ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Boric Acid, Estimation of Minute Amounts of. N. Trinder. (*Analyst*, 1948, **73**, 494.) Conditions have been worked out for the determination of small amounts of boric acid using the dyes alizarin blue S and the unsulphonated dye base of Solway purple (Colour Index No. 1073). Alizarin blue S in strong sulphuric acid changed in colour from purple to brown to green. The final concentration of sulphuric acid for this dye is important, a high acidity being needed. Alizarin blue S is only half as sensitive as the base of Solway purple but it has the advantage that it is stable in sulphuric acid solution and, except for fluorides and nitrates, is unaffected by the presence of large amounts of impurities. The base of Solway purple gives a colour change from pale yellowish-green to deep blue. It has the disadvantage that it sulphonates slowly to a blue compound at high sulphuric acid concentrations and gives a slight colour change if comparatively large amounts of manganese are present. It has the advantage that it is twice as sensitive as alizarin blue S and the dye colour deepens progressively over a wide range of boric acid concentrations. The most suitable dye for use in any particular determination will depend on the impurities present and the concentration of borate. Details are given of the methods using both dyes, the colour estimations being made using a Spekker absorptiometer with Ilford violet filters (No. 601) for alizarin blue S and Ilford Yellow filters (No. 606) for the unsulphonated dye base of Solway purple. R. E. S.

Drying Conditions. A Study of U.S.P. XIII and N.F. VIII. N. L. Deahl, J. L. Powers and M. W. Green. (*Bull. Nat. Form. Comm.*, 1948, **16**, 153.) The results of investigations on the drying conditions of over 1,000 substances contained in the U.S.P. XIII and N.F. VIII are given. Attempts were made in the work to standardise on a few different temperatures and conditions which were consistent with accurate and reproducible results. Another object was to establish the temperature and the time required for the drying of a large class of substances which are at present dried to "constant weight." Where substances gave identical results when dried under a wide range of conditions, the easiest and most acceptable laboratory procedure was recommended; weighings were made to within $\pm 0{\cdot}1$ mg., weighing bottles after removal from the ovens were cooled in desiccators over calcium chloride until they reached room temperature (kept constant at 25°C.); drying over sulphuric acid was done at 25°C. and vacuum drying was carried out at a pressure of less than 1 mm. Hg.; the temperature of drying was controlled to within ± 1°C. of the desired temperature; other precautions taken followed the directions of the U.S.P. XIII; dryings were carried out on two or more samples of each substance. In general drying was carried out wherever possible under "standard conditions" as follows: (1) at 60°C.; (2) at 80°C.; (3) at 105°C.; (4) at 120°C.; (5) at 150°C.; (6) over sulphuric acid at room temperature; (7) in vacuum. The following general results are recommended for drying over sulphuric acid: (1) for the removal

of "surface moisture" only, when the amount present is small and the material is non-hygroscopic in character, 2 to 4 hours drying time was usually sufficient; (2) for the removal of "surface moisture" from materials of a hygroscopic nature, overnight drying (16 to 24 hours) may be required; (3) drying over sulphuric acid for removal of water of hydration was generally unsatisfactory, either because of incomplete dehydration or because of the time involved. It is recommended that substances dried at normal pressure and high temperatures should, in many cases, be dried at 105°C. rather than at 100°C. With drugs such as acacia and sterculia gum, the particle size was found to influence the loss on drying to a considerable extent, indicating that definite specifications for particle size as well as for temperature and time of drying were necessary; thus losses ranged from 10·2 to 14·4 per cent. on the same sample when reduced to different particle sizes. In vacuum drying, the working pressure is important, since greater losses are obtained with a low vacuum. It is recommended that vacuum drying should be carried out at a pressure of less than 5 mm. Hg. Tables are given covering over 1,000 recommended drying conditions: for U.S.P. XIII drugs; for the methods of drying applicable in general tests and analytical processes; and for the methods of drying applicable to U.S.P. XIII reagents. Methods of drying are similarly given for drugs, processes, and reagents of the N.F. VIII, following the principles outlined above. Graphs are included, showing the loss on drying with time of acetophenetidin, cotarnine chloride, emetine hydrochloride, sulphathiazole, digitalis, stibophen, ovary, and liquid glucose, under varying conditions. R. E. S.

Morphine, Ammoniacal Zinc Ferrocyanide Solution as Reagent for. G. Denigès. (*Bull. Soc. Pharm. Bordeaux*, 1947, **85**, 29.) The reduction of ferricyanide by morphine, with subsequent formation of Prussian Blue, forms a familiar test for the alkaloid. Owing to the strong colour of ferric ferricyanide, the reaction is not very sensitive and cannot be employed quantitatively. These objections are removed by employing zinc in place of iron to detect the ferrocyanide formed. The reagent is prepared by mixing immediately before use, equal volumes of a 6 per cent. solution of potassium ferricyanide (free from ferrocyanide) and of a solution of 2·5 g. of zinc sulphate in 50 ml. of water to which is added 20 ml. of ammonia. A few drops of this reagent is added to about 1 ml. of the solution to be tested, when in presence of morphine a turbidity or precipitate appears in a short time, and may be estimated nephelometrically. The method may be applied to any clear preparation of morphine, even if coloured. G. M.

Oxydimorphine in presence of Morphine, Determination of. N. Thörn and A. Ågren. (*Svensk. farm. Tidskr.*, 1949, **53**, 33, 49.) Both morphine and oxydimorphine give colours with aromatic aldehydes in presence of sulphuric acid, but in the former case the extinction is very small at 600μ. The method described below may be used for the determination of oxydimorphine formed in solutions of morphine after storage or sterilisation. For the test, 0·50 ml. of the solution to be tested is transferred to a dry test tube and treated with 10·0 ml. of a solution of 1·0 g. of vanillin in 100 ml. of sulphuric acid (95 ±1 per cent.) The reagent must be added slowly from a pipette, with continuous shaking and cooling. The mixture is stirred with a glass rod, immersed in a water-bath for 20 minutes, and then cooled in running water. A blank test is carried out at the same time using a solution of pure morphine of the same concentration. The difference in the extinctions is measured at 600 mμ. The official solvent of

the Swedish Pharmacopœia for morphine injection contains 5 per cent. of glycerol and 15 per cent. of alcohol, and the method may be applied directly to such solutions, but the extinction is somewhat different, as shown by the table below.

Oxydimorphine in solution per cent.	Extinction	
	in water	in dilute glycerol-alcohol
0·2	0·190	—
0·4	0·372	0·315,0·325
0·6	0·530,0·549	0·456,0·463
1·0	0·894,0·907	0·814,0·844

G. M.

Procaine Penicillin G, Spectrophotometric Determination of Procaine in. C. V. St. John. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 343.) Weigh accurately approximately 50 mg. of procaine penicillin G and transfer to a 100 ml. volumetric flask. Dilute to volume with distilled water and shake until completely dissolved. Transfer 5 ml. of this dilution to a 250 ml. volumetric flask, dilute to volume, and measure the optical density of the resulting solution at 290μ against water in the reference cell. Obtain the concentration of the solution by calculation from an extinction coefficient determined on a sample of pure procaine hydrochloride or a sample of pure procaine penicillin G analysed by the chloroform shake-out titrimetric procedure. The accuracy of the method compares very favourably with that of the conventional method. Care must be taken, however, to clean the cells frequently with dichromate-sulphuric acid cleaning solution and to make careful adjustment of the instrument. Cell calibration should also be checked. The arithmetical average of the readings for several adjustments of the instrument on each of two fillings of the cell should be taken.

S. L. W.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Anti-Pernicious Anæmia Factor, Presence of Cobalt in. E. Lester Smith. (*Nature*, 1948, **162**, 144.) The use of the borax bead test and the specific red colour reaction with nitroso *R* salt reveals the presence of cobalt in the ash of the anti-pernicious anæmia factor recently isolated as red needle-shaped crystals. The crystals, dried in vacuo at 56°C. contain 4·0 per cent. of cobalt, estimated colorimetrically with α-nitroso-β-naphthol. Assuming 8 per cent. loss on drying and one atom of cobalt per molecule, this corresponds to a molecular weight of 1,600, in agreement with the result (1,500 to 1,750) obtained by X-ray crystallography. The different molecular weight (3,000) found by diffusion may be due to errors in the method, impure material, or possibly association in solution. The molecule also contains three atoms of phosphorus. American workers have confirmed the presence of cobalt and phosphorus in vitamin B_{12} isolated by them. G. B.

Bacitracin, Stability of. G. C. Bond, R. E. Himelick and L. H. Macdonald. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 30.)

Bacitracin, the antibiotic produced by the growth of the "Tracy I" strain of *Bacillus subtilis* was studied to ascertain its stability in various pharmaceutical preparations. It was found to parallel penicillin quite closely in its stability, or lack of it, with various substances. The dry substance was quite stable at 37°C., but showed definite decomposition at 56°C. At 80°C. decomposition occurred within 48 hours. Within a *p*H range of 5 to 7, aqueous solutions of bacitracin, with or without buffers, were stable for several months at refrigerator temperature, but lost about 50 per cent. of their activity in a week at room temperatures. Ointments prepared with anhydrous fatty bases showed good stability at room temperature, but attempts to prepare a stable water-miscible ointment were unsuccessful. A lozenge and a powder containing ephedrine for the preparation of nasal solutions were shown to be stable. Assay was by the cylinder-plate method using *Staphylococcus aureus* or *Micrococcus flavus*. G. R. K.

***Lactobacillus casei*, Growth Factors for.** F. W. Chattaway, D. E. Dolby and F. C. Happold. (*Biochem. J.*, 1948, **43**, 567.) The concentration and separation from liver of further factors promoting acid production by *Lactobacillus casei* are described. Importance is attached to the preparation of the casein digest used in the growth medium as some processes do not remove appreciable *L. casei* activity. Growth factors insoluble in saturated baryta were separated from the crude liver extract together with at least three factors soluble in saturated baryta and in silver nitrate at *p*H 1·0; these consist of a filtrate fraction which is not adsorbed on alumina at *p*H 3, and two fractions which are adsorbed, one of which is eluted with a 20 per cent. v/v solution of methyl alcohol or water and the other with 0·5 per cent. w/v solution of ammonia. The three latter components have properties dissimilar to both pteroyl glutamic acid and the folic acid of Mitchell *et al.* (1941). The greatest concentration of folic acid-like material was found in the silver salts insoluble at *p*H 1 (from the baryta-soluble material) which contained none of the components discussed above. The differential action of ninhydrin and nitrous acid, and of esterification and acetylation, upon the activity of the material eluted by a 2 per cent. w/v solution of ammonia for *Streptococcus fæcalis R.* and *L. casei*, confirms that there are two components present in this material. The fact that the above chemical treatment affects the filtrate fraction and the baryta-insoluble fraction dissimilarly to one another, and to the above ammonia-eluate material, is evidence that four separate and distinct factors are present. R. E. S.

Penicillin Activity *in vitro*, Enhancement by Vitamin K_5. Robertson Pratt, J. Dufrenoy and P. P. T. Sah. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **38**, 435.) The addition of vitamin K_5 (2-methyl-4-amino-1-naphthol hydrochloride) in concentrations of 0·1 to 10 mg./l. enhances the effect of pencillin against *Staphylococcus aureus, Proteus vulgaris* and *Escherichia coli* as measured by the cup plate method to a greater extent than would be expected from the sum of the activities of the two components acting separately. The magnitude of the enhancement depended on the concentration of K_5 and of pencillin and on the test organism. The enhancement is particularly marked with E. coli. A. L.

Penicillin, New Absorption Delaying Vehicle. F. H. Buckwalter and H. L. Dickison. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 472.)

In this preliminary report the authors describe experiments which show that peanut oil-aluminium stearate gels are superior to peanut oil or peanut oil-beeswax combinations as repository forms for penicillin. J. W. F.

Penicillin G Sodium in Aqueous Solution, Stability of. T. J. Macek, E. J. Hanus and B. A. Feller. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 322.) Solutions of crystalline sodium benzylpenicillinate (penicillin G sodium) in water lost 50 per cent. of their activity in about 2 days at 25°C. and in about 16 days at 10°C. The rate of decomposition was unchanged when normal saline solution and 5 per cent. dextrose solution were the solvents, and was independent of the initial concentration. It was also observed that the *p*H of the solutions fell from *p*H 6 to *p*H 4 in about the time required for 50 per cent. decomposition, but thereafter rose to about *p*H 4·5, the rise being attributed to the decarboxylation of benzylpenicilloic acid. The greater stability of solutions of partly purified penicillin sodium suggested that the process of purification removed substances capable of acting as buffers and preventing the rapid fall in *p*H, which accelerates the rate of decomposition. By adding various phosphate and citrate buffers to maintain the *p*H at about 6·0, the stability of solutions of sodium benzylpenicillinate was increased so that at 25°C. the time for 50 per cent. decomposition was 15 days and at 10°C. solutions still had 75 per cent. of their original activity after 56 days. The stabilising effect was shown to be due to the buffering action of the phosphate or citrate ion and not to a specific ion effect; it was also shown to increase with increase in the concentration of the buffer mixture. G. R. K.

Streptomycin, Degradative Studies on. M. L. Wolfrom and W. J. Polglase. (*J. Amer. chem. Soc.*, 1948, **70**, 2835.) An inactive product was obtained by degradation of dihydrostreptomycin; this, when acetylated with pyridine and acetic anhydride gave crystalline $C_{16}H_{24}O_{12}N_3.(CH_3C)(COCH_3)_{10}$.(I) m.pt. 261·5° to 262·5°C. $[\alpha]_D^{23°C.} -84$ (*c*, 1; water). Methanolysis of (I), designated deca-acetyldideguanyldihydrostreptomycin with subsequent reacetylation yielded hexa-acetylstreptamine, transition point 250°C. m.pt. 341 to 345°C., and methyl penta-acetyldihydro-α-L-streptobiosamide, m.pt. 194 to 195°C., unchanged on admixture with a specimen prepared from dihydrostreptomycin trihydrochloride, $[\alpha]_D^{23°C.} -120°$ (c,0.5; chloroform). (I) was found to be readily soluble in methyl alchohol, water and hot ethyl alcohol, sparingly so in chloroform, ethyl acetate and ethyl alcohol, and insoluble in benzene and ethyl ether. Aqueous solutions of N,N,N-tetra-acetyldideguanyldihydrostreptomycin (II), N^1,N^3,-diacetyl streptamine (III) and N-acetyldihydro-α-L-streptobiosaminide were prepared by partial deacetylation of the aforementioned acetyl derivatives with 0·05N sodium hydroxide in water-dioxane. Treatment with periodate showed the presence in II of an α-glycol which is not in III, and this α-glycol is present in the streptamine moiety of II. The presence of such a glycol group indicates that streptobiosamine is attached at C4 of streptidine, thus confirming the result of Folkers. R. E. S.

Streptomycin and Mannosidostreptomycin, Crystalline Trihydrochlorides of. L. J. Heuser, M. A. Dolliver and E. T. Stiller. (*J. Amer. chem. Soc.*, 1948, **70**, 2833.) Crystalline streptomycin trichloride dihydrate has been prepared as monoclinic prisms showing birefringence. On heating, gradual decomposition without melting took place. Drying at 55°C. *in vacuo* gave $C_{21}H_{39}N_7O_{12}3HCl,2H_2O$; after drying at 100°C. *in vacuo* a substance was

obtained which gave $[\alpha]_D^{26\cdot6°C.}$ $-86\cdot1°$ (1 per cent. in water) and $C_{21}H_{39}N_7O_{12},3HCl$. When assayed with *Klebsiella pneumoniæ* in a broth-dilution test, the trihydrochloride dihydrate had a potency of 820 units/mg. and on this basis the anhydrous material would have an activity of 891 units/mg. The trihydrochloride of mannosidostreptomycin crystallised in the form of isotropic hexagonal plates. By means of the counter-current distribution method, this material was also shown to be a single entity and to be free of streptomycin. Drying at 55°C. *in vacuo* gave $C_{27}H_{49}N_7O_{17}$, $3HCl,2H_2O$; at 100°C. *in vacuo* analysis showed $C_{27}H_{49}N_7O_{17},3HCl$, $[\alpha]_D^{26\cdot6°C.}$ $-54\cdot1°$ (1 per cent. in water). When assayed with *K. pneumoniæ* in a broth-dilution test, the anhydrous mannosidostreptomycin had a potency of *ca.*210 units/mg. R. E. S.

Vitamin A in Whale-Liver Oil, Chromatographic Estimation of. N. T. Gridgeman, G. P. Gibson and J. P. Savage. (*Analyst*, 1948, **73**, 662.) The method given is based on the fact that the main components of the unsaponifiable fraction of whale-liver oil are selectively adsorbed on weakly active alumina in the order: anhydro-vitamin A<oxidised vitamin A <vitamin A(alcohol)<kitol<sterols<selachyl alcohol. The technique consisted of depositing the material on the column from a non-polar solvent and then developing and eluting the chromatogram with solvents of progressively increasing polarity; the eluate was collected fractionally and Carr-Price spot tests were used to identify the vitamine A portions, from which aliquots were bulked for spectrophotometric estimations. A quantity (approximately 0·35 to 0·40 g.) of the unsaponifiable fraction in light petroleum was used for chromatography and 25 ml. quantities of light petroleum-ether mixture were used to elute the fractions, the proportion of ether being gradually increased in each succeeding fraction. The eluate was collected in 5 ml. fractions and a few drops of solution from individual tubes were tested with Carr-Price reagent to establish the range of tubes containing the vitamin. The tubes corresponding to the zone below the vitamin A usually give a reddish-purple colour with the reagent; while those corresponding to the zone above the vitamin gave a bluish-purple or greenish-purple colour. Both these colours are readily distinguished from the bright blue of the vitamin A solution. Moreover, in a good chromatogram, the set of vitamin A tubes will be separated at either end from the sets containing the adjacent zones by one of two tubes whose eluate content is almost nil; these correspond to the inter-zone regions in the chromatogram and will give only a faint coloration with the Carr-Price reagent. Aliquots drawn from those tubes showing vitamin A reaction were pooled, diluted with cyclohexanol and $E_{1\text{ cm.}}^{1\text{ per cent.}}$ 326 to 328μ was measured; if the fraction is pure, the maximum of the absorption curve will lie between 326μ and 328μ, E 300μ/Eλ_{max} will be not more than 0·63, and E 360μ/λ_{max} not more than 0·35. Details are given of the full analysis by this method of a sample of whale-liver oil. The method could be extended to more normal oils. Chromatography of the unsaponifiable matter of a shark liver oil, a mixed fish-liver oil diluted in vegetable oil, a distilled vitamin A ester concentrate, and a cod-liver oil, showed that of the total absorptions at 326μ (on the whole oil for the first three samples and " via unsap." for the cod-liver oil) the following fractions were due to vitamin A: 85, 92, 91 and 90 per cent., respectively. Recovery experiments on vitamin A acetate dissolved in vegetable oil and in dolphin oil gave results of 97 to 99 per cent. on the unsaponifiable fractions. R. E. S.

BIOCHEMICAL ANALYSIS

Folic Acid, Polarographic Determination of. W. J. Mader and H. A. Frediani. (*Anal. Chem.*, 1948, **20,** 1199.) The determination of folic acid can be made quantitatively and rapidly. The sample is dissolved in 1 per cent. tetramethylammonium hydroxide solution containing cadmium chloride as internal standard with sufficient ammonium chloride to prevent precipitation of the cadmium from alkaline solution. A polarogram of this solution yields two clearly defined waves, one at 0·74 volt (against the saturated calomel electrode) for the cadmium, and one at 0·98 for the folic acid. With known folic acid concentrations (and fixed cadmium concentration) a straight line results on plotting the step-height ratios of cadmium-folic acid against folic acid concentration using log log coordinates. Replicate runs on a fixed sample indicated a reproducibility of $\pm$ 2 per cent. if the drop rate was controlled within 0·7 second, and the temperature within 10°C., variations which exceed the conditions ordinarily encountered in an analytical laboratory. The diffusion current constant as defined by Lingane was calculated to be 1·72. It was found more convenient to utilise the ratio-concentration curve and thus to read off sample concentrations directly in mg./ml. than to calculate sample concentrations. The method could be applied to folic acid tablets and to tablets with vitamin B_6 added, but could not be used in the presence of iron. R. E. S.

Penicillin, Spectroscopic Estimation of. G. H. Twigg. (*Analyst*, 1948, **73**, 211.) The author has reviewed various applications of ultra-violet and infra-red spectroscopy for the estimation of total and individual penicillins, and has discussed the limitations of these methods. The aim of spectroscopy is to discover in the absorption spectrum a band which is characteristic of each penicillin molecule as a whole. This is not attained in practice and the absorptions arising from separate parts of the molecule have to be used. Such a procedure may provide an estimate of total penicillin, but it leads to fundamental difficulties in assaying individual penicillins; impurities and deactivated penicillin products may contain similar molecular groupings and thus have bands almost identical with those of the penicillins. It is, therefore, likely that spectroscopic estimation of individual penicillins can only be applied to the pure material. The ultra-violet analysis of total penicillin depends on the development of an absorption band at 3220 Å when an aqueous solution of penicillin is treated with acid under standard conditions. The band, which is due to an intermediate product, disappears after a time and the reading must be taken at its maximum intensity. The method can be used with impure material and gives an accuracy to within 5 per cent.; chemical methods of estimation are probably more speedy and accurate. The ultra-violet analysis of penicillin G depends on the development of absorption bands in the ultra-violet spectrum due to the phenyl group. Two methods have been developed. One compares the ultra-violet spectrum of the unknown sample with that of a known standard by photography and for pure samples of penicillin G gives an accuracy of $\pm$ 2 per cent. The second method is based on the relative absorption at 2630 Å and 2800 A. Penicillin G has no absorption band at the higher wave-length but impurities and decomposition products have. All measurements of optical density are made with solutions of constant penicillin content (1·8 mg. per ml.) as both penicillin K and F show some absorption at 2630 Å. The optical density difference E_{2630}-E_{2800} is plotted against percentage of penicillin G content and gives a straight line, the analyses being evaluated from this calibration curve. Both these methods suffer from similar defects, phenyl-

containing inactivated materials interfere and concentrations of penicillin X in excess of 1 per cent. will invalidate the results. Infra-red analysis of the individual penicillins is theoretically the ideal method, as the infra-red spectrum of any subtance is unique. Unfortunately serious practical difficulties arise; the penicillin may contain impurities or inactivated materials which have absorption bands in the same position as the bands used for analysis, the penicillin may also be in the form of a salt which is insoluble in all the solvents that are of use in infra-red measurements, and degradation may occur when converting it to the free acid and transferring to a suitable dry solvent. The effect of crystal structure is not known and a separate calibration may be necessary with amorphous material, and it is not easy to measure I_0, the intensity of incident radiation. A suitable internal standard, generally *dl*-alanine, in known concentration has to be mixed with the penicillin. The ratio $R = \log (I_0/I)_{703} \log/(I_0/I)_{851}$ is determined for penicillin G, where I_0 and I are the intensities of incident and transmitted radiations respectively at both 703 $cm.^{-1}$ and 851 $cm.^{-1}$. A calibration curve R against percentage of penicillin G is plotted by diluting the pure penicillin G with magnesium oxide; results using this curve have an accuracy of $\pm$ 2 per cent. For other penicillins different bands are used. L. H. P.

Salicylate in Blood, Fluorophotometric Method for the Estimation of. A. Saltzman. (*J. biol. Chem.*, 1948, **174,** 399.) One ml. of oxalated or citrated plasma is shaken in a test-tube with 9 ml. of a freshly prepared mixture of 1 volume of a 10 per cent. solution of sodium tungstate and 8 volumes of N/12 sulphuric acid, and the precipitated proteins are filtered off after 10 minutes. To 5 ml. of the filtrate 7 ml. of sodium hydroxide solution (40 per cent.) is added and the mixture is placed in a fluorophotometer. The fluorescence is directly measured within 30 minutes using the same filters as in the vitamin B_1 determination. The values are read off a standard reference curve, plotted by adding varying amounts of a standard salicylate solution, containing 1·16 mg. of sodium salicylate in 12 ml. of water to a reagent blank mixture, consisting of 5 ml. of tungstic acid and 7 ml. of sodium hydroxide solution (40 per cent.). These values must be multiplied by 2 to correct for the dilution. Salicylate concentrations of 1 to 2 mg. in 100 ml. are detectable, and both free and combined salicylate is determined. A modification of the ethylene dichloride method for determining salicylate concentration in the blood is also described. L. H. P.

Streptomycin B, Chemical Assay of. W. B. Emery and A. D. Walker. (*Nature*, 1948, **162,** 525.) The use of 0·2 per cent. anthrone, a reduction product of anthraquinone, in 95 per cent. sulphuric acid is described for distinguishing streptomycin B (a mannoside) from streptomycin A; it can also be used for estimating the former in a mixture. The results obtained are in accord with those calculated from biological and chemical assays, making the accepted assumption about the relative biological activities of the two steptomycins. The glucosamine moiety, present in both molecules, does not react with the reagent. R. E. S.

Urea in Blood, An Improved Diacetyl Reaction for the Estimation of. V. R. Wheatley. (*Biochem. J.*, 1948, **43**, 420.) The enhancing effect of a number of substances on the diacetyl-urea reaction has been studied. Phenols were unsatisfactory and produced precipitates; with aromatic amines the colour produced was orange or red, and not yellow, whilst diphenylamine and its derivatives produced an intense magenta colour. The reaction with

N-phenyl anthranilic acid was studied in detail and adopted for the estimation of urea in blood. Investigations of the reaction conditions were made involving a study of the quantities of reagent used, the time of heating, the development of the colour and the specificity of the reaction. Calibration and absorption curves are given for the reaction colour, which obeyed Beer's law up to a urea concentration of 20 μg./ml. Under the conditions prescribed ammonia, histidine, tyrosine, cystine, caffeine, uric acid, barbiturates, acetamide, asparagine, creatine, sulphonamides and thiouracil all give negative reactions. Proteins and monosubstituted ureas give red colorations; semicarbazide gives a magenta colour similar to that obtained with urea, while biuret gives a brown colour. Creatinine gives a positive reaction, but fortunately the sensitivity in this case is only one hundredth of that with urea, so that creatinine will not interfere with the estimation of urea in biological fluids. The method showed fairly good agreement with the manometric hypobromite method except that in 10 per cent. of cases errors of 8 to 10 mg./100 ml. were obtained. The estimation can be performed with as little as 0·2 ml. of blood and compares favourably with other colorimetric urea determinations, although it is not sufficiently accurate for urea clearance tests. R. E. S.

CHEMOTHERAPY

Fungistatic Activity and Structure in a Series of Simple Aromatic Compounds. G. W. K. Cavill, J. N. Phillips and J. M. Vincent. (*J. Soc. chem. Ind., Lond.*, 1949, **68**, 12.) Derivatives of benzene are assessed for activity against *Aspergillus niger*. For comparison, the logarithm of the reciprocal of the millimolar concentration giving 50 per cent. inhibition is used to express fungistatic activity. In the case of ionised substances, this is calculated relative to the un-ionised form as there is some evidence that the ionised forms are not so active fungistatically. Benzene and toluene have a small activity, aniline, benzoic acid, phenol and nitrobenzene are less active, but chlorobenzene is more active. Saturated compounds are considerably less active than the corresponding aromatic substances. In general, halogen substitution increases activity. Substitution of aniline, benzoic acid or phenol with hydroxyl or amino groups nearly always reduces activity, except that $-OH$ substituted *ortho* to carboxyl groups increases activity slightly. *Meta* or *para* substitution of a carboxyl group in phenol or in aniline decreases activity, but *ortho* carboxyl groups cause an increase, or little change, in activity. Nitration of phenol or aniline increases activity (except for picric acid and trinitroresorcinol which are ionised), but nitrobenzoic acid is less active than benzoic acid. The introduction of $-CH_2-$ groups between ring and carboxyl group does not enhance the activity of benzoic acid, and decreases that of 4-aminobenzoic acid; the same applies to the methyl esters. To conform to the conclusions above, 4-hydroxybenzoic acids have to be regarded as substituted benzoic acids, not as substituted phenols. Alkylation, akyl esterification or alkyl etherification generally increases activity. There is some correlation between reciprocal water solubility and activity for a homologous series, but this breaks down if a wider range of compounds is taken. Slightly better agreement is obtained when the logarithm of relative solubility in alcohol and water is compared with activity, but there are considerable deviations. G. B.

Polymethylene *bis*-Quaternary Ammonium Salts, Curare-like action of. R. B. Barlow and H. R. Ing. (*Brit. J. Pharmacol.*, 1948, **3**, 298.) The following series of polymethylene *bis*-quaternary ammonium dibromides

were prepared and tested for curare-like activity on the phrenic-nerve diaphragm preparation of the rat (n = number of carbon atoms in the polymethylene chain):—*bis*-trimethylammonium series, n = 2, 3, 4, 5, 7, 8, 9, 10, 11, 12 and 13; *bis*-triethylammonium series, n = 2, 3, 4, 5, 7, 8, 9, 10 and 13; *bis*-strychninium series, n = 2, 3 and 5; *bis*-quinolinium series, n = 3, 5 and 10; *bis*-(phenyldimethylammonium) series, n = 3 and 5. In the *bis*-trimethyl series, the salt with n = 2 is about twice as active as tetramethylammonium iodide; salts with n = 3, 4 or 5 are only feebly active; activity increases from n = 7 to n = 9; salts with n = 9, 10, 11 and 12 are about 5 to 6 times as active as tetramethylammonium. In the *bis*-triethyl series, salts with n = 2 or 3 are relatively inactive; activity increases from n = 4 to n = 13. None of the members of the other three series was so active as the most active members of the *bis*-trimethyl series. In the rabbit head-drop test the *bis*-trimethyl member with n = 9 was nearly as active as tubocurarine chloride; the member with n = 10 was about 3 times as active. The *bis*-triethyl member with n = 13 was about two-fifths as active as tubocurarine. Some *bis*-onium salts augment the response of the rat diaphragm to maximum stimuli and inhibit the cholinesterase of caudate nucleus (dog). The sensitivity of the rat diaphragm to *bis*-onium salts differs greatly from that of the rabbit, and the rat diaphragm is less sensitive to methylstrychninium and more sensitive to tetramethylammonium iodide than the frog's sartorius, suggesting that synthetic curare-like drugs ought to be tested on a variety of species. S. L. W.

PHARMACY

DISPENSING

Fatty Oils, Neutralisation of, for Injection. C. G. van Arkel and J. J. M. van Sonsbeek. (*Pharm. Weekbl.*, 1949, **84**, 70.) Neutralisation of fatty oils is sometimes carried out by shaking the oil with excess of calcium or magnesium oxide, possibly with the addition of a trace of alcohol. The method was found effective in reducing the natural acid value of an oil from 0·32 to 0·16 (with magnesia) or 0·09 (with lime). Traces of these metals could be detected in the neutralised oils. When using an oil to which fatty acid had been added to give an acid value of 9·63, it was found necessary to add water in order to obtain a satisfactory result, but too much water causes formation of emulsions and difficulty in filtration. The dissolved metal amounted to, for magnesium 0·2 mg./100 ml., and for calcium, 6·5 mg./100 ml. If, however, the filtration is carried out with the aid of heat, larger quantities are dissolved. It is concluded that the method of neutralisation with soda is to be preferred on account of greater reliability and the possibility of filtration at a raised temperature. G. M.

Penicillin Ointment, Stability of. S. H. Culter. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 370.) A number of penicillin ointments, using various types of bases, with sodium, potassium, and calcium salts of varying degrees of potency were prepared. These ointments were stored in collapsible tin tubes at room and refrigerator temperatures and assayed from time to time to determine the stability of the penicillin. It was found that impure sodium penicillin (400 I.U./mg.) is very unstable in an aqueous or a non-aqueous water-miscible ointment base and has only limited stability in an anhydrous petrolatum base. Calcium penicillin (500 to 650 I.U./mg.) is much more stable than the impure sodium salt in the same bases, while the high potency (1583 to 1620 I.U./mg.) crystalline sodium or potassium salts are equal, if

not superior, to the impure calcium salt for ointment purposes in respect of stability. Penicillin ointments are somewhat more stable at refrigerator temperatures than at higher temperatures. The inclusion of sulphadiazine, sulphathiazole, adrenaline, or benzocaine in a penicillin ointment does not materially influence the stability of the penicillin, but water, zinc stearate, aluminium hydroxide gel hasten its deterioration to a marked degree.

S. L. W.

Penicillin Powders, Preparation of. J. Büchi and F. O. Gundersen. (*Pharm. Acta Helvet.*, 1949, **24,** 31.) A penicillin powder, prepared by dilution of penicillin with lactose, had a limited stability, since 15 per cent. of the activity was found to be lost after keeping for 2 months at 4°C. Preparations made according to the following formula were more satisfactory and showed no loss after 4 months at 4°C., provided that the materials were dried thoroughly before use, and the powder was kept over calcium chloride. Crystalline sodium penicillin, 1,000,000 units; hydrogenated arachis oil (m.pt. 37°C.), 0·25 g.; anæsthetic ether, 5 ml.; sodium laurylsulphate, 0·50 g.; de-enzymated gum acacia, 1·50 g.; diluent, to 50·00 g. In the preparation the penicillin is rubbed down with a solution of the hardened arachis oil in the ether, in order to coat the particles of penicillin, and the other substances are then added. The mixture is finally passed through sieve VI (approx. 100 mesh/inch; wire 0·08 mm. diam.). Anhydrous lactose, sulphanilamide or dried milk may be used as diluent. G. M.

Sterility of Chemicals, Employment of Filtration in Testing. O. Bang, G. Bowitz and A. T. Dalsgaard. (*Arch. Pharm. Chemi.*, 1949, **56,** 643.) The authors have examined the method of testing for sterility proposed by Davies and Fishburn (*Quart. J. Pharm. Pharmacol.*, 1946, **19,** 36). Their results show that the risk of infection arising during manipulation cannot be ignored, since out of 113 tests (30 with a dry filter, the others with sterile solutions) 15 gave positive results. Tests were carried out with a number of pure chemicals, and positive results were obtained with a proportion in the cases of ascorbic acid, hexobarbitone, morphine hydrochloride, dextrose, allyl*iso*propyl barbitone, phenobarbitone, oxedrine tartrate, benzocaine, and boric acid. "Sterilised" boric acid powder gave positive results in 9 tests out of 10. Generally the contaminating organism was a Gram-positive rod. The authors consider that the method is worthy of further study and possible official adoption. G. M.

PHARMACOGNOSY

Antimalarial Plants, Chinese. S. T. Yang. (*J. Amer pharm. Ass., Sci. Ed.*, 1948, **37,** 458.) A brief description of five Chinese medicinal plants which may have antimalarial activity is given. 1. *Fraxinus malacophylla* Hensl. This tree occurs in S.E. Yunnan and is known as *Pei Chiang Kan;* the root-bark is used. Recent tests indicate the absence of antimalarial activity. 2. *Fraxinus chinensis* Roxb. Grows in Szechuan and Yunnan; the bark yields fraxetin which earlier workers claimed to possess antimalarial activity; however recent work indicates it is ineffective. 3. *Clerodendron yunansis* Hu. Grows near Kunming where thin slices of the twigs of this tree are sold as *Tien Ch'ang Shan*. No investigations have been conducted on this plant. 4. *Alstonia yunansis* Diel. Also grows near Kunming; the bark, twigs and pods are sold as *Chih Ku Ch'ang Shan.* Several alkaloids and a resinous substance are present, but they show no antimalarial activity. 5. *Dichroa febrifuga* Lour. This is the only plant in the group whose anti-

malarial activity has been established. It is an evergreen shrub growing in Szechuan and Yunnan; the dried roots are known as *Ch'ang Shan.* In Yunnan the leaves and twigs are used and these have been shown to be much more active than the roots. J. W. F.

Belladonna, Indian, Pharmacognosy of. H. W. Youngken and W. E. Hassan, Jr. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 450.) A complete description and comparison of this plant with *Atropa Belladonna* Linn. is given. The materials used were grown by the workers from authenticated seeds and surplus material has been deposited in the Herbarium of the Massachusetts College of Pharmacy. *Leaves:* the following data are reported; *Vein Islet Number*, species average = 10; *Palisade Ratio*, 5·0 – **8·3** – 12·4 (*A. Belladonna*, 4·5 – **6·9** – 9·2), *Stomatal Index*, Upper Surface, 3·4; Lower surface, 17·6; (*A. Belladonna*, 2·9 and 17·6 respectively). *Floral Members;* the Indian variety has larger flowers than European, and the corolla is bright yellow. *Roots and stems:* similar basic structure except that the cells of the Indian variety are larger than those of the European. *Alkaloidal content*: the Indian variety has a high alkaloidal content. Drying under infra-red lamps at 145° C. destroys the alkaloidal content. No significant increase in alkaloidal content resulted from the injection of amino-acids into the growing plant. The authors suggest that Indian belladonna is a variety of the European and should be named *Atropa Belladonna* variety *acuminata.* J. W. F.

PHARMACOLOGY AND THERAPEUTICS

Adrenaline and *nor*Adrenaline, Action on Human Heart-rate. H. Barcroft and H. Konzett. (*Lancet*, 1949, **256**, 147.) The actions of intravenous infusions of *nor*adrenaline and of adrenaline on the heart-rate and arterial blood pressure of normal men and women have been studied. In doses of 10 to 20μg./minute *nor*adrenaline causes bradycardia, whereas adrenaline causes tachycardia. Subjective effects during the adrenaline infusions included mild palpitation, hyperventilation, tightness in the chest, and muscular fatigue; there were usually no subjective symptoms during infusions of *nor*adrenaline. The explanation of the different actions of adrenaline and *nor*adrenaline on the heart-rate is not known, but the authors suggest that two factors are probably concerned—the direct excitatory action of the drug on the pacemaker, and its reflex inhibitory action due to its pressor action on the vascular system. S. L. W.

***Courbonia virgata.* Identification of Toxic Principle as a Tetramethylammonium Salt.** A. J. Henry. (*Brit. J. Pharmacol.*, 1948, **3**, 187.) The tuberous root of *Courbonia virgata* A. Brongn. (Fam. Capparidaceae), a plant occurring in the Southern Sudan, Northern Uganda, Kenya, and French Equatorial Africa, has been found to contain a toxic principle, tetramethylammonium iodide. This was also found in the scaly shoots and the superstructure, and in the leaves of the subsidiary shoots. It has been named tetramine and the fresh root contains about 0·2 per cent. About 0·25 g. of the base taken orally (in the form of the root) has proved lethal to adult human beings within an hour. Lethal dose of the iodide subcutaneously was 0·5 to 1 mg./25 mg. of mouse, the symptoms being convulsive spasms, collapse, and death within 30 minutes. Intravenous injection of 8 mg. of the iodide into a rabbit caused death within 2 minutes. The

toxic principle is not extracted from alkaline solution by organic solvents and unless its presence is suspected and its properties known, it might easily be overlooked. The toxic properties of the plant are well known to the natives. As a qualitative test for tetramine, the crystalline precipitate which it produces with Wagner's reagent can be used. From warm dilute solutions, either acid or neutral, the periodide rapidly separates as well-shaped rhombic crystals which are readily recognised under the microscope. S. L. W.

Dextran as a Plasma Substitute. J. J. Bull, C. Ricketts, J. R. Squire, W. d'A. Maycock, S. J. L. Spooner, P. L. Mollison and J. S. C. Paterson. (*Lancet*, 1949, **256**, 134.) Dextrans are produced by the growth in culture of certain micro-organisms, in particular of *Leuconostoc mesenteroides*, in a substrate of glucose and phosphate. After removal of protein and inorganic salts from the culture fluid dextran is precipitated as a syrupy gum by organic solvents such as acetone; so obtained, it is a polysaccharide composed entirely of glucose units. The molecules of this crude dextran are too large for infusion purposes and preparations of smaller molecular size are produced by partial hydrolysis with acid. In defining a specification of dextran for intravenous use the proportion of dextran of low molecular weight should be kept to a minimum; it is probably also important to define the upper limit of molecular size. Physico-chemical methods for controlling molecular size are described. The solution for infusion is colourless or pale straw colour and of about the same specific gravity and saline concentration as plasma, with a colloid osmotic pressure 1.5 to 2.0 times that of normal plasma. It is well tolerated as an infusion by man and is not pyrogenic, toxic, or antigenic. Immediately after infusion the erythrocyte-sedimentation rate is increased and rouleaux can be observed in smears of blood. No increase in the osmotic fragility of the red cells has been observed. Dextran has proved efficacious as a plasma substiute in cases of burns and has produced a sustained increase in the venous return in patients with surgical shock or hæmorrhage, but as there is still doubt as to the ultimate fate of dextran in the body it cannot yet be recommended unreservedly for intravenous infusion. S. L. W.

Histamine Antagonists, Comparison of. J. J. Reuse. (*Brit. J. Pharmacol.*, 1948, **3**, 174.) Comparison of a number of histamine antagonists on isolated organs placed them in the following order of descending activity: neoantergan, 3277 RP, benadryl, antistin, nupercaine. Besides being the most active of these drugs against histamine, neoantergan is also the most specific; it had the least action against acetylcholine, and its action against nicotine, potassium and adrenaline was much smaller than its action against histamine. It is thus clearly the best of the drugs studied to use in specific tests for histamine in unknown solutions, but it is useless if high concentrations are used. A satisfactory method for carrying out tests of this kind is first to find doses of the tissue extract and of histamine which cause equal effects on a piece of guinea-pig's ileum, and then to continue giving these doses alternately and to study the effect, in a series of responses, of a brief addition (1 minute) of a small dose of neoantergan to the bath. The dose of neoantergan is chosen so as to produce 50 to 70 per cent. inhibition of the subsequent response to histamine. The concentration of neoantergan for this effect is usually about 1/10 the concentration of histamine. A method for the rough biological assay of neoantergan is described which involves the use of only about 0·002 μg. of the drug per dose. The antihistamine drugs

were found to depress the isolated heart and dilate the coronary vessels. The activity of these drugs as local anæsthetics on the frog's lumbar plexus appeared to be more nearly related to their activity against acetylcholine than to their activity against histamine; benadryl and 3277 RP were, in fact, much more potent than antistin and neoantergan both as local anæsthetics and as acetylcholine antagonists. S. L. W.

Isomeric Heptylamines, Comparative Pharmacology of. D. F. Marsh, (*J. Pharmacol.*, 1948, **94,** 225). In this study the vasopressor activity of the isomeric heptylamines in anæsthetised and unanæsthetised dogs was determined and compared with adrenaline. By limiting the investigation primarily to compounds with a total of seven carbon atoms it was possible to determine the relationship between spatial configuration and pharmacological activity without having to consider differences in molecular weight. The most potent of the compounds was 4-methyl-2-heptylamine which is about 1/200 as active as adrenaline and has a long duration of action; orally, the heptylamines have but little pressor action in man. With the exception of the 4-heptylamine, they increased the tone of isolated rabbit jejunum with a concentration of 4 mg./100 ml., caused contraction of the rat uterus and antagonised the relaxant action of adrenaline. In the perfused heart they produced a decrease in rate, force of contraction and outflow of perfusate. They do not antagonise histamine constriction in the perfused guinea-pig lung. S. L. W.

Isuprel in Spontaneous and Induced Asthma. F. C. Lowell, J. J. Currey and I. W. Schiller. (*New Engl. J. Med.*, 1949, **240,** 45.) Isuprel, *l*-(3: 4-Dihydroxyphenyl)-2-*iso*propylaminoethanol, (*iso*propyladrenaline) has been studied for a number of years in Europe under the name of aleudrin, and has been advocated for use in the form of an aerosol for the relief of attacks of bronchial asthma. The authors report their observations of the administration of isuprel to asthmatic subjects in the out-patient clinic, in the wards, and in the laboratory (induced asthma). In most cases the drug was given as an aerosol, in a concentration of 1·0 and 0·5 per cent., but it was subsequently given in tablets sublingually containing 10 mg., or subcutaneously or intramuscularly in a concentration of 0·02 or 0·01 per cent. Given by aerosol it was very effective in relieving mild or moderately severe asthma and appears the most effective agent available for self-medication; in severe and prolonged attacks it was far less satisfactory. In certain cases other medication, particularly aminophylline intravenously, was required; on recovery, isuprel was again effective in the control of milder attacks. Sublingual and parenteral administration of the drug was not very effective. Side-effects were uncommon in the doses used in this study. S. L. W.

Procaine, Influence of Potassium and Calcium Ions on. H. J. Bein. (*Brit. J. Pharmacol.*, 1948, **3,** 251.) The action of procaine on the refractory period of the isolated rabbit auricle was determined in the presence of varying amounts of potassium and calcium. The influence of potassium and calcium was shown to be antagonistic; increasing the amount of potassium or decreasing the amount of calcium both potentiated the action of procaine, about the same potential change being obtained by raising the K by 50 per cent. or by lowering the Ca by 50 per cent. A reduction of the potassium or an increase of the calcium content produced the same effect qualitatively but not quantitatively. The action of procaine was depressed, but to obtain

the same degree of depression as that obtained by reducing the K concentration by 50 per cent. it was necessary to increase the Ca concentration by 600 per cent. The determining factor is therefore not the ratio K/Ca but the absolute amount of potassium present, though the presence of at least the normal amount of Ca is necessary. S. L. W.

Proguanil (Paludrine), Intravenous. R. N. Chaudhuri and H. Chakravarti. (*Brit. med. J.*, 1949, **1**, 91.) Proguanil acetate was administered by intravenous injection to 11 patients ranging in age from 9 to 60 years, in 8 of whom *Plasmodium falciparum* infection was predominant, while 2 had *P. vivax* infection and 1, mixed infection. Four patients were gravely ill with pernicious symptoms, 4 had heavy parasitic infection with frequent vomiting, and 3 had "ordinary" malaria. Doses varied from 25 to 400 mg., and were repeated in a few cases, the total amount injected ranging from 200 to 600 mg. Although the series was too small to determine the best dosage, in the majority of cases 200 to 400 mg. produced a striking effect, controlling the temperature and clearing the peripheral blood of asexual parasites in 2 or 3 days. One patient admitted in a moribund state, died; another, with typical cerebral malaria, remained unconscious for several days and later developed signs and symptoms of encephalitis, from which, however, he recovered completely. The injections were well tolerated, although 2 patients complained of pain along the injected vein, possibly due to some local phlebitis. A footnote to this paper reports that proguanil lactate is more soluble and less irritant than the acetate, and is being used by intramuscular injection. G. R. K.

Proguanil (Paludrine) in Prophylaxis and Treatment of Malarial Infections caused by a West African Strain of *Plasmodium falciparum*. G. Covell, W. D. Nicol, P. G. Shute and M. Maryon. (*Brit. med. J.*, 1949, **1**, 88.) Proguanil was found to act as a true causal prophylactic of infections of the strain of *Plasmodium falciparum* used, and the prophylactic dose recommended for non-immune adults exposed to malarial infection in West Africa is 100 mg. daily. Although it controlled the clinical attack caused by infections with the same strain, its action in this respect and in clearing asexual parasites from the peripheral blood was somewhat less rapid than that of mepacrine or quinine, and by itself, it did not effect a radical cure. Nevertheless, a course of 300 mg. twice daily for 10 days effectively sterilised the gametocytes and rendered them non-infective to mosquitos for as long as they continued to be present in the peripheral blood in sufficient numbers for infection to occur. Proguanil also has the lowest toxicity of any known antimalarial drug. In the treatment of *P. falciparum* malaria infections, rapid termination of the clinical attack, a high radical cure rate, sterilisation of the gametocytes and minimum risk of injurious side-effects is achieved by a course of 300 mg. of proguanil twice daily for 10 days with 900 mg. of mepacrine given in 3 doses on the first day, followed by a maintenance dose of 100 mg. of proguanil for the ensuing 6 weeks. G. R. K.

Pyrogens and Fever of Acute Infection. J. L. Bennett. (*J. exp. Med.*, 1948, **88**, 267, 279.) The possibility that the fever accompanying acute infections is a response to a pyrogen produced by the infecting organism was investigated in rabbits. Animals surviving dermal pneumococcal infections, or peritonitis due to *Escherichia coli*, were given intravenous injections of typhoid or *E. coli* vaccine. They showed no tolerance to the fever-promoting

effect of these pyrogenic materials, indicating that the pyrogen produced by the organisms is not a significant factor in the production of fever. It was, however, found that tolerance developed on repeated injection of pyrogenic material during fever, showing again that the pyrogen produced by *E. coli* is not the main factor in the causation of a raised temperature. It is suggested that perhaps a product of cell injury is the cause of the fever. Similar experiments were carried out with the injection of sterile exudates of acute inflammation, the exudates being produced by the intrapleural injection of turpentine in dogs. Daily injection of exudate produced no tolerance to its fever-producing effect. Animals tolerant to pyrogens remained fully responsive to exudate. The fever-producing property of exudates is not therefore due to the presence of bacterial pyrogen. H. T. B.

β-Pyrrolidine-ethyl-phenothiazine (Pyrrolazote), Pharmacology of. M. J. Vander Brook, K. J. Olson, M. T. Richmond and M. H. Kuizenga. (*J. Pharmacol.*, 1948, **94**, 197). This compound was compared with pyribenzamine and was shown to be highly specific as a histamine antagonist. It appears to be effective for a longer period of time than pyribenzamine as judged by both the activity it exhibits against histamine spasms of smooth muscle *in vitro* and the protection it affords against fatal histamine intoxication by aerosol *in vivo*. It possesses anti-anaphylactic properties similar to those of pyribenzamine. Pyrrolazote has no effect on the pressor responses to adrenaline; in this respect it differs from pyribenzamine, benadryl and neoantergan, all of which enhance the pressor response. Acute toxicity experiments show that pyrrolazote is considerably less toxic than pyribenzamine in mice, rats and rabbits, and chronic toxicity studies in rats showed that a dose of 10 mg./kg. orally 5 days each week for 10 weeks produced no gross pathology, and growth was not impaired. Histopathology limited to degenerative fatty infiltration of the liver occurred at doses of 25 mg./kg. and greater. S. L. W.

Quinine Methiodide, Pharmacology of. F. H. Shaw, P. Keogh and M. MacCallum. (*Austral. J. exp. Biol.*, 1948, **26**, 147). The authors show that while quinine methiodide retains many of the properties of quinine it has also a curare action on the neuromuscular junction and sympathetic ganglia. It weakens the depressor action of adrenaline and in this respect is the complement of yohimbine and ergotoxine. Because of its extreme toxicity towards the respiratory centre it would be an unsuitable clinical substitute for curare. Intravenous doses of as low as 10 mg./kg. in the cat or dog nearly always resulted in immediate cessation of respiration. It is suggested that it may provide another useful pharmacological test for adrenaline. S. L. W.

Thyroid Activity, Biological Assay of. D. E. Hutcheon (*J. Pharmacol.*, 1948, **94**, 308). The results of two relatively simple methods for estimating the physiological potency of thyroxine are presented. One is a quantal response type of assay depending on the decreased resistance to anoxia of mice treated with thyroxine. For this, adult mice, weighing from 20 to 25 g., were divided into 4 groups of 10 animals, one group serving as a control while the other 3 were given thyroxine 2·5, 5·0 ano 10·0μg. subcutaneously daily for 7 days. 48 hours after the last injection the mice were all placed in an air-tight chamber of 32 litres capacity containing soda-lime. When approximately half the mice had died the survivors were removed and the mortality-rate of each group noted. The method of calculating the

(*Continued on page* 421)