ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Carvone, Determination of. D. H. E. Tattje. (*Pharm. Weekbl.*, 1956, **91**, 733.) This colorimetric method requires less time than the dinitrophenylhydrazine method and less material than the sulphite method: 4 ml. of a solution of carvone (at least 0·1 per cent) in aldehyde-free alcohol is mixed with 5 ml. of 2 per cent solution of ethyl 3:5-dinitrobenzoate in acetone-free methanol, followed by 1 ml. of 0·7N sodium hydroxide, and after 3·5 minutes the colour is measured in a 1 cm. cell at 537·5 mμ, using a blank of ethanol, reagent and sodium hydroxide. A fresh blank should be made for each estimation, and the reaction should be carried out at 20°. Between the concentrations of 0·05 and 0·4 per cent the calibration curve is a straight line—E (1 per cent, 1 cm.) = 2·55. The method is suitable for oil of caraway. G. M.

Chloramphenicol in Water-containing Preparations, Determination of the Hydrolytic Decomposition of. A. Brunzell. (*Svensk farm. Tidskr.*, 1957, **6**, 129.) Chloramphenicol yields on hydrolysis 1:3-dihydroxy-1-(*p*-nitrophenyl)propyl-2-amine, which may be detected or determined quantitatively by means of a colour reaction with sodium naphthoquinone sulphonate. To 1 to 5 ml. of a neutral solution equivalent to 0·2 to 2 mg. of hydrolysed chloramphenicol is added 10 ml. of buffer solution (Clark and Lubs), pH 8, and 5 ml. of naphthoquinone sulphonate solution. After 15 minutes, 2 ml. of 0·5N acetic acid and 2 ml. of ascorbic acid solution (10 per cent) are added to remove the excess of reagent, and the solution diluted to 25 ml. The light absorption is determined at 440 mμ against a reagent blank, 10 minutes after the addition of the acetic acid. The result is calculated from the datum that E(1 per cent, 1 cm.) = 162 for the amino alcohol, equivalent to 125 for decomposed chloramphenicol. The reagent is prepared freshly by dissolving 1 g. of sodium 1:2-naphthoquinone-4-sulphonate in 50 ml. of water and diluting to 100 ml. with ethanol. Propylene glycol, glycerol, lactose and undecomposed chloramphenicol do not interfere in the determination of the decomposition product. Solutions of chloramphenicol in water were found to lose about half their activity in 290 days at 20 to 22°. When buffered to pH 7·4 with borax and boric acid (as in oculoguttae chloramphenicoli Ph. Dan.) about 14 per cent of the activity was lost under these conditions. Propylene glycol solutions were relatively stable unless they contained much water. Aqueous solutions decomposed to the extent of about 3 per cent on heating at 100° for 15 minutes. G. B.

Iodine and Iodides, Control of Purity of. A. Berka and J. Zýka. (*Československ. Farm.*, 1957, **6**, 110.) Iodides and iodine are determined by titration against 0·01M *N*-bromosuccinimide; in solutions containing 15 per cent of HCl, the reagent oxidises these to iodine chloride. For the determination of iodine, 0·1 g. is dissolved in about 30 ml. of ethanol, 1 g. of NaCl is added and the solution is made up to 100 ml. Sufficient hydrochloric acid is added to a 10 ml. aliquot, which is then titrated against the reagent, the end point being determined

potentiometrically. Iodine chloride, sometimes present as an impurity in iodine, does not interfere. For iodides, a 5 to 10 mg. sample is dissolved in water, hydrochloric acid is added and the solution is titrated in the same way. Bromides and chlorides do not interfere. The method can be used for the determination of iodides in ointments. The reagent can also be used for the volumetric determination of arsenites and hydrazine and its derivatives, including isoniazid. E. H.

Methadone Hydrochloride, Ephedrine Hydrochloride and Hyoscine Hydrobromide, Separation of, by Paper Chromatography. F. Abaffy and S. Kveder. (*Acta pharm. Jug.*, 1956, **6**, 209.) Whatman No. 1 filter paper was used, with a mixture of butanol 10, glacial acetic acid 1 and water 4 as developing solvent. Ninhydrin was used for the detection of ephedrine and the modified Dragendorff reagent of Munier and Macheboeuf for the detection of methadone and hyoscine on the same paper. The R_F values obtained by the circular, ascending and descending techniques are given. G. B.

Morphine, Extraction of, from Poppy Capsules and its Recovery by Ion Exchange. C. L. Mehltretter and F. B. Weakley. (*J. Amer. pharm. Ass., Sci. Ed.*, 1957, **46**, 193.) Dried poppy capsules were extracted with 2 quantities of *iso*butanol saturated with water, containing 0·23 per cent of ammonia. The morphine was removed from the extract by passing it through a bed of cation exchange resin, from which it was readily eluted with dilute aqueous alkali, and purified. Zeo-Karb H and Duolite C–10, sulphonated cation exchange resins containing few cross-links were employed, and Zeo-Karb H was found to have 55 per cent greater adsorptive capacity for morphine than Duolite C–10. The *iso*butanol solvent was recycled after the addition of a suitable quantity of ammonia. G. B.

Morphine Hydrochloride and the Main Non-phenolic Opium Alkaloids, Use of Ion Exchangers with. A. Jindra and J. Böswart. (*Československ. Farm.*, 1957, **6**, 77.) Morphine in both methanolic and aqueous solution is quantitatively retained by the anion exchangers Amberlite IRA-400, Lewatit MN and Wofatit L150 or L160, but it could be quantitatively eluted only from the first two; 0·5N acetic acid or N hydrochloric acid is used as eluent. On these resins morphine can be separated from codeine, narcotine, thebaine, papaverine and narceine. Of a number of cation exchangers tested, only Wofatit F proved to be suitable for the quantitative absorption and elution of morphine; a 3 to 5 per cent solution of ammonia is used as eluent. Strong alkalias attack the resin and with solutions of salts of strong bases the morphine is only partially recovered. E. H.

Morphine, Identification of, in the Presence of Chlorpromazine in the Urine. B. Košir and J. Košir. (*Acta pharm. Jug.*, 1956, **6**, 181.) A method is described in which morphine and chlorpromazine are extracted from urine, concentrated by evaporation and rendered slightly alkaline, by shaking with a 4:1 mixture of chloroform and ethanol. Chlorpromazine is removed by shaking the hydrochlorides of the extracted bases in hydrochloric acid solution with chloroform, in which the chlorpromazine salt is soluble, but the morphine salt is not. The presence of morphine in the extract can then be confirmed by

chromatographic analysis. The colour tests given below can be used for distinguishing between morphine and chlorpromazine.

Reagent	Morphine	Chlorpromazine
Ferric chloride	purple	red
Hydrochloric acid (concentrated)	no colour	red
Potassium ferricyanide-ferric chloride reagent (Ph.Jug.II)..	blue	purple

G. B.

Neovitamin A_1, Separation of, from All-*Trans* Vitamin A_1 by Chromatography. B. Barnholdt. (*Nature, Lond.*, 1956, **178**, 1401.) Good separation of the isomeric neovitamin A_1 and all-*trans* vitamin A_1 is obtained by chromatography on alumina prepared from fine-grained aluminium hydroxide. 1·5 mg. of a mixture was chromatographed on a column of alumina (75 cm. × 9 cm.), prepared from aluminium hydroxide, analytical grade (Baker's analysed), with a grain size distribution characterised in that 100 per cent passes sieve DIN 80, while 97 per cent passes sieve DIN 100 and 70 per cent passes sieve DIN 150 E. Aluminium hydroxide was heated to 800° for seven hours, cooled in a vacuum desiccator, and mixed with distilled water (6·5 g./100 g.) before use. Elution was with ether and light petroleum, the content of the former being gradually increased from 2–25 per cent by volume. The eluate was collected in 4 ml. fractions, the vitamin content of each determined by measurement of the ultra-violet absorption at 325 mμ. Complete separation was achieved. J. B. S.

Potassium Precipitants as Alkaloidal Reagents. L. Rosenthaler and F. Lüdy-Tenger. (*Pharm. Acta Helvet.*, 1957, **32**, 35.) By analogy of ammonium salts and organic bases with potassium, the authors have investigated the effect of nine of the best known potassium precipitants on 37 plant alkaloids and 8 synthetic bases, 1 per cent solutions being used. In 47·7 per cent of the 400 reactions tried there was precipitation, among which 28 per cent were crystalline. Some of these were characteristic enough to be used as auxiliary microchemical tests for identification purposes. For quantitative purposes however, only one reagent, viz., potassium tetraphenylboron appeared to be suitable. Some sodium precipitants were also tried, but rarely produced precipitate. D. B. C.

Protoveratrines A and B, Separation of, from Associated Alkaloids. J. Levine and H. Fischbach. (*J. Amer. pharm. Ass., Sci. Ed.*, 1957, **46**, 191.) Chromatography on paper moistened with buffer solution, pH 3·5 was used to separate the alkaloids from commercial protoveratrines. A sharp separation of germitetrine, protoveratrine A and protoveratrine B was obtained using a mixture of ethylene chloride 15 ml., ethoxyethyl acetate 10 ml. and pyridine 1 ml. as the developing solvent, the R_F values of the alkaloids being 0·5, 0·7 and 0·25. The position of the alkaloids on the paper was detected with the aid of bromophenol blue. Column chromatography on Celite columns containing a mixture of McIlvaine's buffer solution (pH 3·0) and ethylene glycol was found suitable for the separation of protoveratrine A, protoveratrine B and other alkaloids by the use of a series of solvents of increasing solvent power. A suitable series consisted of (1) carbon tetrachloride 3, ethylene chloride 2, (2) ethylene chloride, (3) chloroform 2, ethylene chloride 1 and (4) chloroform. In this series protoveratrine was first eluted, followed by a fraction more hydrophilic than protoveratrine A and less than protoveratrine B. Protoveratrine B was obtained as a third fraction, and the final chloroform eluate contained alkaloids more hydrophilic than protoveratrine B. G. B.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

***Digitalis purpurea*, Effect of Fermentation on Glycosides of.** D. H. E. Tattje. (*Pharm. Weekbl.*, 1956, **91**, 937.) Digitalis leaves were examined after drying in the field, on screens and after fermentation in a heap at about 30°. In the case of the fermented samples the total aglycone content was significantly increased, and the gitoxigenin content very significantly. This phenomenon is the more striking since the content of aglycones combined with digitoxose is not increased. The explanation of this is not clear, but possibly during the fermentation a glycoside is formed which has no digitoxose in the molecule. The enzyme digipurpurase was not destroyed in the fermentation, and also the primary glycosides were not converted to secondary glycosides to any greater extent than with the other samples. G. M.

***Digitalis purpurea*, Inheritance of Glycosidal Composition in.** F. H. L. van Os and J. H. Stehouwer. (*Pharm. Weekbl.*, 1956, **91**, 942.) Strains of *Digitalis purpurea*, which were originally examined for botanical differences, were found to vary in the composition of their glycosides. These chemical characters can be fixed in the strains. Three types are described, with their characters and the composition of their glycosides. Distinction is made between the digipurpurin type, a stropeside type and a digitoxin type. This discovery is important since it shows that a determination of the total glycoside content gives no indication of physiological action. Most cultures consist of a mixture of strains, and the biological standard sample is also a mixture. In the case of samples from selected cultures or from certain natural regions it is necessary to establish this relation before it is possible to standardise them chemically. G. M.

Digitoxin, Estimation of, by Paper Chromatography. E. Fujiwara. (*Acta med. biol., Japan*, 1956, **4**, 137.) The apparatus consisted of a wire frame inserted through the lid of an hermetically sealed glass cylinder. On this frame two sheets of Whatman No. 1 paper (8 × 34 cm.) were hung; to each sheet four spots containing digitoxin in methanol were transferred. The sheets were suspended above the solvent at the bottom of the cylinder for 24 hours: the frame was then pushed down till the lower ends of the sheets were immersed in the solvent. By this means eight tests could be carried out under identical conditions. The solvent was a chloroform-water-methanol (10:10:2) mixture and the temperature used was 17°. After development the papers were dried, sprayed with a fresh mixture of 3 per cent aqueous chloramine-T and 25 per cent ethanolic trichloroacetic acid and heated at 120° for ten minutes. The digitoxin was identified by its yellow fluorescence in ultra-violet light. A "zone of flow" rather than a discrete spot was formed, and the author shows that the lengths of these zones, relative to the solvent front distance, are proportional to the concentrations of digitoxin. The results with solutions of pure digitoxin only are described in this paper. J. W. F.

Gitoside—A New Digitalis Glycoside. J. E. Murphy. (*J. Amer. pharm. Ass., Sci. Ed.*, 1957, **46**, 170.) Gitoside was isolated from residues obtained in the processing of digoxin from *Digitalis lanata*, but has also been detected in other *Digitalis* species, including *D. purpurea*. The residues were treated with acetone, in which gitoside is relatively soluble, to remove most of the digoxin

The concentrate was placed on a Celite column containing formamide and water as the stationary phase and developed with chloroform:benzene (3:1). Further purification was achieved using formamide:water (2:1) as the stationary phase and ethylene chloride:benzene:acetone (10:10:2) as the developing solvent, and finally repeating with chloroform containing 10 per cent of heptane as the mobile phase. Hydrolysis of gitoside yielded one molecule of gitoxigenin and one molecule of digitoxose. It was noted that on hydrolysing glycosides containing digitoxose, part of the digitoxose formed another compound which still gave a reaction for 2-desoxy sugars when the paper chromatograms were sprayed with trichloroacetic acid in ethanol. G. B.

Flavonol Glycosides, Occurrence in Hips. D. Öiseth and A. Nordal. (*Pharm. Acta Helvet.*, 1957, **32**, 109.) Flavonol glycosides were isolated from the fruits of wild rose (mainly *R. canina*) by adsorption on Amberlite IRC–50, followed by separation by paper chromatography. Two of the glycosides were identified as *iso*quercitrin and campherol-3-glucoside respectively. The third glycoside gave on hydrolysis quercitron and glucose, and the fourth gave campherol, glucose and an unidentified component. The aglycones quercetin and campherol were found to be present also in leaves and buds of *R. canina*, and in the fruits of *R. afzeliana*, *R. cinnamomea*, *R. moyesi*, *R. rugosa* and *R. villosa*. G. M.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Bracken Poisoning of Cattle—Nature of the Poison. A. J. Thomas, I. A. Evans and W. C. Evans. (*Biochem. J.*, 1957, **65**, 5P.) Finely powdered, low temperature-dried bracken was exhaustively extracted with (*a*) acetone, (*b*) ether and (*c*) water. The extracts were concentrated under reduced pressure, and the dry residues fed to three yearling Welsh Black cattle, at the rate of 5 lb./day, mixed with bran; the rest of the diet was hay. Acetone, ether and water extracts, each equivalent to 5 lb. milled bracken, were given by rumen fistula to another three animals daily. No poisoning occurred with the ether and acetone extracts. Cattle receiving the residues developed clinical bracken poisoning within a month. Cattle receiving water extracts and residues gave equivocal results. The water extract led to a greater fluctuation in leucocytes and platelets than is usual with normal animals. J. B. S.

Fluorocarbon, Purification of Poliovirus with. L. A. Manson, E. L. Rothstein and G. W. Rake. (*Science*, 1957, **125**, 546.) Poliomyelitis virus (type II, strain MEF-1) suspension was mixed with an equal volume of a solution of 1:2-difluorotetrachloroethane in *n*-heptane (s.g. 1·30), cooled to 4° and blended in a Servall Omnimixer at 14,500 r.p.m. After standing for 10 minutes to allow any aerosols to settle, the homogenate was centrifuged for 10 minutes at 1000 *g*, when three layers were obtained, (a) upper aqueous layer containing the virus, (b) lower fluorocarbon mixture, (*c*) protein accumulated at the interface. Repetition of this treatment permits the removal of up to 90 per cent of the protein. Homogenisation for much shorter periods (1 to 2 minutes) was found to be almost as effective as the longer blending time; increase in the proportion of the fluorocarbon mixture also increased the efficiency of the method. Assay of the residual infectious virus concentrations after successive fluorocarbon treatments shows that there is no significant decrease of titre after three such treatments. The purification procedure has been applied to

other viral suspensions, such as coliphage T5; poliovirus types 1 and 3; feline pneumonitis virus, and certain members of the adenovirus group. J. B. S.

BIOCHEMICAL ANALYSIS

Carbohydrates, Determination of, in Biological Material using the Thymol-Sulphuric Acid Reaction. M. R. Shetlar and Y. F. Masters. (*Analyt. Chem.*, 1957, **29**, 402.) An account is given of the different absorption curves given by nearly all sugars when they react with thymol in the presence of strong sulphuric acid. It was shown that while glycosidic linkages did not affect the reaction either qualitatively or quantitatively, the presence of protein had a slight effect on the absorption curves apparently due to the reaction of the sulphuric acid with the protein itself. The reaction could be used, however, to estimate carbohydrate bound to protein in serum, and was found to correlate closely with the method using tryptophan of Shetlar, Foster and Everett (*Proc. Soc. exp. Biol. N.Y.*, 1948, **67**, 125). Using a Beckman spectrophotometer, the complex was found to obey the Beer-Lambert law for samples between 10 and 100 μg. under the conditions described. For serum protein samples, 0·2 ml. of a one in five dilution sufficed. No limits of error are stated. D. B. C.

Iodide, Determination of, in Urine. H. L. Helwig, W. A. Reilly and J. N. Castle. (*J. Lab. clin. Med.*, 1957, **49**, 490.) A method is described for concentrating and analysing urinary iodide. To concentrate the urinary iodide add to 200 ml. of urine carrier free ^{131}I that has been reduced with sodium sulphite to give a suitable counting rate of 500 counts per ml. Accurately determine the actual counts/sec./ml. of urine. Acidify the urine to pH 5 with sulphuric acid and heat to 95°, filter to remove protein, cool and while stirring add 0·5 ml. of concentrated sulphuric acid until the urine is 0·1N with acid. Pour the cooled urine through a AgCl-cellulose column prepared in 1 cm. bore glass tubing 10 cm. long. [The column is prepared by adding in succession from the tip upwards a 1 cm. layer of glass wool, a 0·5 cm. layer of cellulose powder suspended in 0·1N sulphuric acid, a layer of AgCl-cellulose powder and a 1 cm. layer of glass wool. The AgCl-cellulose powder is prepared by adding 0·1 g. of silver nitrate to 1 g. of Whatman cellulose powder in 100 ml. distilled water. The suspension is acidified with sulphuric acid and a slight excess of sodium chloride is added while stirring. 33 ml. of the suspension is used in preparing the column and the column is washed with 100 ml. of 0·1N sulphuric acid to remove excess Cl and any free AgCl colloid.] Discard the effluent, wash the column with 200 ml. of 0·1N sulphuric acid and discard the washings. The iodide is removed as iodate from the column by adding 15 ml. of bromine saturated 0·05N sulphuric acid. To determine the urinary iodide heat the eluate from the column containing $Br_2{}^{127}_{131}I\ O_3$ in a water bath for one hour to remove bromine. Continue heating, add one drop of saturated potassium permanganate solution to oxidize any further unknown reducing substances present and after ten minutes add 1 to 2 drops of 10 per cent sodium nitrite to destroy oxides of manganese. After another ten minutes heating add solid urea to destroy traces of nitrous acid. Maintain the volume during boiling by the addition of water. Adjust the pH to 2·8 with sodium bicarbonate and add 1 ml. of citrate-phosphate buffer of pH 2·8 (16·83 ml. of 0·1N citric acid plus 3·17 ml. of 0·2M disodium phosphate). Place some of the solution in the cuvette of a spectrophotometer, adjust to zero optical density at 352 mμ. Add a drop of 50 per cent aqueous potassium iodide and measure the optical density of the iodine released. Determine the concentration of stable iodine from a standard curve relating optical

density to concentration of iodine as iodate. Pipette an aliquot of the reaction mixture in the cuvette and measure its radioactivity.

$$\text{Urinary I} - (\mu\text{g./ml.}) = \frac{\mu\text{g. I/ml. in cuvette}}{\frac{^{131}\text{I c./s./ml. in cuvette}}{^{131}\text{I c./s./ml in urine}}}$$

G. F. S.

Mercury, Determination of, in Urine. V. L. Miller and F. Swanberg Jr. (*Analyt. Chem.*, 1957, **29**, 391.) The method described is rapid and specific, and consists of catalytically oxidising the sample with hydrogen peroxide, reaction of the Hg^{++} with excess ditolyl mercury according to the equation: $Hg^{++} + R_2Hg = 2RHg^+$ (R = tolyl), extraction of the RHg^+ with chloroform and colorimetric determination of the mercury by the dithizone reaction. As little as 1 μg. of Hg/100 ml. of sample may be determined, the error being about ± 10 per cent. The procedure may also be applied to very dilute solutions of mercury. Silver and bismuth interfere by causing a precipitate during the oxidation procedure, which must be removed by filtration. There is no interference in the presence of 1000 μg. of cobalt, nickel, copper, zinc, cadmium, lead and manganese. D. B. C.

Morphine in Blood and Tissues, Determination of. J. C. Szerb, D. P. MacLeod, F. Moya and D. H. McCurdy. (*Arch. int. Pharmacodyn.*, 1957, **109**, 99.) A method is described to detect morphine in very low concentrations. It depends on the elimination of impurities by precipitation with benzene and the adsorption of the morphine on an ion exchange resin. The morphine is then determined colorimetrically with the aid of Folin-Ciocalteu's phenol reagent. The blue colour formed depends on the presence of free phenolic hydroxyl groups and is stable for over twenty-four hours. The lower limit of sensitivity of the method is three μg. of morphine free base per ml. of blood or gram of tissue and 0·6 μg./ml. of plasma. Recovery, after addition of 3 to 5 μg. of morphine to either blood, plasma or tissue homogenates gave an average of 80 per cent. Other morphine derivatives tested and recovered in proportions similar to morphine were: levorphanol, methyldihydromorphinan, nalorphine and levallorphan. Diacetylmorphine is hydrolysed during the procedure and may be estimated by the method. Normorphine, a metabolite of morphine, could only be recovered to the extent of 30 per cent since it is largely removed in the initial zinc sulphate-barium hydroxide precipitation.

G. P.

Urinary Indoles, Separation and Characterization of. R. Rodnight. (*Biochem. J.*, 1956, **64**, 621.) Examination of human urine using the ion exchange resin Zeo-Karb 227, followed by paper chromatography and tests on guinea pig ileum has shown the presence of two indoles. There was strong evidence that these were 5-hydroxytryptamine and tryptamine. *N*-Methyl-5-hydroxytryptamine or bufotenin was not detected. The excretion of "urinary 5-hydroxytryptamine" in twelve normal adults ranged from 45 to 120 μg. 24 hours and similar values were found for "urinary tryptamine" in six subjects. Addition of extra tryptophane to the diet nearly doubled the excretion of 5-hydroxytryptamine. Using the method described the recovery of 5-hydroxytryptamine, *N*-methyl-5-hydroxytryptamine, bufotenin and tryptamine added to urine was about 70 per cent. G. F. S.

CHEMOTHERAPY

Chlorophyll, Antibacterial Activity of. S. Mowbray. (*Brit. med. J.*, 1957, **1**, 268.) The water-soluble chlorophyll preparation used in this investigation was sodium potassium copper chlorophyllin. A fresh 10 per cent solution (the maximum possible concentration) was prepared daily in sterile distilled water and dilutions of this were used throughout. The sensitivity of various organisms to chlorophyll was determined using a plate method. The highest concentration of chlorophyll incorporated in solid media was 1/100. Chlorophyll sensitivity was also determined by making bacterial counts of organisms growing in fluid media. Some bacteriostatic action was shown against certain Gram-positive organisms, including *Staph. aureus*, *Str. pyogenes*, the pneumococcus, and the pathogenic clostridia. After a period of bacteriostasis bacterial multiplication proceeded in the presence of all concentrations of chlorophyll tested. After repeated culture in the presence of chlorophyll *Staph. aureus* becomes resistant to the temporary bacteriostatic effect. The possible potentiating effect of antibiotics on the antibacterial activity of chlorophyll was also investigated, using a routine plate assay method. The activity of penicillin, oxytetracycline, and streptomycin was shown to be markedly potentiated by the presence in the medium of subinhibitory concentrations of chlorophyll. S. L. W.

Di- and Triphenylmethane Dyes, Relation between Chemical Structure and Bacteriostatic Activity of. E. Fischer. (*Arzneimitt.-Forsch.*, 1957, **7**, 192.) This communication deals with basic di- and triphenylmethane dyes from their bacteriostatic aspect only, since many of these compounds behave quite differently when their bactericidal effects are compared. For an optimum bacteriostatic effect there must be three six-membered rings and at least two amino groups. These latter should be substituted by two ethyl radicals since it was found that methyl or phenyl substitution produced marked loss of activity. It is probable too, that propyl, butyl and *iso*butyl substitution causes loss of activity, but the author questions the purity of the products. Their activity was less than that of the methyl substitution products. D. B. C.

PHARMACY

Antacid Evaluation, An Approach to. R. H. Schleif. (*J. Amer. pharm. Ass., Sci. Ed.*, 1957, **46**, 179.) An apparatus is described which facilitates the testing of antacids *in vitro*. To carry out an examination of an antacid preparation 50 ml. of simulated gastric juice (U.S. Pharmacopeia) and 50 ml. of water are placed in a beaker and warmed in a water bath to the appropriate temperature for the test (37°). The solution is stirred at a constant rate with a magnetic stirrer and the sample to be tested is released into the beaker by a timing mechanism. Throughout the experiment the reaction of the solution is recorded automatically. 50 seconds after the sample is released, the timing mechanism operates an automatic pipette which delivers simulated gastric juice at the rate of 4 ml./minute for 1 or 2 hours. It is claimed that the close control of experimental conditions which is achieved by the automatic operation of the equipment provides good reproducibility, and that the results are in agreement with that of another method involving continuous recording (that of Rossett and Flexner) which had previously been shown to yield results in agreement with those of *in vivo* methods. From the recordings the following figures are obtained: (1) speed of action, the time taken to reach pH 3 under the test conditions, (2) duration of action, the time taken to return to pH 2. G. B.

Atropine Solutions, Aged, Bioassay of. E. J. Huycke. (*J. Amer. pharm. Ass., Sci. Ed.*, 1957, **46**, 160.) Commercial solutions of atropine sulphate and tartrate for injection which had been stored for some time under warehouse conditions (protected from light) were assayed for atropine. Dilutions of these solutions and of a standard atropine solution were injected intraperitoneally into groups of mice and the dilatation of their pupils determined under standard lighting conditions, after an interval of 15 to 18 minutes. The fiducial limits were estimated to be $\pm 15{\cdot}6$ per cent ($P = 0{\cdot}95$) using suitable dilutions of the solutions and 6 mice at each dose level. Tropine in quantities likely to be present did not affect the pupil. The method of instillation into the eyes of cats was less suitable owing to the difficulty of establishing narrow fiducial limits with small numbers of cats. Determinations were carried out by instilling the solutions into human eyes. The sensitivity depended upon the colour of the iris, but this difficulty could be overcome by instilling the standard solution into one eye and the solution under investigation into the other eye of the same subject. The experiments showed that atropine solutions slowly deteriorate, the amount of decomposition being approximately 35 per cent in 5 years. G. B.

B-Complex Vitamins and Ascorbic Acid in Aqueous Solutions. A. S. Gambier and E. P. G. Rahn. (*J. Amer. pharm. Ass., Sci. Ed.*, 1957, **46**, 134). In solutions of vitamin B-complex without vitamin B_{12}, the rate of decomposition was increased by making the solutions more acid, increasing the concentration of riboflavine or increasing the quantity of air in the vials. The rate of decomposition of riboflavine was increased by making the solution more alkaline, which also decreased the stability of pantothenates. Decomposition of aneurine was accompanied by the precipitation of thiochrome, and that of riboflavine by the precipitation of chloroflavine, when the aneurine concentration was 15 mg. or more/ml. and that of riboflavine 0·5 to 1 mg./ml. B-complex solutions were not usually stable for long periods of time, but it was possible to prepare "dosage forms" of 2 to 5 ml. of solution containing in each ml. aneurine hydrochloride 6 mg., riboflavine 0·5 mg., pyridoxine hydrochloride 1·1 mg., nicotinamide 26·25 mg. and sodium pantothenate 3·75 mg., and provided that ingredients of high purity were employed they could be stored for 18 months at room temperature without the development of turbidity. The addition of ascorbic acid to B-complex solutions displaced the oxidation/reduction equilibrium, preventing the precipitation of thiochrome, while increasing the tendency for chloroflavine to precipitate. The replacement of riboflavine by riboflavine 5-phosphate resulted in preparations which remained stable without precipitation during long periods at room temperature. In solutions of vitamin B-complex containing 0·5 to 1 mg. of cyanocobalamin per ml., a correlation was established between losses of aneurine and cyanocobalamin within the limits of pH 3·3 to 6·5. The optimum pH was that for aneurine solutions, pH 3·3. The stability of cyanocobalamin appeared to be reduced by decomposition products, particularly the thiazole moiety of aneurine. A solution containing aneurine hydrochloride 60 mg., nicotinamide 55 mg., and cyanocobalamin 1 mg./ml. at pH 4·5 lost 3·6 per cent of its cyanocobalamin content on storage for 12 months at room temperature. The best formulation containing vitamins B_1, B_6 and B_{12} was prepared from aneurine hydrochloride 60 mg., pyridoxine hydrochloride 27·5 mg., and cyanocobalamin 1 mg./ml. at pH 3·3. Decomposition tests at elevated temperatures were found to be unreliable owing to the thermolabile nature of the substances under investigation. G. B.

Rubber Closures, Extraction Tests for. E. J. Morrisey Jr. and W. L. Hartop Jr. (*Drug Standards*, 1957, **25**, 1.) A number of rubber caps varying from 2 to 20 according to the surface area, is placed with 200 ml. of distilled water in a 300 ml. flask, and autoclaved together with a similar flask containing only 200 ml. of distilled water, at 121° for 30 minutes. After allowing to cool, the liquid is decanted from both flasks and rejected. The flasks and rubber caps are rinsed and a further 200 ml. of distilled water added to each flask. After autoclaving at 121° for 2 hours the flasks are allowed to stand at room temperature for 3 to 5 hours. The extract is examined, using the contents of the other flask as a control. A high turbidity indicates the excessive use of dusting agents such as zinc stearate. Titration with 0·01N iodine may reveal the presence of reducing agents such as mercaptobenzothiazole, and a change in pH indicates the presence of acid or alkaline ingredients, usually alkaline substances used in accelerating rubber cure. The test is useful for the preliminary examination of rubber caps and rejection of unsuitable batches, and for obtaining evidence of uniformity of quality in a series of batches. G. B.

Solubilising Agents in the Preparation of Stable Calcium Gluconate Solution for Parenteral Use. D. C. Chakravarty and J. W. Jones. (*Drug Standards*, 1957, **25**, 4.) The stability of a simple 10 per cent solution of calcium gluconate was compared with that of a similar solution containing a small proportion of calcium saccharate (commonly used as a stabilising agent), and a number of solutions equivalent in calcium content, approximately half the calcium being in the form of another salt. The solutions were submitted to alternate freezing and thawing and daily shaking for 55 minutes in the presence of sand, which accelerated the rate of precipitation in the plain calcium gluconate solution, and the same solution containing a small amount of calcium saccharate or ethylenediaminetetra-acetic acid. Solutions in which about half the calcium was present as lactobionate, glucoheptonate or laevulinate withstood this treatment without the formation of a precipitate. Changes in pH and the presence of carbon dioxide had no effect on the stability of any of the solutions. G. B.

PHARMACOLOGY AND THERAPEUTICS

Aerosols in Chronic Bronchitis. K. N. V. Palmer. (*Lancet*, 1957, **272**, 611.) This is the report of a clinical study to assess the value of an aerosol solution in reducing the viscosity and aiding the expectoration of sputum in chronic bronchitis. The solution under investigation was Alevaire which contains 0·125 per cent of a detergent superinone (Triton WR 1339) in a sterile aqueous solution containing 2 per cent sodium bicarbonate and 5 per cent glycerol. As a control, a solution the same as Alevaire was employed except that it did not contain the detergent. Twenty-five patients with chronic bronchitis were studied over a period of 3 consecutive weeks; (1) a week with no treatment; (2) a week of daily inhalations of Alevaire; (3) a week of daily inhalations of the control solution. The solutions were given for an hour thrice daily, being nebulised at the rate of about 15 ml. an hour; the nebulisers were attached to a suitable air-compressor or oxygen supply. The results showed that aerosols of Alevaire and of the control solution were equally effective in bringing about considerable symptomatic improvement, the main effect being the greater ease of expectoration. A few patients showed objective improvement and changes in the viscosity of the sputum with either aerosol. A further

experiment with 2 patients, using on consecutive days Alevaire, the control solution, normal saline solution, or water, showed that the reduction in the viscosity of the sputum was about the same with all four aerosols. It would appear that the effect is due to the hydration of the viscid sputum, and it is suggested that a simple water aerosol given by an efficient nebuliser would be equally effective as the detergent aerosol. S. L. W.

Amphetamine Poisoning. R. Greenwood and R. S. Peachey. (*Brit. med. J.*, 1957, **1**, 742.) Three examples of acute amphetamine poisoning are described. One was due to excessive use of a benzedrine inhaler, a second to the swallowing of the contents of one and a half inhalers, and a third to the swallowing of 50 tablets of dexamphetamine. One case developed an acute psychosis, a common feature of severe amphetamine intoxication. All the patients recovered. Soluble phenobarbitone, 0·2 to 0·4 g., was given intramuscularly six-hourly; 2 of the patients were given in addition paraldehyde 10 ml. intramuscularly. These 3 cases bring the total number of cases of acute amphetamine poisoning reported in this country to 11, in 7 of which the source of the drug was an amphetamine-containing inhaler. S. L. W.

Antihaemophilic Factor; Clinical Trial in Haemophilia. R. A. Kekwick and P. Wolf. (*Lancet*, 1957, **272**, 647.) This paper describes the preparation of a concentrate of human antihaemophilic factor and its use in the treatment of 6 cases of haemophilia. The antihaemophilic activity of the concentrate was not less than 85 per cent of the fresh citrated normal human plasma used as the starting material, and the potency in terms of activity per mg. of protein was 20 to 25 times that of fresh plasma. The product is dissolved in a volume such that the resulting solution has 10 times the activity of normal plasma; the total protein content is about 2·2 g./100 ml. and the viscosity so low as to present no problem during administration. Such solutions can be held frozen at $-20°$ or dried from the frozen state with negligible loss in activity. Freeze-dried material has been stored at room temperature for 6 months with only slight loss in activity, and redissolves completely in 20 to 25 minutes when the appropriate volume of distilled water is added at room temperature. The dangers of circulatory overloading during treatment are largely avoided with this preparation since 100 ml. is equivalent in antihaemophilic activity to 1000 ml. of fresh plasma. The preparation was used with very satisfactory results in six cases, including single and multiple tooth extractions, haemorrhages into the stomach and joints, and radical surgery of the buttock and rectum, the dosage varying between 100 and 300 ml. given by transfusion. S. L. W.

Bracken Poisoning in Cattle—Therapeutic Treatment. W. C. Evans, I. A. Evans, C. M. Edwards and A. J. Thomas. (*Biochem. J.*, 1957, **65**, 6P). Bracken poisoning in Welsh Black bullocks was treated with subcutaneous injections of DL-batyl alcohol (D-α-octadecylglyceryl ether) in olive oil (1 g. in 10 ml.) on five consecutive days. Control animals receiving olive oil alone died within 3 days. Treated animals showed a leucocyte response, pyrexia abated and the animal recovered. In further experiments DL-batyl alcohol was solubilised with Tween 80 and administered by slow intravenous injection (25–50 ml. of 2 per cent solution) daily at the critical stage of poisoning. Treatment, coupled with wide-span antibiotic therapy, raised the leucocyte counts from 2000–2500/mm.^{3} to 4000/mm.^{3} and platelet counts from 60,000 and 65,000/mm.^{3} to 120,000 and 70,000/mm.^{3}, within four days. J. B. S.

Tranquillising Drugs in the Treatment of Allergic Conditions. B. C. Eisenberg. (*J. Amer. med. Ass.*, 1957, **163**, 934.) Clinical observations were made on three groups of allergic patients receiving tranquillising drugs. Patients were selected for study who exhibited undue anxiety, mental depression or tension, together with individuals whose symptoms failed to respond to the usual types of allergy treatment. The nature of the drugs used was unknown to the patients and they were given daily in the usual therapeutic dosage over a period of 6 weeks. Chlorpromazine was found of some benefit to 19/59 patients; meprobamate was of benefit in 32/83 patients; reserpine was of benefit in 11/52 patients. Mephenesin, used in lieu of control placebos in the meprobamate series, benefited about 10 per cent. None of the patients was "cured" but the sedative and relaxing effects appear to reduce the intensity of the allergic symptoms. Four of the patients in the chlorpromazine series had to discontinue the therapy because of weakness and sleepiness, and in the meprobamate series six patients had to discontinue therapy, one because of extreme drowsiness, one because of dizziness and headache, and four because of erythematous eruption with urticaria and angioneurotic oedema of the face and neck. S. L. W.

APPLIED BACTERIOLOGY

Haemolytic Material from Aerobic Sporing Bacilli. G. R. Williams. (*J. gen. Microbiol.*, 1957, **16**, 16.) In an investigation of the haemolytic activities of members of the genus *Bacillus* which do not produce lecithinase, 10 strongly haemolytic strains were identified. One strain of *Bacillus subtilis* was especially active and isolation of the haemolytic material produced by this strain was attempted. The culture filtrate was 4/5 saturated with ammonium sulphate. Purification of the resulting precipitate yielded a protein preparation only 6 to 7 times more active haemolytically per mg. of protein than the original culture filtrate. A more highly active preparation resulted from suspension of the ammonium sulphate precipitated material in acetone followed by acidification with 5N HCl. Inactive protein was filtered off and the acetone solution concentrated under reduced pressure and added to 5 volumes of water, the pH adjusted to 3·5 and the resulting precipitate dissolved in pH 8·0 buffer. This acidic material showed a maximum absorption at 275 mμ and was referred to as "275-acid". It gave a slow developing biuret reaction, a weak ninhydrin reaction and was found to have a N content of *c.* 1 per cent. It could be obtained from the haemolytic protein fraction at any stage of purification: as purification of the protein proceeded, the relative content of "275-acid" increased. Examination of culture filtrates of 7 other haemolytic strains of aerobic bacilli revealed that in only two of the strains could haemolytic activity be not attributed to "275-acid". The kinetics of haemolysis with "275-acid" were shown to resemble those of tyrocidine rather than those of most other haemolysins and the preparation was found to have very low activity compared with saponin. The author considers that "275-acid" is responsible for haemolysis, but it is so associated with protein in the culture filtrate as to modify its solubility properties. B. A. W.