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1949, **141**, 391.) Choline chloride, trimethylhydroxyethylammonium chloride, $CH_2OH.CH_2.N(CH_3)_3.Cl$, is white, crystalline and deliquescent, with an amine-like odour. It is soluble in water and alcohol, and insoluble in benzene, chloroform and ether; a 10 per cent. aqueous solution has a *p*H of about 4·65. It gives a pale yellow precipitate with mercuric potassium iodide, and an emerald-green colour with cobaltous chloride and potassium ferrocyanide. If free from neurine it gives a white curdy precipitate with phosphotungstic acid and no precipitate with tannic acid; heavy metals are limited to 30 p.p.m., ash to 0·05 per cent. and moisture to 0·5 per cent. The content of choline chloride is 97 to 101 per cent., calculated with reference to the dried substance, and is determined by titration with silver nitrate and spectrophotometrically. Choline chloride is given by mouth in daily doses of 1·5 to 3 g., but the precise dosage is not yet established. It has the lipotropic action and uses of other choline salts and is also recognised for clinical trial in the treatment of fatty infiltration of the liver. G. R. K.

Furtrethonium Iodide (Furmethide Iodide). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1949, **141,** 264.) Furtrethonium iodide, furfuryltrimethylammonium iodide, ($C_4H_3O.CH_2.N(CH_3)_3.I$) is a white- to cream-coloured crystalline powder, soluble in water and alcohol but insoluble in benzene; m.pt. 115° to 119°C. A 1 per cent. aqueous solution has *p*H5·3 to 6·0. With an acidified saturated solution of trinitrophenol it gives a yellow precipitate which melts at 167° to 170°C. It is assayed for nitrogen and iodide and contains 97 to 101 per cent. of furtrethonium iodide. When dried *in vacuo* over phosphorus pentoxide for 4 hours, the loss in weight is not more than 0·5 per cent. Standards are also given for tablets and ampoules. Furtrethonium idodide is similar in action to acetyl-β-methyl choline but is more effective when given by mouth and less active, when given by injection, on the cardiovascular, respiratory and gastrointestinal systems. It is given by mouth or by subcutaneous injection to stimulate micturition; it should not be given intravenously. For normal adults an initial dose of 3 mg. is usually effective within 15 minutes.

G. R. K.

Mephobarbital (Mebarol). (*New and Nonofficial Remedies: J. Amer. med. Ass.*, 1949, **141,** 920). Mephobarbital, *N*-methyl-5-ethyl-5-phenylbarbituric acid is methylphenobarbitone. It occurs as white, tasteless, odourless crystals, m.pt. 177° to 181°C. and soluble in chloroform and solutions of alkali hydroxides and carbonates, but only slightly soluble in water, alcohol and ether. The precipitate obtained on adding silver nitrate or mercuric chloride to a solution in sodium hydroxide is soluble in ammonia. It may be distinguished from barbitone by heating in a water-bath for 30 minutes with potassium nitrate and sulphuric acid, cooling, diluting with water, adding sufficient ammonia to make alkaline, heating to expel gas, cooling, filtering and adding to the filtrate ammonium sulphide, when a dark brown-red ring forms; the precipitate obtained when the filtrate obtained in the same manner is treated with dilute sulphuric acid melts at about 220°C. (distinction from phenobarbitone). When assayed by dissolving in alcohol and titrating electrometrically against sodium hydroxide, it contains 99 to 101 per cent. of mephobarbital; the nitrogen content determined by the Kjeldahl methods is 11·15 to 11·50 per cent. Mephobarbital is a long-acting barbiturate with a comparatively mild hypnotic action and is therefore more suitable as a sedative than as a sporific. It is chiefly used as an anticonvulsant in the treatment of epilepsy of the grand mal and petit mal types. G. R. K.

Naprylate Caprylic Compound. (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1949, **141,** 992.) Caprylic compound is a mixture of sodium caprylate and zinc caprylate used for the treatment of superficial fungus infections of the skin and accessible mucous membranes. It is effective against infection due to trichophytons, microsporons and *Monilia albicans.* In moderate concentrations, it does not irritate the skin and is not absorbed from the skin or mucous membranes. Sodium caprylate occurs as cream-coloured granules soluble in water and sparingly soluble in alcohol. It contains 98 to 102 per cent. of sodium caprylate when assayed by the method for sodium propionate N.F.; when dried at 105°C. for 2 hours it loses not more than 2·5 per cent. of its weight. Zinc caprylate occurs as a fine white powder, insoluble in water and alcohol. When dissolved in dilute sulphuric acid and assayed by the method for zinc sulphate U.S.P., it contains 95 to 100 per cent. of zinc caprylate, calculated with reference to the dried substance; the loss on drying at 105°C. for 2 hours is not more than 3 per cent.

Naprylate is supplied as an ointment and powder containing 10 per cent. of sodium caprylate and 5 per cent. of zinc caprylate in a water miscible base and a mixture of starch and talc respectively, and as suppositories. G. R. K.

Phenylpropylmethylamine Hydrochloride (Vonedrine Hydrochloride). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1949, **141,** 133.) Phenylpropylmethylamine hydrochloride, *dl*-l-methylamino-2-phenylpropane hydrochloride, $C_6H_5.CH(CH_3).CH_2.NH.CH_3,HCl$, is not available in the dry state. It is supplied as a clear, colourless, nearly odourless, aqueous solution with *p*H 5·5 to 6·5, from which the hydrochloride may be prepared as fine white crystals, by the following method. Make the solution alkaline with sodium hydroxide, extract with ether, thoroughly dry with anhydrous sodium sulphate, remove the ether, dissolve the residue in benzene, dry the solution with anhydrous sodium sulphate, pass in dry hydrogen chloride and allow to crystallise. After filtration, washing with dry benzene and dry ether, and drying by air suction, the crystals melt at 147° to 150°C.; they are soluble in water, alcohol, ether and hot benzene. Phenylpropylmethylamine hydrochloride is a local vasoconstrictor with little or no stimulating action on the cardiovascular or central nervous system. It is usually applied as a 2·8 per cent. solution as nasal drops, spray, or tampons. G. R. K.

PHARMACOGNOSY

Belladonna and Datura Species, Post-Harvest Alkaloidal Movement in. W. R. Brewer and L. D. Hiner. (*J. Amer. pharm. Ass.*, 1949, **38,** 541.) An investigation has been made into the differences observed in the alkaloidal content of leaves cured after separation, or on intact belladonna plants; and also to assess the value of the normal drying method of hanging whole herbs. Studies were carried out from 1942 to 1947 on *Atropa belladonna*, L., the plants after harvesting providing leaf and stem parts which were separated and cured on drying trays, and also parts which were cured intact by impaling the whole plant on racks and separating leaf from stem after drying. The possibility that alkaloids could be transferred from the roots to other parts of the plant was investigated by dividing a harvest into two parts. The whole plants from one part were cured by hanging the entire plant, including roots, stems and leaves, tops downward from drying racks. Those from the other part of the same harvest were cured by drying their roots, stems and leaves in three different drying trays. Plants were also cured while remaining in an upright position, to investigate the action of gravity on any alkaloidal transference. All the results obtained were statistically analysed and it was found that the drying of intact leaves on the plant increased their alkaloidal content as compared with leaves which were dried separately; this effect was not due to the position of the plant during the drying procedure nor to gravity. The increase in alkaloidal content of the leaf was accompanied by a decrease in the alkaloids of the root or stem, suggesting relocation. The effect was found in at least 4 species of Solanaceous plants, *Atropa belladonna, Datura stramonium, Datura inermis,* and *Datura tatula.* R. E. S.

PHARMACOLOGY AND THERAPEUTICS

Aconite Tincture, Biological Assay of. M. Barr and J. W. Nelson. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38,** 518.) A method is described for the quantitative assay of aconite tincture, using intravenous injections in mice, in which the comparison of LD50 doses of the Reference Standard

Aconitine solution and of diluted tinctures was used as a basis. The standard error of each series of assays was found to be less than 2 per cent. Aconitine is one of the most potent alkaloids known. Hence, any assay procedure giving a product of variable toxicity is dangerous. This is largely responsible for the fact that aconite has fallen into disuse. The tincture was chosen to be studied since it is the most used aconite preparation in medicine. Objections to the official guinea-pig method, though recommended for its constancy, were its lack of sensitivity and the time factor and expense involved. With mice, a simple and economical biological assay is possible, and the use of a 90-minute observation period reduces the time required to perform a complete assay as compared to the present N.F.VIII method. It is more accurate than the present guinea-pig method, and should also be applicable to other preparations of aconite. G. R. K.

9-Aminoacridinepenicillin, Toxicity of. D. C. Brodie and Elizabeth Lowenhaupt. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38,** 498.) 9-Aminoacridinepenicillin is a bright yellow, crystalline powder, almost insoluble in water and soluble in alcohol, glycerin and propylene glycol. One mg. contains about 1,000 units of penicillin and 0·3 mg. of 9-aminoacridine. In general it resembles 9-aminoacridine in its toxic effects, although there is some modification. Thus, its LD50, when given to mice subcutaneously, is 250 mg./kg. expressed as 9-aminoacridine hydrochloride compared with 70 to 80 mg./kg. for 9-aminoacridine. Similarly, when given orally, the LD50 is equivalent to 100 mg. of 9-aminoacridine hydrochloride per kg. as compared with 78 mg./kg. for 9-aminoacridine. This may be due to the lower water-solubility of 9-aminoacridinepenicillin, to 9-aminoacridine being less readily available to the tissues, to the existence of the drug in solution as a colloidal electrolyte, or to a modification in the inherent toxicity of 9-aminoacridine. 9-aminoacridinepenicillin produces local tissue necrosis with œdema and inflammation when administered intramuscularly, subcutaneously or to scarified skin. G. R. K.

Ammonium Nitrate for Premenstrual Intoxication. E. J. Stieglitz and S. T. Kimble. (*Amer. J. med. Sci.*, 1949, **218,** 616.) Many authors have published suggestions as to the ætiology of a premenstrual syndrome characterised by one or more of the following symptoms: depression, irritability, abdominal fullness, headache, nausea, backache or mammary hyperæsthesia. While disagreement exists as to the specific physiological mechanism involved, excessive production of ovarian steroids or increased sensitivity to them is considered to be the main factor and on this basis the authors administered as a diuretic enteric coated capsules of ammonium nitrate, each containing 1 g., 1 capsule being given 3 times a day with a liberal fluid intake. The nitrate was given because of its greater diuretic efficiency than the chloride and to avoid increasing the chloride content of the tissues. Treatment was given for the 10 days preceding each menstrual period. Dramatic improvement occurred in 61 out of 67 patients treated, the maximum benefit frequently being observed after 2 or 3 months. H. T. B.

Artane: A New Antispasmodic. M. Canelis, F. J. Farnell and T. H. McGavack. (*Amer. J. med. Sci.*, 1949, **218,** 655.) Artane is 3-(1-piperidyl)-1-phenyl-1-cyclohexyl-1-propanol hydrochloride and belongs to a new series of antispasmodic compounds. Its pharmacological properties in animals suggested trial in parkinsonism. On isolated intestinal loops its relaxant effect is 20 times that of trasentin and half that of atropine, but its excitant action on the central nervous system is much less than that of atropine. It has a feeble antihistamine activity. Twenty-three patients with

parkinsonism were treated with the new compound for periods varying from 10 to 46 weeks, their age varying from 30 to 78 years. All but two received 2 mg. of artane three times daily; the two received respectively 2·5 mg. and 3 mg. Within 3 days 21 of the 23 were much improved, and those previously taking atropine or hyoscine were as well as on maximum tolerated doses of the alkaloids. Within 2 weeks there was improvement over all previous treatments but thereafter no increased benefit resulted even with increased dosage, although the improvement was maintained indefinitely, or at least for the 10 months of the trial without signs of tolerance appearing. Accompanying the objective improvement was an increase in the sense of well-being in 19 patients. Three were not improved in emotional status and one became much confused. Replacement of the drug by a placebo caused dramatic relapse within 3 days but the former condition was restored on resuming the drug. The effective safe daily dose is between 6 and 15 mg.; larger doses tend to produce blurring of vision. H. T. B.

***l*-Ephedrine Salt of Penicillin G (Tersavin).** G. Gunberg, L. O. Randall and R. J. Schnitzer. (*J. Pharmacol.*, 1949, **95,** 336.) Tersavin, which has the empirical formula, $C_{16}H_{18}O_4N_2S$, $C_{10}H_{15}ON$, is a white crystalline powder with a melting-range of 135° to 137°C. (decomp.). The specific optical rotation $[\alpha]_D^{26°C.}$ in a 2·4 per cent. aqueous solution is +190°. It is highly soluble in water (60 per cent. at room temperature) and 1·5 per cent. aqueous solution has a *p*H of 6·2 The calculated unitage per mg. is 1187 units (this was confirmed by assay), and the potency of 1·4 mg. therefore corresponds to that of 1 mg. of crystalline sodium penicillin G. Tersavin is only slightly more toxic than ephedrine hydrochloride in mice and rats but about equally toxic in rabbits; its toxicity may be ascribed therefore to its ephedrine content. The vasoconstrictor potencies of tersavin and ephedrine hydrochloride are identical on a molecular basis and each compound produces tachyplaxis to the other; the bronchodilator effects of the two compounds were indistinguishable. Tersavin exhibited the characteristic antibacterial properties of penicillin G. There does not seem to be either an antagonism or a synergism between the two components. Two modifications might however be attributed to the presence of the vasoconstrictor. One is an influence on the blood level which is prolonged and higher with tersavin than with penicillin G; the other is an experimental local streptococcal infection. The authors conclude that tersavin offers the advantages of both the antibacterial properties of penicillin G and the vasoconstrictor effect of ephedrine by the administration of a single chemically defined compound. S. L. W.

Heparin Preparations, Sheep Plasma Method for the Bioassay of. O. F. Swoap and M. H. Kuizenga. (*J. Amer. pharm. Ass.*, 1949, **38,** 563.) The potency of an unknown heparin is determined by a simultaneous comparison of the minimum amounts of standard and unknown heparin which when contained in a volume of 0·3 ml. will keep 1 ml. of recalcified sheep plasma more than 50 per cent. fluid for 1 hour at room temperature. The sheep plasma is obtained by drawing blood from the animal into a sterile vessel containing 50 ml. of a 4 per cent. sodium citrate solution per 500 ml. of blood. The blood and sodium citrate solution is mixed by gentle agitation and by centrifuging. Such plasma can be stored at −20° C. for as long as one year without deterioration. The amount of calcium chloride necessary for recalcification need be determined only once for each lot of frozen plasma. One ml. samples of the strained plasma in each of 4 test tubes are added to 0·05, 0·10, 0·15, and 0·20 ml. quantities of a 2 per cent. solution of calcium chloride. The optimum amount of calcium chloride

solution is that which, in the shortest time, produces a firm clot which cannot be removed from the tube by inverting it. The coagulation tubes for the test are prepared by measuring varying amounts of the heparin dilution (in 0·9 per cent. sodium chloride solution) and the standard into a series of test tubes, the final volume being adjusted in each case to 0·3 ml. To each of these tubes is added 1·0 ml. of the strained sheep plasma and then, noting the time, the optimum amount of calcium chloride solution as determined above. Each tube is immediately stoppered and the contents are mixed by inverting the tube three times. The tubes thus prepared are kept at room temperature, and exactly 1 hour after recalcification are examined for coagulation. Any tube showing partial clotting is shaken sufficiently to estimate whether the clot is more, or less, than 50 per cent. of the total contents of the tube. If the clot is greater than 50 per cent. of the total volume, the tube is regarded as clotted, the end-point being taken as the minimum amount of heparin necessary to maintain the tube in a condition of less than 50 per cent. clot. It is considered that 20 to 25 samples may be assayed daily by this method; calculation of results is rapid and simple and the dosage response curve is steeper than that found with other methods or with other plasmas, while the end-point is easy to determine.

R. E. S.

Hetrazan (Banocide) in Treatment of Loiasis. F. Murgatroyd and A. W. Woodruff. (*Lancet*, 1949, **257**, 147.) Seventeen European patients were treated with hetrazan (1-diethylcarbamyl-4-methylpiperazine hydrogen dicitrate). At first 2 mg./kg. of bodyweight per day was given, but later this was increased to 4 mg./kg. for some patients and then to 6 mg./kg. This daily dose was given orally in three equal separate portions after breakfast, luncheon and supper. The total amounts of drug given varied from 1·2 to 10·5 g. After the first day's treatment 6 patients complained of itching and 3 of these had rashes. The rashes were transitory, lasting 48 hours, but the irritation persisted for 3 to 4 days and in one case 16 days. It was alleviated by giving 50 mg./kg. of benadryl or 100 mg./kg. of anthisan twice daily. In three patents cutaneous thickenings or nodules appeared which disappeared in 2 or 3 days. Three others showed cutaneous serpiginous linear swellings, the appearance of which suggested reactions around adult worms and in two patients specimens of dead adult *L. loa* were extracted. The embryos of *L. loa* rapidly disappeared from the peripheral blood. Embryos of *Acanthocheilonema perstans* in the blood of one patient were insensitive to the drug. During treatment some of the patients complained of nausea and headache. With one exception all patients remained entirely free from symptoms, 9 of them for 6 months or more after treatment. One showed a recurrence of calobar swelling possibly due to reinfection. Two healthy persons who received 6 mg./kg. of bodyweight of the drug daily for 14 days, showed none of the side-reactions indicating that they were probably due to the action of the drug on the parasites. These two people also showed no alterations in the total and differential leucocyte counts. A. D. O.

Hetrazan, Action on Filariasis and Onchocerciasis. F. Hawking and W. Laurie. (*Lancet*, 1949, **257**, 146.) Hetrazan (1-diethylcarbanyl-4-methylpiperazine hydrogen dicitrate) apparently acts like an opsonin by modifying the micro-filariæ so that they are destroyed in the reticulo-endothelial system. A short and simple dose scheme was used, usually consisting of one dose per day for 7 days. The maximum tolerated dose was about 20 mg./kg. of body-weight per day. Doses as low as 1 mg./kg. twice daily or 0·5 mg./kg. 3 times daily, removed almost all the micro-filariæ from

the blood but doses of 0·2 mg./kg. twice daily did not completely clear the bloodstream. With larger doses, microfilariæ disappeared from the blood in 3 days or less, but in a few cases persisted for 3 months. Single doses of 20 mg./kg. removed all or most of the micro-filariæ from the blood in most of the patients treated; the possibility of mass treatment by such short high-dosage courses is being investigated. It was found that if the microfilariæ were not in contact with large phagocytes, e.g. in a serious cavity, they are apparently not destroyed by hetrazan. Follow-up of the patients after treatment showed that hetrazan has a marked action on the microfilariæ of *Onchocerca volvulus* but this is less rapid and less permanent than the action on the micro-filariæ of *Wuchereria bancrofti*. The effect on adult worms, however, was less sure. Hetrazan was well tolerated by patients with filariasis due to *W. bancrofti* but caused violent allergic reactions in patients infected with *O. volvulus*. A. D. O.

Mercury, Absorption of, Through the Skin. A. Bass and E. D. Robinson. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 659.) The penetration of mercury through the skin of rabbits was compared by determination of the concentration of mercury in the kidney tissue. 10 mg. of ointment was inuncted over an area of 30 sq. inches of closely clipped skin, the area covered with a rubber sheet and left for 24 hours after which the animals were killed. The kidneys were removed, weighed and analysed for mercury, using the dithizone method with a photoelectric colorimeter. The following ointments were compared:—(a) boric acid, 2; anhydrous lanoline, 20; beeswax, 10; resorcinol, 4; red mercuric oxide, 5; water, 8; soft paraffin to 100, and (b) the same, but without the resorcinol. The concentration of mercury in the kidney tissue was 6 times higher in the case of the ointment containing no resorcinol. It appears that resorcinol interferes with either the penetration of the mercury compound through the skin, or its storage in the kidney. When the ointments were compared for bacteriostatic power by the Food and Drug Administration agar cup-plate method, the zone of inhibition was more than twice as wide when the ointment contained resorcinol. This may have been due to the diffusion of resorcinol into the aqueous agor medium, in which it is soluble. G. B.

Octin (Methyl-*iso*-octenylamine) in the Treatment of Vasodilating Headaches. G. A. Peters and W. W. Zeller. (*Proc. Mayo Clin.*, 1949, **24**, 565.) Octin relieves the pain of vasodilating headaches by acting as a vasoconstricting agent. It may be administered by either the intramuscular or the oral route, but not intravenously. The best results may be expected in the treatment of the typical migraine attack and more variable results may be obtained in the treatment of headache due to nervous tension. Of 59 headaches in 26 patients, 48 were partially or completely relieved by intramuscular injection of 0·5 to 1 ml. of octin hydrochloride in a concentration of 100 mg./ml. of solution (repeated if necessary after 30 minutes). With oral administration, using octin mucate in a dose of 2 gr. every 30 minutes until relief was obtained, only 15 of 26 headaches in 16 patients were relieved. None of the patients in this study experienced serious side-reactions from the use of the drug. It is a valuable adjunct of particular value in those patients who may have abused the use of the ergotamine preparations and in those for whom the use of ergotamine may be contraindicated. S. L. W.

Orthoxine. Evaluation for Bronchospasm. E. Bresnick, J. F. Beakey, L. Levinson and M. S. Segal. (*J. clin. Invest.*, 1949, **28**, 1182.) Orthoxine is *o*-methoxy-β-phenyl*iso*propylmethylamine hydrochloride and differs from ephedrine in the presence of an *o*-methoxy group and in the

absence of a β-hydroxy group. Clinical trial, using doses of 100 to 200 mg. 3 or 4 times daily, showed that it is similar to ephedrine as an anti-asthmatic. Side effects were numerous and included anorexia, nausea, light-headedness and drowsiness. Administered as a syrup the drug gave clinical relief from troublesome cough in some cases. H. T. B.

Propylthiouracil for Exophthalmic Goitre in Children. A. S. Jackson and H. B. Haley. (*Amer. J. med. Sci.*, 1949, **218**, 493.) Exophthalmic goitre in children is accompanied by various signs and symptoms additional to those occurring with the same disease in adults, including gastro-intestinal symptoms, high susceptibility to upper respiratory infections, sleep disturbances, epistaxis and marked personality changes. In 8 cases ranging in age from 8 to 16 years it was found that propylthiouracil in doses of 0·025 or 0·05 mg. twice or three times a day was most useful for pre-operative preparation. The individual response was uncertain but in general the toxic manifestations of the disease were relieved. In only one case was the progress of the disease apparently permanently arrested so that operation is still the method of choice in young patients. H. T. B.

Protamine Zinc Insulin, Precision of the U.S.P. Assay for. C. I. Bliss (*J. Amer. pharm. Ass.*, 1949, **38**, 560.) The U.S.P. XIII assay (in which each rabbit is injected twice; once with the standard preparation and then with the "unknown" after an interval of about one week) is used in accordance with the Food, Drug, and Cosmetic Act, to assay each new batch of protamine zinc insulin against a specially prepared standard preparation in two or more rabbit cross-over tests in two different laboratories; it must pass both tests before it can be released. At the request of the U.S.P. Insulin Advisory Committee, a statistical analysis of the results obtained in parallel assays in different laboratories of 24 preparations of protamine zinc insulin has been made to test the precision of the U.S.P. XIII procedure. Both the mean difference over 6 periodic bleedings and the trend of these differences with time proved significantly greater than the residual or random sampling error in the two series of tests. The mean differences from two independent assays of the same preparation were not correlated. Within the official tolerance limits therefore the mean difference could be attributed to minor variations in laboratory procedure and not to actual differences in potency. The trend with time, however, was correlated significantly in parallel assays from different laboratories and therefore partly represents a real characteristic of a given preparation of protamine zinc insulin. It is considered that the assay for protamine zinc insulin is capable of distinguishing finer differences than it is called upon to make in actual practice. R. E. S.

Rutin, Effect of on the Biological Potency of Vitamin C. E. W Crampton and L. E. Lloyd. (*Science*, 1949, **110**, 18.) This note is a preliminary summary of an experiment to ascertain the biological value of vitamin C in two natural sources of the vitamin, and to test the effect of added rutin on the apparent vitamin C potency of these substances, as well as on synthetic ascorbic acid. Of three equal groups of guinea-pigs the first received crystalline ascorbic acid, the second canned orange-grape fruit juice, and the third dehydrated potato. Each assay material was given at four different levels of vitamin C content, namely 0·5, 0·79, 1·26 and 2·00 mg. of ascorbic acid daily. The amounts of orange-grape fruit juice and dehydrated potato to be fed were based on chemical analyses of samples of the two materials. Half the animals on each assay material were given orally 100 mg. per day of crystalline rutin. Using the odontoblast method of assay it was found that the rutin treatment gave significantly higher figures on the response-

dose curve at the 0·5, 0·79, and 1·26 mg. levels of vitamin C intake for both the synthetic ascorbic acid and orange-grape fruit juice assays. At the lower levels of vitamin C intake it appears that rutin either makes more available or delays *in vivo* destruction of ascorbic acid in the original source. A further possibility is that rutin forms the base for synthesis by the animals of additional ascorbic acid. The authors tentatively conclude that rutin present in some natural vitamin C sources may be the factor responsible for enhancing the apparent biological potency of vitamin C. S. L. W.

Suramin and Antrycide, Action on Enzymes. B. W. Town, E. D. Wills and A. Wormall. (*Nature*, 1949, **164**, 233.) Hexokinase, urease, yeast decarboxylase, succinic dehydrogenase and trypsin are all strongly inhibited by concentrations of suramin similar to those maintained in the blood plasma of man and other animals for at least several hours after the intravenous injection of a normal dose (1 to 2 g.) of the drug. No evidence is yet available to show whether or not enzyme inhibition plays any part in the action of suramin on trypanosomes, but the possibility of such an effect cannot be excluded. Antrycide, on the other hand, in concentrations as high as M/500, has no inhibitory effect on urease, succinic dehydrogenase and the system of yeast juice enzymes required for the fermentation of glucose, and the hydrolysis of casein by trypsin is not significantly inhibited by M/2400 antrycide. Some of these enzymes are actually mildly stimulated by high concentrations of antrycide. These results do not show that suramin and antrycide exert their trypanocidal actions by different mechanisms, but they would furnish proof of such a difference if it should be eventually established that the inhibition of glucose-metabolising and/or proteolytic enzymes is an essential feature of the trypanocidal action of suramin. S. L. W.

Sympathin. G. B. West. (*Nature*, 1949, **163**, 721.) Recent studies on the substance or substances liberated at the nerve endings when sympathetic nerve fibres are stimulated show that in some cases this is adrenaline while in others the indications are that it is noradrenaline. In cats, under chloralose and cocaine, after double vagotomy, the author was able to demonstrate that in at least two cases the nature of sympathin (as shown by effects produced in different parts of the animal) depends on the length of time the nerve has been sectioned before being stimulated. It appears that sectioning of some sympathetic nerves may be linked with the inhibition of methylation of the primary amine, so that the stimulation produces more noradrenaline and less adrenaline. In these cases the normal adrenergic mediator may be a mixture of varying proportions of noradrenaline and adrenaline. Blood analyses at various stages may serve to identify the substance or substances liberated. S. L. W.

BACTERIOLOGY AND CLINICAL TESTS

Essential Oils, A Method for Comparing the Antibacterial Activity of. M. P. Schroeder and A. M. Messing. (*Bull. nat. Form. Comm.*, 1949, **17**, 213.) The antibacterial activity of certain water-insoluble substances against 14 different micro-organisms was evaluated by the following method. A 12·5 mm. filter-paper disc was placed at the centre of an agar plate, previously seeded with the test organism, 0·1 ml. of a dilution of the substance in alcohol was placed on the disc, and the zone of inhibition was measured after incubation for 24 and 72 hours. The production of a significantly large zone of inhibition after 24 hours, maintained after 72 hours' incubation was the criterion used in assessing bacteriostasis. The test was repeated for several

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