ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Morphine and Codeine, 2-Anthraquinonesulphonate Derivatives of. M. Feldstein, N. C. Klendshoj and A. Sprague. (*Anal. Chem.*, 1949, **21**, 1580.) A solution of sodium 2-anthraquinonesulphonate (1 g. in 20 ml. of water containing 2 ml. of 3N hydrochloric acid) formed characteristic crystals with morphine and codeine, the melting-points of these derivatives being sufficiently far apart to afford a means of identification. Out of 19 alkaloids tested (cocaine, pontocaine, procaine, metycaine, atropine, homatropine, aconitine, scopolamine, strychnine, brucine, caffeine, narceine, dilaudid, quinine, ergamine, pseudopelletierine and nicotine) morphine and codeine only gave a crystalline precipitate. In performing the test about 1 to 2 mg. of the substance was placed on a slide and dissolved in one drop of 6N sulphuric acid. One drop of the reagent was added and the preparation was allowed to stand for 10 to 15 minutes or until crystallisation occurred. Excess of solution was decanted from the preparation by means of a capillary pipette and the decantation and washing repeated; the slide was dried at 100°C., and the melting-point determined. The morphine derivative melted at 198° to 199°C.; its solubility in water at 20°C., was 0·85 mg. per ml. The codeine derivative melted at 175° to 176°C.; its solubility in water at 20°C., was 0·87 mg. per ml. Both derivatives were deep yellow in colour when prepared in large amounts but appeared colourless in minute amounts on a slide. Photographs of the crystals formed with morphine and codeine are given. R. E. S.

ANALYTICAL

Aconite, Assay of. H. Mühlemann and R. Weil. (*Pharm. Acta Helvet*, 1949, **24**, 419.) 5 g. of powdered aconite tuber is mixed with 10 ml. of water, 15 ml. of 25 per cent. ammonia and 100 g. of ether. After shaking for 30 minutes, 5 g. of tragacanth is added, and the ethereal solution is filtered and weighed. The ether is shaken out 5 times with 10 ml. quantities of 0·1 N sulphuric acid, and the acid solution is made alkaline with 2·5 ml. of 25 per cent. ammonia. This solution is shaken 5 times with 20 ml. quantities of ether, and the ethereal solution is evaporated on the water-bath. The residue is again evaporated down with 5 ml. of ether, then with 5 ml. of alcohol. After dissolving, with the aid of gentle heat, in 10 ml. of alcohol, the residue is treated with 10 ml. of 0·1 N sulphuric acid and titrated back with sodium hydroxide, using methyl red-methylene blue indicator. 1 ml. of 0·1 N acid is equivalent to 0·064538 g. of total alkaloids, calculated as aconitine. The titrated solution is then made alkaline with 10 ml. of 5 per cent. sodium hydroxide, and heated under a reflux condenser for 30 minutes on the water-bath. The solution is transferred to a flask, treated with 35 ml. of phosphoric acid (d = 1·7) and 3 ml. of liquid paraffin, and distilled in steam, the distillate being collected in water, previously neutralised to phenolphthalein, containing 1 drop of 0·1 N sodium hydroxide in excess. When about 1200 ml. of distillate has been collected, it is titrated with 0·1 N sodium hydroxide. Calculation proceeds from the fact

that aconitine gives on saponification one molecule each of acetic and benzoic acids, while benzoylaconine gives one molecule of benzoic acid only. G. M.

Antihistamine Drugs, Colorimetric Estimation of. H. M. Jones and E. S. Brady. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 579.) N-(2-pyridyl) substituted antihistamines give coloured compounds when treated with cyanogen bromide and aniline which obey Beer's Law and can be quantitatively determined. To 3 ml. of the solution to be assayed, containing 0·5 to 3 mg. of antihistamine, 1 ml. of a 4 per cent. aqueous solution of cyanogen bromide and 5 ml. of a 2 per cent. aqueous solution of potassium acid phthalate are added, followed after 15 minutes by 1 ml. of a 4 per cent. solution of aniline in alcohol. The colour is measured in a photo-electric colorimeter using a suitable filter. The method was successfully adapted to the assay of creams, elixirs, ointments and tablets, and was also applied to urine, although here interference by other pyridine compounds such as nicotinic acid must be guarded against. Compounds for which the method is suitable include tripelennamine (pyribenzamine), thenylpyramine (histadyl), methapyrilene (thenylene), pyranisamine (neo-antergan), prophenpyridamine (trimeton) and doxylamine (decapryl). G. R. K.

Iodides, Detection of Traces of. G. Denigès. (*Bull. Trav. Soc. Pharm. Bordeaux*, 1949, **87**, 65.) The reagent is prepared by mixing 20 ml. of water, 2 ml. of ammonia and 0·5 ml. of 0·1 N solution of mercuric chloride. The mixture contains a precipitate of aminomercuric chloride, and should be shaken before use. If to 5 ml. is added 10 ml. of a solution containing a trace of iodide, and a similar tube is prepared without the iodide as a control, then, after shaking, a yellowish tint may be perceived in the tube containing the iodide, when observed along the axis of the tubes. The difference is more conspicuous if the mixtures are allowed to settle for 1 hour, and the precipitate is observed. A distinct reaction is obtained at a concentration of 1 mg. of iodide per l. G. M.

Ketohexoses, Specific Qualitative Colour Test. H. Tauber. (*J. biol. Chem.*, 1950, **182**, 605.) To 0·5 ml. of sulphuric acid, 0·2 ml. of a 2·5 per cent. aqueous solution of aminoguanidine sulphate monohydrate is added without mixing, followed by 0·2 ml. of the test solution containing 0·4 mg. of fructose, sorbose or inulin. The liquids are well mixed and a bright reddish purple colour forms in about 1 minute and increases in intensity for some time. Sucrose, 0·8 mg., or raffinose, 1·2 mg., give an identical colour. The following substances produce no colour under similar conditions: arabinose, xylose, ribose, glucose, galactose, mannose, lactose, maltose, melibiose, dextrin, starch, glycogen, formaldehyde, pepsin, trypsin and chymotrypsin. Furfural gives a yellow colour, furfuryl alcohol a brown colour and acetone, methyl ethyl ketone and lævulinic acid a very slight yellow colour. G. R. K.

Kurchi Bases, Labile Nature of, and the Assay of Kurchi Bismuth Iodide. N. K. Basu and N. N. Battacharya. (*Indian J. Pharm.*, 1949, **11**, 157.) The alkaloidal content of an extract of Kurchi Bark was determined by dissolving in standard acid and back titration with alkali. A further quantity of the extract was dissolved in alcohol, the solvent evaporated on a water-bath, the residue dissolved in chloroform and the solvent again evaporated on a water-bath. On titration after this treatment, the alkaloidal content was found to be less. A similar result was obtained with a more highly purified sample consisting mainly of conessine. Assay methods involving evaporation of solutions of the bases are therefore not reliable. The following

method, in which evaporation of the chloroform extract is avoided, is recommended for the assay of Kurchi bismuth iodide. Triturate 0·3 g. of Kurchi bismuth iodide with 5 ml. of sodium hydroxide solution and extract with successive 10 ml. quantities of chloroform, washing each chloroform solution with the same 10 ml. of water. Filter the mixed chloroform solutions and shake with 0·1N sulphuric acid. Wash the chloroform solution with two 10 ml. quantities of water, add these to the aqueous acid liquid and titrate with 0·05N sodium hydroxide, using methylene blue-methyl red solution as indicator. Each ml. of 0·1N sulphuric acid is equivalent to 0·01657 g. of alkaloids. G. B.

Senna Leaves, Spectrophotometric Method of Assay for. B. V. Christensen and I. A. Abdel-Latif. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 589). 10 g. of powdered and dried leaves is refluxed for 30 minutes with 75 ml. of 10 per cent. alcoholic potassium hydroxide. The liquid is filtered, the marc washed and the filtrate adjusted to 100 ml. with alcohol. 25 ml. of this solution is diluted with 25 ml. of water and sufficient dilute hydrochloric acid to adjust the reaction to *p*H 2, and extracted with one 30-ml. and five 20-ml. quantities of ether. The ether extracts are combined and washed with a mixture of 5 ml. of dilute hydrochloric acid and 10 ml. of water. The wash-liquid is itself washed with 15 ml. of ether and the total ether extract and washing adjusted to 200 ml. with ether. 30 ml. of the ether solution is shaken with 10 ml. of ammonia and centrifuged. The ammonia layer is removed and the red colour which develops in it measured spectrophotometrically at 670 mμ. The extinction varies directly with the concentration of the anthraquinone derivatives. The method is rapid, accurate and sensitive and may be used comparatively until reference standards are set up. It should be applicable to all emodin-containing drugs. G. R. K.

Sodium *p*-aminosalicylate, Determination of. H. A. M. van Steenbergen. (*Pharm. Weekbl.*, 1949, **84**, 797.) In view of the ease of decarboxylation of *p*-aminosalicylic acid, it is essential that any method of estimation should not be affected by *m*-aminophenol. Moreover, decomposition during the estimation must be avoided by use of a low temperature and low degree of acidity. Details of the method recommended are as follows. A quantity of solution, corresponding to about 0·3 g. of the acid, is placed in a stoppered flask, diluted, if necessary, to 10 ml., and treated with 40 ml. of ether and sufficient 0·5 N sulphuric acid to bring the *p*H to between 1 and 2 (indicator paper). The mixture is transferred to a continuous extractor, being washed in with several quantities of ether. After extraction, the ether is distilled off, care being taken that the temperature of the residue is not allowed to exceed 40°C. The residue is dissolved in 25 ml. of alcohol and titrated with 0·1 N sodium hydroxide, using phenol red as indicator. The first adjustment of the *p*H may also be carried out before the addition of the ether, but in this case the coarser precipitate of the acid requires vigorous shaking to cause it to dissolve. G. M.

Thiourea as Reagent in Inorganic Analysis. G. Denigès. (*Bull. Trav. Soc. Pharm. Bordeaux.*, 1949, **87**, 67.) Thiourea may be used for the formation of sulphides in inorganic analysis. If, for example, about 5 cg. of thiourea is mixed with 1 to 2 cg. of an arsenite and heated until the reagent fuses and begins to decompose, the mass assumes a strong orange colour owing to formation of red arsenic sulphide (As_2S_2). By extraction with water, the sulphide may be separated and identified by its solubility in ammonia. With arsenates, the product obtained is yellow (arsenious

sulphide) and also soluble in ammonia. Antimonious compounds, treated in a similar manner, give a black residue, while with potassium antimonate the colour, after lixiviation with water, is a brownish green. Calomel, triturated with thiourea in the cold, gives a black mass, but mercuric chloride does not react. G. M.

FIXED OILS, FATS AND WAXES

Lard, Preservation of. E. Sandell and B. Spross. (*Svensk farm. Tidskr.* 1950, **54**, 61.) Lard, if freshly prepared and of low peroxide content, may be effectively preserved by the addition of 0·005 per cent. of hydroquinol, nordihydroguaiaretic acid, or propyl gallate. Benzoin, at a concentration of 0·3 per cent., is also satisfactory, and better than guaiacum resin at the same concentration. The preservative action of diphenylamine, hardened arachis oil, or of α-tocopherol was only slight, while α-tocopherol, which showed a preservative action under accelerated test, was found to give, under storage conditions, no better stability than lard without any addition. When water was incorporated with the lard a larger proportion of anti-oxidant was necessary (at least 0·03 per cent.). In alkaline solution (e.g., in presence of borax), these anti-oxidants cannot be used, as hydroquinonol is ineffective and nordihydroguaiaretic acid and propyl gallate, although giving some protection at high concentrations, cause discoloration. In this investigation the question of possible toxicity of the stabilisers was not considered. G. M.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Blood and Blood Plasma, Chemical Sterilisation of. F. W. Hartmann, G. H. Mangun, N. Feeley and E. Jackson. (*Proc. Soc. exp. Biol., N.Y.*, 1949, **70**, 248.) Plasma, serum or whole blood which has been contaminated with *Bact. coli, Pseudomonas æruginosa,* hæmolytic streptococci or vesicular stamatitis, is sterilised by methyl-bis(β-chloroethyl)amine hydrochloride in a concentration of 500 mg. per litre, provided that the *p*H lies between 6·7 and 7·2. After 5 days, no toxic products remain, unless the *p*H is too low to permit degradation of the ethylammonium ion. There is no evidence of antigenic or other toxic reactions. Complement, immune bodies, phosphatase, fibrinogen and red cell fragility are only slightly altered by the treatment but there is a marked increase in the prothrombin time. G. B.

Progesterone, Solubility in Water and in Saline Solution. A. L. Haskins, Jr. (*Proc. Soc. exp. Biol., N.Y.*, 1949, **70**, 228.) Synthetic α-progesterone m.pt. 128°C. has a characteristic ultra-violet absorption band with a maximum at 240 mμ in alcohol (95 per cent.) and at 248 mμ in aqueous solution. The solubility at room temperature is 16·8 μg./ml. in water and 15·1 μg./ml. in 0·9 per cent. sodium chloride solution. These solubilities have been determined by evaporating a small quantity of an alcoholic solution, warming the residue with the solvent, allowing to stand and measuring the concentration of progesterone in the supernatant liquid spectrophotometrically at intervals. Under these conditions, solution is complete in 72 hours. G. B.

Vitamin B_{12}, Synthesis of, in the Digestive System of the Sheep. P. H. Abelson and H. H. Darby. (*Science*, 1949, **110**, 566.) In an experiment to determine the possibility of a relationship between cobalt deficiency disease in sheep and the fact that vitamin B_{12} contains cobalt, the authors

administered radioactive cobalt in tracer doses to sheep. Examination of the fæces showed more than half the traced cobalt had been incorporated into an organically bound form, and treatment with acid enabled most of the active cobalt to be extracted. Biological assay, using *Lactobacillus lactis* Dorner, and *Lactobacillus leichmannii* indicated the presence in the fæces of large amounts of vitamin B_{12}. It is known that the growth of certain bacteria in the rumen of sheep is stimulated by the administration of cobalt and it is possible that these rumen bacteria synthesise the B_{12}. E. N. I.

BIOCHEMICAL ANALYSIS

Adrenaline—norAdrenaline Mixtures, Estimation of. J. H. Burn, D. E. Hutcheon and R. H. D. Parker. (*Brit. J. Pharmacol.*, 1950, **5,** 142.) The authors describe a modification of the nictitating membrane method which has a much greater accuracy and does not require a cat with a denervated nictitating membrane. The contractions of a normal nictitating membrane and the blood pressure are recorded in a spinal cat. The ratio of the height of the membrane contraction to the rise in blood pressure bears an almost linear relation to the percentage of adrenaline present, provided that the solutions injected cause about the same rise of blood pressure. The method, which has a high degree of accuracy, is applicable to total amounts of adrenaline and noradrenaline not less than 10 to 30 μg. in 1 to 3 ml. S. L. W.

norAdrenaline in presence of Adrenaline, Colorimetric Estimation of. U. S. von Euler and U. Hamburg. (*Science,* 1949, **110,** 561.) The colorimetric method is based on the formation of noradrenochrome and adrenochrome on oxidation with iodine. At *p*H 4·0, adrenochrome formation is complete in 1½ minutes after treatment with iodine, but only about 10 per cent. of the noradrenaline is transformed into noradrenochrome under the same conditions. On 3 minutes treatment with iodine at *p*H 6·0, maximal formation of noradrenochrome and adrenochrome is attained. The procedure is as follows. The extract, buffered to *p*H 4 is treated with 0·1N iodine. After precisely 1½ minutes, excess of iodine is removed by means of sodium thiosulphate. The colour is read within 5 minutes against a blank without iodine at wave-length 529 mμ. The procedure is repeated with a second sample buffered to *p*H 6 and 3 minutes iodine treatment. Standard readings are made with 100 μg. of adrenaline and noradrenaline at *p*H 4 (1½ minutes) and *p*H 6 (3 minutes), giving the calibration factors for both substances and the percentage of noradrenaline oxidised at *p*H 4 in 1½ minutes. At 529 mμ, the adrenochrome figure is the same on oxidation at *p*H 4 and *p*H 6. Results agree well with those obtained by biological methods. E. N. I.

Calcium and Magnesium in Small Amounts of Biological Material, Colorimetric Determination of. G. D. Michaels, C. T. Anderson, S. Margen and L. W. Kinsell. (*J. biol. Chem.*, 1949, **180,** 175.) The determination depends on the formation and colorimetric determination of calcium and magnesium phosphate; the total phosphate is determined (calcium and magnesium), the calcium is precipitated as oxalate, and the magnesium is determined (after separation) as magnesium ammonium phosphate; the calcium is then redissolved and reprecipitated as calcium phosphate. Both phosphates are then determined and the sum of the two should equal the

total phosphate found previously. For urine a sample is just acidified with hydrochloric acid, ammonium phosphate and ammonium hydroxide solutions are added, the precipitate is removed by centrifuging, washed with alcohol and dissolved in 1:4 hydrochloric acid, the solution being made up to a standard volume. An aliquot portion of this solution is taken for the determination of total phosphate; to a further aliquot is added oxalic acid and sodium acetate to *p*H 4·0 thus precipitating calcium oxalate but not magnesium. The calcium oxalate precipitate is washed with dilute ammonia solution and the supernatant liquid and washings are combined for the determination of magnesium. The calcium oxalate precipitate is dissolved in hydrochloric acid and reprecipitated as phosphate with ammonium hydroxide and ammonium phosphate; the calcium phosphate precipitate is then washed with alcohol and redissolved in hydrochloric acid for the determination of phosphate. To the supernatant liquid (containing the magnesium) ammonium phosphate and ammonia solution are added and the precipitated magnesium ammonium phosphate is washed with alcohol and redissolved in hydrochloric acid for the determination of phosphate. The actual phosphate determinations were performed colorimetrically by the standard method of Fiske and Subbarow (*J. biol. Chem.*, 1925, **66**, 375). Analysis of known mixtures of calcium and magnesium in water gave the constant ratio calcium-phosphorus of 1·62:1 and magnesium-phosphorus of 0·782:1, indicating the formation of magnesium ammonium phosphate, whereas, in the case of calcium, apparently an equal mixture of tricalcium phosphate and ammonium calcium phosphate results. Recovery of total phosphate ranged from 100·0 to 99·3 per cent.; experiments with known amounts of calcium and magnesium in urine gave equally good recoveries. R. E. S.

Chloride in Blood, Polarographic Microdetermination of. W. J. Zimmerman and W. M. Layton, Jr., (*J. biol. Chem.*, 1949, **181**, 141.) A polarographic method for the determination of blood chloride was developed, depending on the fact that, using 0·1M potassium nitrate as supporting electrolyte, the diffusion current of the anodic depolarisation wave produced at the dropping mercury electrode is directly proportional to chloride concentrations between 10^{-4} and 2×10^{-3} equivalent per l. 0·05 ml. samples of whole blood, serum or plasma are delivered into 4·0 ml. of approximately 0·15M phosphoric acid in a test-tube, and 1·0 ml. of 3 per cent. sodium tungstate solution is added to precipitate proteins; the solution is mixed, centrifuged for approximately 1 minute at 2,000 r.p.m., and the clear supernatant solution is transferred to the electrolytic cell. The current is measured at a single fixed, applied voltage and in the procedure described a setting of $-0{\cdot}6$ volt against the saturated mercurous sulphate reference electrode was found suitable (0·34 volt versus the saturated calomel electrode); it was unnecessary to remove oxygen from the solutions since it is reduced at potentials negative to the chloride wave. Minimum and maximum galvanometer readings were recorded on sample solutions and a sufficient number of standard chloride solutions in phosphotungstic acid to cover the range of chloride values encountered were run simultaneously. Details of the electrolytic cell used and the electrode assembly are given. In nine experiments in which 16·0 m.eq. per l. of chloride as potassium chloride was added to 0·05 ml. samples of plasma and whole blood, the mean error in recovery was 0·9 m.eq. per l., the maximum error being 1·5 m.eq. per l. The values obtained by this method when applied to 0·05 ml. samples of serum, plasma, and whole blood were compared with the values obtained by titration; 28 parallel determinations in duplicate gave a mean deviation

between the values obtained by the two methods of 1·0 m.eq. per l.; 86 per cent. of the polarographic values agreed with iodimetric values within 2·0 m.eq. per l., and none differed by as much as 3·0 m.eq. per l. R. E. S.

Citric Acid, Microcolorimetric Determination of. H. H. Taussky. (*J. biol. Chem.*, 1949, **181,** 195.) A method is described which avoids the use of hydrazine sulphate in the procedure based on conversion of citric acid to pentabromoacetone, and on the subsequent reaction between pentabromoacetone and alcoholic sodium iodide with the development of a yellow colour complex; hydrazine sulphate had been utilised at two stages in this procedure for the reduction of free bromine and manganese dioxide. The previous method (*J. biol. Chem.*, 1947, **169,** 103) was therefore modified so that ferrous sulphate replaced hydrazine sulphate. Preliminary bromination is carried until the point at which excess of free bromine has to be removed by reduction. After the mixture has reached room temperature, the bromine fumes are removed by suction, 2 ml. of saturated ferrous sulphate solution is added and the mixture is shaken; the procedure is then continued as previously described (*ibid.*). In the oxidation of citric acid to pentabromoacetone, 2·5 ml. of potassium permangenate solution is added instead of 2·0 ml. as originally described, the larger amount resulting in a more rapid formation of the manganese suspension. After standing in the water-bath at about 18°C. for 30 minutes, bromine fumes are removed by suction and 6 ml. of ferrous sulphate solution is added, the mixture being shaken and allowed to stand for about 3 minutes. Similar recovery results were obtained with ferrous sulphate as with hydrazine sulphate, although in blood and urine lower values were found with ferrous sulphate, suggesting that the latter is more specific. R. E. S.

Copper, Determination of traces of, by means of *Penicillium*. J. Keilling, A. Camus, P. Foulet and J. Burdin-Steeg. (*C.R. Acad. Sci., Paris,* 1949, **228,** 2059.) For the determination of traces of copper, *Penicillium glaucum* is to be preferred to *Aspergillus niger* on account of the greater ease of appreciation of the scale of dosage from the colouration of the conida. The material under examination, or the ash, is sterilised and added, in increasing quantities, to flasks containing 40 ml. of nutrient medium, free from copper, which is then inoculated with a suspension of the conidia. Four days is allowed for growth. The minimum quantity which ensures a green colouration of the mould contains 1 μg of copper. Alternatively the colour may be compared with standards. It should be noted that apparatus should only be flamed with a glass burner. The appearances observed are as follows:

Copper, μg per litre	Appearance
0	white.
0·2	very few green points.
0·5	slight green stain.
1·0	definite green

G. M.

Dicoumarol in Biological Fluids, Estimation of. J. Axelrod, J. R. Cooper and B. B. Brodie. (*Proc. Soc. exp. Biol., N.Y.*, 70, **4,** 693.) A simple and sensitive spectrophotometric method is described. 1 to 3 ml. of plasma or urine, containing up to 50 μg. of dicoumarol and 0·5 ml. of 3N hydrochloric acid are shaken with 20 ml. of heptane for 30 minutes, after adjusting the aqueous volume to about 3·5 ml. The mixture

is centrifuged, 15 ml. of the heptane phase shaken with 4 ml. of 2·5N sodium hydroxide for 5 minutes and the organic phase removed. 3 ml. of the aqueous phase is transferred to a quartz cuvette and the optical density determined in a spectrophotometer (Beckman) at 315 mμ. The distribution of dicoumarol in a heptane-acidified water system is such that at room temperature, with volumes of 20 ml. and 3·5 ml. respectively, about 95 per cent. of dicoumarol is in the organic phase. Known amounts of dicoumarol gave optical densities proportional to the concentration. The method does not include metabolic products of the drug. Basic organic drugs do not interfere since they are not extracted at an acid *p*H. Of a wide range of acidic and neutral substances tested, only pentothal and salicylates interfered. E. N. I.

Digitoxin, Polarographic Determination of. J. G. Hilton. (*Science*, 1949, **110**, 526.) A study of the polarographic properties of digitoxin showed that it could be determined in amounts as low as 0·1 μg. in 50 per cent. alcohol solution, and could also be extracted by means of suitable solvents from complex mixtures and determined in similar low concentrations. Varying amounts of pure digitoxin in 0·5 ml. of 0·2N tetraethylammonium hydroxide in a Heyrovsky reaction vessel were diluted to 5 ml. total volume with a 50 per cent. alcoholic solution in order to study the half-wave potential and height of break at different concentrations. Nitrogen was bubbled through the prepared solutions for a period of 15 to 20 minutes and the polarogram recorded, the process being repeated until a curve of satisfactory height and concentration had been determined and the average half-wave potential calculated. Digitoxin in blood was extracted by shaking with 2·5 times the total volume of light petroleum followed by careful separation. After evaporation the residue was dissolved in 2·5 ml. of absolute alcohol and decanted to remove all alcohol-insoluble components; this solution was diluted to 5 ml. with distilled water and 2·5 ml. was placed in a Heyrovsky reaction vessel with 2·0 ml. of a 50 per cent. alcohol solution and 0·5 ml. of 0·2N tetraethylammonium hydroxide. Nitrogen was bubbled through the solution for 15 to 20 minutes and the polarogram recorded. Graphs of height and concentration are given and it is claimed that digitoxin can be determined in concentrations as low as 0·1 μg. with an error of ±0·02 μg. between values from 0·1 to 0·4 μg. of digitoxin. The average half-wave potentials were found to be −1·965 in alcoholic solution and −1·958 when extracted from blood. R. E. S.

Œstrogens in Urine: Determination by a Micro-fluorimetric Method. M. Finkelstein. (*Proc. Soc. exp. Biol., N.Y.*, 1948, **69**, 181.) The method permits of the accurate determination of the œstriol fraction and of the œstradiol-œstrone fraction in as little as 10 ml. of urine. The sample (10 ml.) is hydrolysed under a reflux condenser for 1 hour with 0·7 ml. of hydrochloric acid. After cooling 5·6 g. of sodium chloride and 10 ml. of water are added and the liquid extracted 5 times with 20 ml. of benzene. The benzene extracts are washed with 3 ml. of 9 per cent. sodium bicarbonate solution, evaporated to 30 ml. and again washed with 30, 15 and 15 ml. of 9 per cent. sodium carbonate solution. The œstradiol-œstrone fraction remains in the benzene while the œstriol fraction is almost quantitatively removed by the sodium carbonate solution. The pooled carbonate extract is adjusted to *p*H6, extracted three times with 35 ml. of ether, and the ether extract washed twice with 10 ml. of 9 per cent. sodium bicarbonate solution and twice with 10 ml. of water. The ether is then evaporated and the residue

taken up in 10 to 20 ml. of alcohol (96 per cent.), aliquots of which are used for fluorimetry. If the ether residue is coloured a further purification procedure can be applied. For the œstradiol-œstrone fraction the benzene extract is washed with a little dilute sulphuric acid and then twice with 15 ml. of water. It is then extracted 4 times with an equal volume of N/1 sodium hydroxide. The alkaline extract is acidified to a *p*H of less than 5 and extracted three times with 50 ml. of ether. The ethereal extract is concentrated to about 50 ml., washed with 10 ml. of dilute sulphuric acid, then twice with 20 ml. of 9 per cent. sodium carbonate solution, and twice with 20 ml. of water, and evaporated to dryness, the residue being taken up in alcohol. To make the determination, an aliquot of the alcohol solution is evaporated to dryness at 120°C. and the residue cooled. 7 ml. of phosphoric acid is added and the mixture heated in a boiling water-bath in the dark for 30 minutes. After cooling, the fluorescence is measured in a Coleman fluorimeter using filters P_2 and PC_2. The instrument is calibrated against the pure œstrogens. The best recoveries (above 80 per cent.) in the extraction process are obtained with œstradiol, and as little as 1 μg. added to 10 ml. of urine could be demonstrated, with 60 per cent. recovery. The recoveries of œstrone were about 70 per cent. and of œstriol 50 to 60 per cent. The latter should be determined only in dilute solution. H. T. B.

Organic Acids, Qualitative Analysis of, by Filter Paper Partition Chromatography. K. Fink and R. M. Fink. (*Proc. Soc. exp. Biol., N.Y.*, 70, 654.) The separation of both volatile and non-volatile organic acids, with chain lengths of about 8 carbon atoms or less, is described. The hydroxamate derivatives are prepared by reacting the methyl ester of the acid with about a two-fold excess of potassium hydroxide and hydroxylamine in methyl alcohol. The chromatogram is developed by suitable solvents and then sprayed with ferric chloride solution to make the derivatives visible as purple spots on a yellow background. The colour reaction will detect acid present in the order of 10^{-7} mol., and a rough quantitative estimation may be made of the amount present by judging from the size of the spot and the intensity of the colour. Isobutyric acid and phenol were the best solvents tried for two-dimensional chromatograms of the dicarboxylic acids. R_F values for a number of hydroxamate derivatives are given for several solvents. E. N. I.

Vitamin A in Milk. A. E. Sobel and A. A. Rosenberg. (*Anal. Chem.*, 1949, **21**, 1540.) A new method has been devised for the determination of vitamin A and carotene in milk using activated glycerol dichlorhydrin. One ml. of milk is pipetted into a test tube, 1 ml. of N potassium hydroxide in ethyl alcohol (90 per cent.) is added, the contents of the tube are mixed and the tube is placed in a water bath at 60°C., for 35 minutes. The tube is removed, allowed to cool to room temperature, 2 ml. of light petroleum is added, and the tube is stoppered with a stopper pre-extracted with light petroleum. After shaking for 10 minutes the tube is centrifuged for approximately 30 seconds, or until complete separation has occurred, when the supernatant light petroleum extract is removed with a fine tipped rubber bulb dropper. The saponified milk sample is shaken with two succeeding 1 ml. aliquots of light petroleum allowing 5 minutes for each shaking, the supernatant ether layer being collected as before and bulked with the previous extract in a small test-tube. The mixed light petroleum extracts are evaporated to dryness in a water-bath at 40° to 50°C. in a slow stream of nitrogen. One ml.

of chloroform is added to bring the dried extract into solution and 4 ml. of activated glycerol dichlorhydrin is added at 25°C. After 2 minutes the solution is transferred to a cell and the absorption read at 550 mμ against a blank consisting of 1 ml. of chloroform and 4 ml. of reagent; following this a reading is taken at 800 mμ. The absorption at 800 mμ will give the carotene content from a carotene calibration chart, while this permits the evaluation of the interference due to carotene at 555 mμ from a carotene interference chart; the optical density due to carotene interference at 555 mμ is then subtracted from the total optical density at this wavelength the resulting optical density giving the vitamin A content per ml. from a vitamin A calibration curve. Methods are given for the preparation of the various calibration graphs. Values agreed closely with those obtained using the Carr-Price reagent. In the estimation of serum vitamin A, approximately the same results are obtained with saponification as without; saponification of a milk sample is a necessary step, however, since without saponification practically none of the vitamin A is extracted with light petroleum. It is suggested that vitamin A-containing lipids in fresh milk are bound more firmly to proteins than in blood. In experiments with added vitamin A the precision of the recovery was $100 \pm 3{\cdot}5$ per cent. of the calculated value. R. E. S.

Vitamin B_6, Some Reactions of. H. Laubie. (*Bull. Trav. Soc. Pharm. Bordeaux*, 1949, **87**, 119.) The following reactions are given. 1. A few drops of Denigès sulpho-titanic reagent added to 1 mg. of the vitamin gives a yellow colouration. 2. By adding 6 drops of 3 per cent. solution of copper sulphate to 1 ml. of a solution of the vitamin, followed by 1 drop of saturated solution of ammonium thiocyanate, a green precipitate insoluble in chloroform is obtained. 3. To 1 ml. of an alkaline solution a few drops of copper sulphate solution are added, a green precipitate is obtained. 4. To 1 ml. of a solution of the vitamin 2 drops of 5 per cent. solution of uranium acetate is added; an intense yellow colour is produced. G. M.

Vitamin B_{12}, Microbiological Assay of. C. E. Hoffmann, E. L. R. Stokstad, B. L. Hutchings, A. C. Dornbush and T. H. Jukes. (*J. biol. Chem.*, 1949, **181**, 635.) The use of two strains of *Lactobacillus leichmannii*, designated respectively strains 313 (ATCC 7830 and ATCC 4797) for the assay of vitamin B_{12} in liver extracts is described. Both strains gave satisfactory results; the former grows more rapidly, enabling the use of a short assay period. Details of the medium are given, the assays being carried out in 12 × 100 mm. tubes in a total volume of 2·0 ml.; crystalline vitamin B_{12} was used for obtaining standard response curves. In preliminary experiments the response obtained was increased when the period of autoclaving the assay tube was prolonged although, in contrast, little growth was obtained when the medium was sterilised by steaming even though large amounts of vitamin B_{12} were added; an investigation of this finding by adding the samples to the medium *after* autoclaving showed that vitamin B_{12} was partially destroyed by autoclaving and that a second growth factor was produced in the medium during the autoclaving process. When thioglycollic acid was included in the medium, similar results were obtained when the liver extract samples were added before autoclaving and when the samples were added aseptically to autoclaved medium, indicating that thioglycollic acid protected vitamin B_{12} during the assay autoclaving process. An investigation of the growth factor produced in the medium during autoclaving revealed that it was due to interaction

between the glucose and the other constituents of the media with the production of a reducing agent. Glucose did not form a reducing agent unless it was autoclaved in contact with the rest of the medium, and it was found that sucrose did not form a reducing agent even when autoclaved together with the medium. Further results indicated that ascorbic acid and cysteine could replace thioglycollic acid as reducing agents. It was found that the addition of the basal medium to a liver extract accelerated the decrease in potency and the addition of thioglycollic acid to a diluted liver extract did not completely protect the vitamin B_{12} as indicated by the microbiological activity of the samples; the addition of thioglycollic acid to liver extract diluted with the basal medium, however, gave good protection. An extract of asparagus was found to have an effect similar to thioglycollic acid. It was found that vitamin B_{12} was destroyed by heating with 0·2 N sodium hydroxide at 100°C. for 30 minutes and that under these conditions the desoxyribosides of thymine, guanine and hypoxanthine were not affected; liver extract was assayed before and after this treatment with alkali, and the difference between the two assay values was used to express the vitamin B_{12} content; usually about 97 per cent. of the total microbiological activity of liver extract was found to disappear upon treatment with alkali, indicating that desoxyribosides were responsible for only a small fraction of the microbiological activity of the extracts. R. E. S.

CHEMOTHERAPY

Analgesics, New Series of. D. W. Adamson and A. F. Green. (*Nature*, 1950, **165**, 122.) A series of potent analgesics, as active as morphine in the rat, has been discovered in the 3-tertiary-1 : 1-(2′-thienyl)-but-1-enes, the analgesic/toxicity ratios being not greatly different from that of amidone. The analgesic dose in dogs was 5 to 10 mg./kg. compared with 1 to 2 mg./kg. for amidone, but the effect was not accompanied by the gastro-intestinal disturbances common with amidone or morphine. As with other analgesics, however, analgesia was accompanied by rise of temperature and respiratory depression and by sedation. An excitatory phase was manifest, particularly in the cat, and high doses cause spasticity of the muscles. The compounds also exhibited considerable antihistamine, spasmolytic and local anæsthetic activity. F. H.

Benzene Derivatives and Simple Phenols in the Chemotherapy of Tuberculosis. F. A. French and B. L. Freedlander. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 343.) 37 compounds were tested *in vitro* against *M. tuberculosis* H 37 Rv. The 4-alkyl ethers of *p*-hydroxybenzoic acid and 2 : 4-dihydroxybenzoic acid showed a rise in bacteriostatic activity with increase in the weight of the ether group. Simple phenols were mildly inhibitory. Some of the compounds also inhibited the growth *in vitro* of *M. tuberculosis* 607, *M. leprae*, and *Trichophyton gypseum*. All compounds tested were devoid of *in vivo* activity in guinea-pigs, except for 2 : 2′-dihydroxybenzophenone and 2-hydroxy-3:5-dichlorobenzophenone which gave low positive results. Lack of *in vivo* activity was probably due to unfavourable absorption-distribution-excretion characteristics. *p*-Anisic acid, which is a constituent of the lipoid fraction of virulent tubercle bacilli, appears to stimulate growth slightly. Replacement of the methyl group by higher alkyl groups gives rise to inhibitors, and the analogous *p*-alkyl ethers of 2 : 4-dihydroxybenzoic acid inhibit at almost exactly the same concentrations.

Vinyl analogues shows the same or somewhat increased activity as compared with the parent compound, for example, umbelliferone and β-methylumbelliferone compared with 2:4-dihydroxybenzoic acid, and hydroxybenzophenones compared with salicylic and benzoic acids. Following the observation that salicylic acid, after administration, is partly converted into gentisic acid, which can enter into rapid reversible oxidation-reduction systems, it is suggested that the presence of drugs having quinoid oxidation-reduction systems may interfere with the growth of tubercle bacilli, and that this may be the mode of action in such cases as *p*-aminosalicylic acid and *p*-alkoxybenzenes. G. B.

PHARMACY

DISPENSING

Phenobarbitone, in Ethyl alcohol-water systems, Solubility of. E. E. Levallen. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **10**, 722.) Incompatibility between phenobarbitone sodium and acids in dispensing mixtures can be avoided by adding alcohol to the mixture to effect solution of the precipitated phenobarbitone. The author has determined the solubility of phenobarbitone in a series of water-alcohol systems, and has expressed the results in the form of solubility curves. By reference to these curves the amount of alcohol required to dissolve any given amount of phenobarbitone can be calculated, within the limits of the curves. Examples of the calculations which are involved when other substances, likely to influence the solubility of phenobarbitone are present in the mixture, are also given. E. N. I.

Pills, Preparation of, by the Drop Method. F. Ernefeldt and E. Sandell. (*Farm. Revy*, 1950, **49**, 41.) The method is based on dropping melted fat (m.pt. 38° to 40°C.) into alcohol (about 65 per cent.), the density of the latter being so adjusted that the molten fat falls slowly while solidifying. In the apparatus described the melted fat (e.g. hardened arachis oil) is kept at constant level in a small beaker fed from a constant level device. This beaker is connected, by means of a small syphon, with a second beaker to ensure a greater constancy of level. From this last beaker the liquid drops from a syphon on to a funnel and thence to the dropping tube. The latter consists of a 2 ml. ampoule with two holes blown near the lower end, and the upper end cut off. The liquid passes through the holes and drops from the base of the ampoule. The whole of this apparatus is kept in an air bath at 60°C. It is important that the lower surface of the ampoule should be horizontal. A drop rate of about 90 drops per minute is used, and in the manufacture of a large number of pills the relative standard deviation in weight was 0·6 per cent. The temperature of the oil should be about 58°C. as if too low the shape of the pills is unsatisfactory. G. M.

Sulphadiazine-Penicillin Mixtures, Sterilisation of. O. Lennert-Petersen. (*Dansk. Tidsskr. Farm.*, 1950, **24**, 33.) Sterilisation of mixtures of sulphadiazine and penicillin is important because such materials may be used as dusting powders during surgical operations. A mixture of sulphadiazine (previously dried at 110°C. for 3 hours) and potassium benzylpenicillin (1650 U/mg.) may be heated at 140°C. for 3 hours without appreciable loss of penicillin, although 160°C. produces complete inactivation in 1 hour. On the other hand, with the sodium salt of benzylpenicillin,

destruction was rapid even at 140°C. Penicillin cream, of the same formula as that of the British Pharmacopœia, infected by the addition of earth containing *B. subtilis*, was sterile after heating for 20 minutes at 120°C. G. M.

GALENICAL PHARMACY

Resorcinol, Stabilisation of, for Topical Preparations. A. Halpern and H. A. Getz. (*J. Amer. pharm., Ass., Pract. Pharm. Ed.*, 1950, **11**, 24.) Varying proportions of ascorbic acid, sodium formaldehyde sulphoxylate, sodium metabisulphite and α-tocopherol were added to ointment bases, lotions and emulsions containing 5 per cent. of resorcinol, and the preparations suitably exposed to light and air until discoloration occurred. Ascorbic acid, 1·4 per cent., delayed discoloration in ointment bases for about 140 hours, α-tocopherol, 1·0 per cent., for about 130 hours, sodium metabisulphite, 1·0 per cent., for about 30 to 40 hours, and sodium formaldehyde sulphoxylate, 1·0 per cent., for 30 to 60 hours. In the fluid preparations, the order of efficiency was ascorbic acid, sodium metabisulphite, sodium formaldehyde sulphoxylate, and α-tocopherol, except that when an oily phase was present, α-tocopherol was the best antioxidant. Sodium metabisulphite and sodium formaldehyde sulphoxylate had the disadvantage that they were incompatible with certain of the preparations used. Similar experiments in which the aqueous phase of water-containing preparations was buffered at different *p*H levels confirmed the view that an alkaline *p*H accelerates discoloration. G. R. K.

NOTES AND FORMULÆ

Antazoline Hydrochloride (Antistine Hydrochloride). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **142**, 258.) Antazoline hydrochloride is 2-(*N*-phenyl-*N*-benzylaminomethyl)-imidazoline hydrochloride, $C_6H_5.N(CH_2.C_6H_5).CH_2.C_3H_5N_2,HCl$. It occurs as white, odourless crystals with a bitter taste, m.pt. 232° to 238°C. with decomposition, sparingly soluble in water and in alcohol and insoluble in ether and in benzene; a 1 per cent. aqueous solution has *p*H 5·6 to 6·6. The base liberated on adding sodium hydroxide to an aqueous solution melts at 114° to 118°C.; with nitric acid antazoline hydrochloride gives a red colour which becomes green on standing; with trinitrophenol it gives a crystalline picrate which melts at 155° to 159°C. When dried at 105°C. for 4 hours, it loses not more than 0·5 per cent. of its weight; sulphated ash is not more than 0·2 per cent. When assayed by precipitation as the picrate it contains 97 to 103 per cent. of antazoline hydrochloride. A 0·001 per cent. solution exhibits an ultraviolet absorption maximum at 2420Å with an extinction coefficient of 520 ± 5. Assay processes are also described for solution and tablets. Antazoline hydrochloride is milder and less irritating to the tissues than other antihistamine substances. G. R. K.

Theophylline Sodium Glycinate. (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1949, **139**, 1149.) Theophylline sodium glycinate contains slightly more than 2 g.-mol. of glycine to 1 of theophylline sodium, and 49 to 50 per cent. of theophylline. It is a white odourless powder with a bitter taste. It decomposes between 190° and 210°C. and is soluble in water and decomposed by acids. It has the action of theophylline sodium acetate and theophylline with ethylenediamine, but is more stable in air and less irritating to the stomach. It is given by mouth as powder, tablets, elixir and syrup in a dose of 0·3 to 1 g. every 4 to 6 hours, or as suppositories containing

0·78 g. For children, the oral dose is 0·15 to 0·4 g. (over 12 years), or 0·1 to 0·2 g. (6 to 12 years) every 4 to 6 hours. It may be administered as an aerosol with oxygen in bronchial asthma; 2 ml. of a 5 to 10 per cent. solution every 4 hours is usually effective, but severe dyspnœa may require continuous therapy or alternation with inhalation of penicillin or other antibiotic.

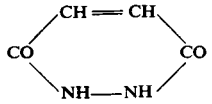
G. R. K.

PHARMACOGNOSY

Anthraquinone derivatives, a new Microchemical Outside Indicator. B. V. Christensen and I. A. Abdel-Latif. (*J. Amer. pharm. Ass.*, 1949, **38,** 651.) Potassium hydroxide pellets are used to detect traces of anthraquinone derivatives in any extract of the emodin drugs or in any solvent. A drop of the liquid is placed on the pellet which immediately develops a red colour. J. W. F.

Aloin, Distribution Chromatography of. A. B. Svendsen and K. B. Jensen. (*Scientia Pharm.* 1949, **17,** 118.) Two samples of commercial barbaloin were examined, one dating from 1898, the other a recent one. A column of kieselguhr, with 30 to 40 per cent. of water, was used; the mobile phase being composed of ether, chloroform, isopropyl alcohol or mixtures of the latter two liquids. The results showed that after prolonged storage (not well protected from light and air) a small amount of anthranol or anthrone is converted into emodin. There is also formed an appreciable quantity of a red-violet substance which does not give either the Bornträger or the Schonteten reaction, but gives a distinct positive reaction for sugar. A similar substance may be obtained by repeated heating of aloin on the water bath. G. M.

Maleic Hydrazide; A selective Herbicide. H. B. Currier and A. S. Crafts. (*Science,* 1950, **111,** 152.) A solution of the diethanolamine salt of maleic hydrazide,



containing the equivalent of 0·2 per cent. of maleic hydrazide, and in some instances a wetting agent, was sprayed on to 2-weeks-old barley and 5-weeks-old cotton. A few days after treatment the leaves of the barley turned dark green and slowly died back from the tips; in about 6 weeks the barley was dead. In contrast the cotton was completely unaffected and in no way different from control cotton plants. Subsequent tests have shown that various types of plants react quite differently to the compound, and that age is critical, young plants responding to a much greater extent. Cotton treated in the cotyledonous stage was severely inhibited whereas plants 16 in. in height showed no apparent response. Young water grass and Johnson grass stopped growing, developed anthocyanin pigmentation and died; older plants showed some response but survived. Results already obtained seem to justify very thorough testing of this compound. G. R. K.

Senna Leaves (Alexandrian), a new Fluorometric Method of Assay. B. V. Christensen and I. A. Abdel-Latif. (*J. Amer. pharm. Ass.*, 1949, **38,** 652.) An ether extract of senna leaves is made according to the procedure previously described (*J. Amer. pharm. Ass.*, 1949, **38,** 589.) This extract is shaken with ammonia solution and the fluorescence of the ammonia layer

is measured in a " Lumetron " fluorometer, which is described. The fluorescence was found to be inversely proportional to the concentration of senna extract. The method is only comparative as no reference standard for senna leaves has been established. J. W. F.

PHARMACOLOGY AND THERAPEUTICS

Antihistamines, Dermatitis following use of. W. B. Sherman and R. A. Cooke (*J. Allergy*, 1949, **21,** 63). Exacerbations of contact dermatitis of the eyelids, face and neck are reported following the use of pyribenzamine ointment in 2 cases, and of antistin eye-drops in 1 case. In the last patient who showed reactions to many antihistamine drugs there was also a maculopapular rash of the trunk apparently due to oral use of pyribenzamine. In the case of the pyribenzamine ointment there was no reason to suspect the base as a causative agent, and control patch tests with powdered pyribenzamine and antistin eye-drops on 3 normal persons showed no reaction. All 3 patients affected gave past histories of allergic manifestations. The 3 patients illustrated striking variations in sensitivity to different antihistamines; one patient reacted on patch tests to pyribenzamine but not to other drugs of the group; the second reacted to pyribenzamine, thenylene and neo-antergan but not to 6 other antihistamine drugs; and the third reacted to 8 antihistamine drugs but not to thephorin which differs in chemical structure. S. L. W.

Artane Therapy for Parkinsonism. L. J. Doshay and K. Constable. (*J. Amer. med. Ass.*, 1949, **140,** 1317.) Pharmacological tests established that the action of artane mildly resembles that of atropine in the control of sialorrhœa, in cycloplegic effects and cerebral stimulation, but that it is entirely free from the toxic effects of atropine on the cardiac vagus, blood pressure and circulation. Clinical studies in 117 patients have established its usefulness against parkinsonism and its freedom from disturbing side reactions. Though the usual dosage is between 6 and 10 mg. a day, 15 to 20 mg. is easily tolerated, and doses of 30 to 50 mg. have not had deleterious effects. Hypertension, cyanotic induration of the liver or renal disorders do not contraindicate the use of the drug. The investigation was conducted with 47 post-encephalitic, 33 idiopathic and 37 arteriosclerotic patients for periods ranging from 6 months to 2 years. In this series, 76.1 per cent. of the patients were improved and 23.9 per cent. unimproved. A favourable response was observed more frequently among members of the arteriosclerotic and idiopathic groups than among the post-encephalitic. In addition to the physical improvement in rigidity, tremor and oculogyria, it has a cerebral-stimulating action which is particularly effective in combating the depression and inertia. S. L. W.

Boric Acid as a Poison. E. G. Young, R. P. Smith, and O. C. MacIntosh. (*Canad. med. Ass., J.*, 1949, **61,** 447.) The deaths of 6 babies, 6 to 11 days old, are reported as the result of one feeding of milk which had been diluted with a 2·5 per cent. aqueous solution of boric acid in error for sterile water. The deaths occurred in from 19 hours to 5½ days. It was estimated that the amount of boric acid ingested was less than 3 g. The clinical diagnosis of death was intoxication with respiratory failure. Associated symptoms of poisoning which developed progressively in this group of infants were: (1) vomiting and diarrhœa, increasing in intensity

after the first day, (2) excoriations of the skin on the second and subsequent days, (3) convulsive movements, especially of the facial muscles and the extremities, (4) cyanosis and collapse as terminal picture. The authors emphasise that solutions of boric acid or borax should be labelled "poison." S. L. W.

Cation Exchange Resin for Treatment of Œdema. L. Irwin, E. Y. Berger, B. Rosenberg and R. Jackenthal. (*J. clin. Invest.*, 1949, **28**, 1403.) A study was made of the electrolyte interchange between the diet and a cation exchange resin, liquonex CRW, a sulphonated polystyrene. The resin was given orally in gelatine capsules in doses sufficient to neutralise from 100 to 250 molar equivalents of base per day, in three equal doses. The general effect was to immobilise sodium in the gut, thus causing a decrease in the urinary sodium and an increase in the fæcal sodium. With a dosage of resin sufficient to neutralise 150 molar equivalents of sodium (6 g. salt), the daily urinary excretion was reduced to 50 molar equivalents, while a dosage of resin equivalent to 250 molar equivalents reduced the urinary excretion to 8 molar equivalents. Administration of the resin enabled a patient with congestive heart failure to take a relatively normal diet without absorbing an excessive amount of sodium. In two œdematous patients there was a diuresis of existing depots of fluid and no reaccumulation. Calcium and potassium are preferentially taken up by the resin and a deficiency of either or both may theoretically occur although not noticed in this study. The liberation of acid in the gut may create an acidosis. The principal disadvantage of the treatment is the large number of capsules, 20 to 25, to be taken with each meal. H. T. B.

Chloramphenicol, Observations on. J. D. Gray. (*Lancet*, 1950, **258**, 150.) Chloramphenicol, given by mouth, is effective against a wide variety of organisms. Bacteria do not acquire a resistance to the drug, and a "chloramphenicolase" has not yet been encountered; the substance seems to be inactivated only when it is exerting its antibiotic effect. In patients being treated with chloramphenicol, the mucous surfaces of the upper respiratory tract become completely sterile, and the sterile condition is maintained for 2 or 3 days after the last dose is given. This may be of value in surgery, especially if sterilisation is found to extend into the lungs. An urticarial reaction to a single dose is reported; this may have been due to massive bacterial death. Large doses of chloramphenicol hasten the onset of fatigue in skeletal muscles and also give rise to a rapid fatigue of accommodation of the eyes on reading, with recovery after a short rest. Owing to the intensely bitter taste of this substance, chloramphenicol may have to be administered to young children by stomach tube; rectal administration is not successful. Preliminary trials suggest that chloramphenicol may be effective in the treatment of whooping-cough. G. B.

Chloramphenicol in Paratyphoid B Fever. M. Curtin. (*Brit. med. J.*, 1949, **2**, 1504.) In view of previous favourable clinical reports, chloramphenicol was tried in 6 patients in whom a diagnosis of paratyphoid B fever was confirmed by culture of *Salmonella paratyphi B*. All had been ill for some days before treatment was instituted. The drug was always given four-hourly but dosage and duration of treatment were varied. Usually an initial dose of 1 or 2 g. was followed by 0·5 g. for 1 to 4 days after which dosage was reduced to 0·25 g. for a further 4 to 5 days. Prompt improvement occurred in all cases, usually within 24 hours, and was followed by clinical recovery. The temperature returned to normal in a maximum of 3 to 4 days. Recovery was more rapid when the treatment was instituted in the early days of the

infection. In 4 cases a convalescent carrier state developed persisting for 6 to 9 weeks. Three apparently healthy fæcal carriers were treated with 0·5 g. four-hourly for 4 days and then 0·25 g. four-hourly for 3 days. This dosage had no effect on the carrier state. H. T. B.

Curare Preparations; Assay by the Rabbit Head-drop method. N. K. Dutta and F. C. MacIntosh. (*Analyst*, 1949, **74**, 588.) A solution of the drug is infused into the ear veins of a rabbit until the animal is so weakened that it can no longer hold up its head. The volume of solution required to produce this end-point is noted. The unknown preparation is compared with the standard in a two-day cross-over test. The apparatus employed by the authors (which is described, with a diagram) enables 4 rabbits to be treated simultaneously. The error of a cross-over test on 8 animals is usually ± 10 per cent. (limits for P = 0·95). S. L. W.

Decamethonium Bromide in Anaesthesia. D. A. Holaday, A. M. Harvey and D. Grob. (*New Engl. J. Med.*, 1949, **241**, 816.) Bis-trimethylammonium decane dibromide (C10) was employed as a curarising agent during 172 anæsthesias. It was found capable of producing adequate abdominal relaxation but was not without some interference with respiration, observable respiratory depression being encountered in 55 per cent. of the patients. Its effect was of considerably briefer duration than that of *d*-tubocurarine, necessitating more frequent injections. On the other hand, a standard dosage range may be used regardless of the anæsthetic agent employed, since no potentiating effect is observed as when *d*-tubocurarine is administered to patients under ether anæsthesia. The most satisfactory method of administration was to give 1 to 2 mg. of the dibromide, in a solution containing 1 mg./ml., intravenously at a rate not exceeding 1 mg./minute. Doses of 0·5 to 0·1 mg. are then injected every 5 or 10 minutes for as long as curarisation is required, with a maximum total dose of 10 mg. S. L. W.

Deoxycortone and Ascorbic Acid, Effect of, on Formaldehyde-induced Arthritis in Normal and Adrenalectomised Rats. G. Brownlee. (*Lancet*, 1950, **258**, 157.) Experimental arthritis can be induced in rats by the injection of formaldehyde. Normal and adrenalectomised rats can be protected against this arthritis by parenteral administration of deoxycortone (1 mg.) and ascorbic acid (20 mg.) daily. Parenteral administration of ascorbic acid only gives some degree of protection to normal rats but not to adrenalectomised rats. Deoxycortone, administered alone, aggravates the arthritis in normal rats and has little effect on adrenalectomised rats. A possible explanation is that both deoxycortone and ascorbic acid are components of an essential enzyme system, or alternatively that the deoxycortone has to be changed chemically by the ascorbic acid before it is effective. The aggravating effect of deoxycortone by itself seems to suggest that unaltered deoxycortone is not anti-arthritic, but that it can compete as a building unit with the anti-arthritic sterols, a mechanism analogous to the antagonism of sulphanilamide and *p*-aminobenzoic acid. G. B.

Diamino-diphenyl sulphone, Oral Treatment of Leprosy with. J. Lowe. (*Lancet*, 1950, **258**, 145.) Diamino-diphenyl sulphone (D.A.D.P.S.) is almost completely absorbed from the gut, and only slowly eliminated by the kidney; a high concentration can be maintained in the blood by administration of small oral doses, daily or twice weekly. Toxic effects, previously reported,

are due to excessively high dosage and are not produced by a starting dose of 100 mg. daily, gradually increased during 5 weeks, to 300 mg. This drug is at least as effective against lepromatous and tuberculoid forms of leprosy as promin, diasone or sulphetrone. The activity of these drugs may be attributed to diamino-diphenyl sulphone formed by their hydrolysis. Absorption and hydrolysis of these more complicated sulphones is incomplete, and the cost of treatment is reduced to about 1/20th when diamino-diphenyl sulphone is used. The drug is well tolerated by patients with tuberculosis of the lungs, and may possibly be of value in this condition. G. B.

Digitoxin, Quantitative Detection of Minute Concentrations of. R. Bine and M. Friedman. (*Proc. Soc. exp. Biol. N.Y.*, 1949, **69**, 487.) Studies were made of various concentrations of digitoxin upon the duck heart immersed in (1) Tyrode's solution, (2) rat serum, and (3) human serum. The method of quantitative determination employed was the same as that previously described by these authors (*Proc. Soc. exp. Biol. N.Y.*, 1947, **64**, 162; *Amer. J. med. Sci.*, 1948, **216**, 534). It was found that the embryonic duck heart was not only extraordinarily sensitive to minute quantities of digitoxin but also offered a means whereby the concentration of digitoxin could be assessed in a quantitative fashion. The embryonic heart preparation was able to detect the presence of 0·005μg. of digitoxin in 1 ml. of Tyrode's solution, and with increasing concentrations of the drug there was a progressive reduction in the time taken for the occurrence of the digitalis effect. In rat serum the action of digitoxin was much less effective, only quantities of 0·2μg. or more per ml. being detected. Human serum was even more inhibitory, only quantities of 0·6μg. or more of digitoxin per ml. being detected. S. L. W.

Dimercaprol and Parathyroid Extract; Effects on Distribution of Lead in Rabbits. K. R. Adam, M. Ginsburg and M. Weatherall. (*Brit. J. Pharmacol.*, 1949, **4**, 351.) The distribution of lead in the tissues of rabbits 13 and 21 days after the intravenous injection of lead acetate (2·07 g. Pb/kg.) was studied by use of the isotope Pb^{210} (radium D). Some of the rabbits were treated with dimercaprol, or parathyroid extract, or both, for some days during the week before they were killed. Parathyroid extract was employed for its reputed effect in mobilising lead from bones, since at this phase in the distribution of a single intravenous dose of lead most of the lead remaining in the body would be found in the bones and it would be unlikely that dimercaprol would have much effect on lead so deposited. Apart from a transient increase in the urinary excretion of lead after dimercaprol none of the treatments caused any substantial change in the distribution or the excretion of lead. About 50 per cent. of the lead was excreted in 21 days. The bones contained about 25 per cent. of the dose 21 days after injection, the bone marrow and liver being the only other tissues consistently containing more than 1 per cent. The authors conclude that dimercaprol and parathyroid extract have no useful effect in rabbits subacutely poisoned with lead. S. L. W.

Fat Emulsion for Intravenous Feeding. S. R. Lerner, I. L. Charkoff and C. Entenman. (*Proc. Soc. exp. Biol., N.Y.*, **70**, 388.) A stable olive oil emulsion was prepared as follows. Two parts of olive oil and one part of glycerol monostearate were heated, with stirring, to give a clear solution. This was diluted with glucose (5 per cent.) to yield a final concentration of 10 per cent. of fat. By high pressure homogenisation, an

emulsion with most of the fat particles of diameter 1μ or less, and *p*H 7·0, was obtained, which could be sterilised by autoclaving at 5 lbs. for 30 minutes. The emulsion is stable for several months. It is well tolerated by dogs when injected intravenously at rates of 1 ml. per minute per kg. and is rapidly removed from the bloodstream. Although dogs that had received as many as 31 injections were not free of tissue responses, the reactions were quite mild. E. N. I.

Gantrisin: Studies on Solubility, Absorption and Excretion. F. A. Svec, P. S. Rhoads and J. H. Rohr (*Arch. intern. Med.*, 1950, **85,** 83). This compound, 3:4-dimethyl-5-sulphanilamido-isoxazole (formerly known as NU445) shows promise as a sulphonamide, particularly in the treatment of coliform infections of the urinary tract. Toxic manifestations have been similar to those produced with other sulphonamides except for the absence of renal complications. The solubility of gantrisin in urine rapidly increases from 60 mg./100 ml. at *p*H 5·4 to 327 mg./100 ml. at *p*H 6·14, whereas the solubility of sulphadiazine at *p*H 6·3 is only 12·9 mg./100 ml. and approaches that of gantrisin only after the *p*H of the urine is raised to 7·5 or 8. Administration of 1 g. 4-hourly orally or intramuscularly, or 2 g. 8-hourly intravenously, maintains adequate average blood levels. Up to 12 g. daily has been given without untoward effect, and the intramuscular or intravenous administration of a 40 per cent. solution of the ethanolamine or diethanolamine salts of the drug produces no reaction. From 88 to 90 per cent. of the drug is excreted in the urine within 48 hours after administration of a single dose by any of the three routes, the most rapid excretion occurring during the first 8 hours. From 28 to 35 per cent. of the drug appears in the urine in the conjugated form, regardless of the route of administration, and a similar degree of acetylation occurs in the blood. Levels of the drug in the spinal fluid are about one-third those in the blood. S. L. W.

Leptazol and Strychnine for Testing Anticonvulsant Drugs. H. L. Williams and C. C. Pfeiffer. (*Proc. Soc. exp. biol., N.Y.*, 1949, **70,** 254). A method of evaluation of anticonvulsant drugs depends upon the determination of the degree of protection conferred against leptazol or strychnine. The anticonvulsant is administered intraperitoneally and the mouse placed in a cone-shaped holder of plexi-glass, the tail being drawn out through a slit in the holder. Leptazol (0·5 per cent.) or strychnine sulphate (0·01 per cent.) is injected at the rate of 0·05 ml. every 10 seconds. The degree of protection is judged by comparison of the time of the first twitch, pseudo-convulsion, persistent convulsion and death, and of the type of seizure and percentage mortality. By use of this method in which the effect of graded dosage of convulsant is observed in each animal, a statistical analysis of results is possible when a smaller number of animals is used than is the case when a test involving a fixed subcutaneous dose of convulsant is used. G. B.

Methadone and its Isomers, Side effects of. J. E. Denton and H. E. Beecher. (*J. Amer. med. Ass.*, 1949, **141,** 1148.) The incidence of side effects following the injection of *dl*-methadone, *l*-methadone, *dl-iso*-methadone, *l-iso*methadone and morphine was observed in two groups of healthy young men, as it was found impossible to distinguish between the side effects of these drugs and the after effects of anæsthesia and surgery in post-operative patients. The drugs were administered subcutaneously as solutions of the hydrochlorides, and isotonic sodium chloride solution was

given as a control. Records of the nature, incidence and duration of signs and symptoms, respiratory rate, pulse rate and blood pressure were made before, and for 5 hours following an injection. *dl*-Methadone, *l*-methadone and *dl-iso*methadone, in comparable analgesic doses, are as toxic as morphine in respect to the occurrence and duration of side effects. *l-iso*Methadone produces less nausea than morphine. All four forms of methadone slow the pulse rate and depress the respiratory rate to the same degree as morphine. None of the drugs tested alters systolic or diastolic blood pressure.

G. R. B.

Procaine-Penicillin G, Prophylaxis of Tetanus with. M. Novak, M. Goldin and W. I. Taylor. (*Proc. Soc. exp. Biol., N.Y.*, **70,** 573.) The possible prophylactic value of penicillin in tetanus was determined by injecting mice with an LD100 infective dose of *Clostridium tetani* spores, detoxified by heating at 80°C. for 30 minutes, followed at varying intervals by similar injections of penicillin G procaine in oil containing 2 per cent. of aluminium monostearate. A statistical analysis of data on mortality and time of death of the mice receiving penicillin prophylaxis and untreated controls showed a significant decrease in mortality and prolongation of life in the treated mice. The lowest mortalities resulted when doses of 150 and 300 units of penicillin were given immediately after the injection of the spores and when 150 units were given after 3 and 6 hours' delay. The series having low mortalities were also observed to have the greatest time lapse before symptoms and deaths. The results also show that penicillin injected into the necrotic areas is more effective than the same unitage injected at a different site. E. N. I.

Phenadoxone, Pharmacology of. B. Basil, N. D. Edge and G. F. Somers (*Brit. J. Pharmacol.*, 1950, **5,** 125). Phenadoxone (heptalgin, *dl*-6-morpholino-4 : 4-diphenyl-heptan-3-one hydrochloride, CB11) is a colourless, odourless crystalline solid. It is freely soluble in water at *p*H 4; above *p*H 4 the free base begins to come out of solution, precipitation being complete in weakly alkaline solutions. It can be autoclaved without decomposition in acid solution at *p*H 2·5, but at *p*H 3·5 precipitation may occur unless the solution is suitably buffered. Phenadoxone is the most active of some 40 amino-ketones and amino-esters related to amidone examined for their analgesic activities. It has been shown to be more active on rats than morphine, amidone, pethidine or Hoechst 10581 (the hexane analogue). In spite of this its acute toxicity to mice is lower than that of amidone and its therapeutic index is therefore correspondingy higher. Its pharmacological properties closely resemble those of amidone. At therapeutic dose levels undesirable effects such as cardiac depression and vasomotor disturbances are absent, but it has a strong respiratory depressant action when given in high doses and should be used with special caution intravenously. It has a weak spasmolytic action on smooth muscle and has some surface analgesic action. No irritant action was observed after injection by the subcutaneous, intramuscular or intravenous route. There was no evidence of tolerance developing in rats dosed daily for 3 weeks, though it has been reported that dogs dosed twice daily developed analgesic tolerance in about a month.

S. L. W.

Procaine Penicillin, with Aluminium Monostearate. E. J. Wayne, J. Colquhoun and J. Burke. (*Brit. med. J.*, 1949, **2,** 1319.) Serum penicillin levels were estimated at frequent intervals for the first 24

hours and then 12 hourly for 2 days in 10 adults who received single doses of 300,000 units of procaine penicillin in arachis oil with 2 per cent. of aluminium stearate, and in 33 adults who received 600,000 units. The minimum bacteriostatic level was taken as 0·06 μ/ml., a level above this figure was maintained for 24 hours in all but one of the 10 cases receiving doses of 300,000 units, and in every case receiving the higher dosage. In the latter, the average level remained above 0·06 μ/ml. for 72 hours. Preparations with three different sizes of particle were given to 15 patients in doses of 600,000 units daily for 4 days. Constant serum levels were obtained with one preparation in which 95 per cent. of the particles were below 5 μ in diameter and with another preparation in which the majority of crystals were below 5 μ in size but which contained some rather large crystals. With the third preparation, containing particles 5 to 20 μ in size, the levels were not so constant. Detectable quantities were present in the blood 72 hours after injections ceased. Serum pencillin levels were estimated in one patient who received caronamide before and after a single dose of 600,000 units. During caronamide administration, significantly higher blood levels were noted 36, 48 and 96 hours after penicillin had been given. Serum penicillin levels were estimated by dilution in phenol-red-glucose-serum medium inoculated with *Streptococcus pyogenes* and comparison with similarly inoculated dilutions of standard penicillin in the same medium. G. R. B.

Steroid Metabolism, Abnormality in, Associated with Rheumatoid Arthritis. I. F. Sommerville, J. J. R. Duthie, G. F. Marrian and R. J. G. Sinclair. (*Lancet,* 1950, **258,** 116.) The authors have discovered an abnormality in the metabolism of progesterone in rheumatoid arthritis, a clinical condition which responds to treatment with 11-dehydro-17-hydroxy-corticosterone (cortisone), a closely related steroid. Two successive daily doses of 60 mg. of progesterone in arachis oil were injected intramuscularly into a group of men and post-menopausal women suffering from rheumatoid arthritis, and into a similar control group. Daily urine specimens were collected from each subject 2 days before injection, the 2 days when the hormone was being administered and for the following 4 days. Pregnanediol determinations were made on the samples and the results clearly showed that rheumatoid arthritics of both sexes excrete in the urine as pregnanediol an abnormally high proportion of the administered progesterone. Further work is needed to prove whether this abnormality is specific to rheumatoid arthritis and related conditions, but some support is given to the hypothesis that rheumatoid arthritis may be associated with an analogous abnormality in the metabolism of the adrenocortical steroid hormones. E. N. I.

Tetraethylthiuram disulphide and Apomorphine, Action of, in Alcoholism. R. Lecoq, P. Chauchard and H. Mazoué. (*C.R. Acad. Sci. Paris,* 1949, **229,** 1261.) Tetraethylthiuram disulphide, like alcohol, has a diphasic effect on chronaxia, which first decreases, then finally increases. When both are administered, either together or in succession, the phase of diminution disappears and the phase of augmentation is amplified and prolonged. Thus the compound has the effect of exaggerating the action of the alcohol, being in this respect quite different from substances such as vitamin B which minimise the chronaxial effects of alcohol. The latter effect is produced even after preliminary treatment with tetraethylthiuram disulphide, showing that this compound produces an accumulation of intermediate products of the catabolism of alcohol. Apomorphine acts similarly. It is this effect which causes

conditioned reflexes resulting in distaste for alcohol, but, on account of it, the use of apomorphine and tetraethylthiuram disulphide is not entirely free from danger. G. M.

Theophylline Ethylenediamine and Dihydroxypropyltheophylline, Comparative Toxicity of. K. B. Jensen. (*Arch. Pharm. Chemi*, 1949, **56**, 741.) In view of the low solubility of theophylline, and of the objection to the use of more soluble compounds such as theophylline ethylenediamine, it has been proposed to use a soluble derivative in the form of dihydroxypropyltheophylline. The toxicity tests previously recorded with the latter compound must, however, be considered as only preliminary. The two compounds have now been compared by injection into the peritoneal cavity of mice, using 15 mice at each dose level. The results show that theophylline ethylenediamine is about 5 times as toxic as dihydroxypropyltheophylline, the values for LD50 being respectively 1450 and 261 mg./kg. G. M.

Thyroid Preparations, Acetonitrile Test for Control of. P. Laland and K. F. Støa. (*Acta pharmacol.*, 1949, **5**, 1.) The test is still valuable as a biological control, supplementary to chemical estimations. For routine tests the present procedure is to use 3 groups of male mice, each mouse weighing 18 to 20 g. and fed on an ordinary diet. The first group is used to determine the LD50 of a 10 per cent. solution of acetonitrile, and the second and third the LD50 of the same solution after administration of thyroxine and thyroid respectively on the previous day. The acetonitrile solution is given by intravenous injection into the tail, the thyroxine by subcutaneous injection and the thyroid by stomach tube as a 4 per cent. suspension in water. There are in each group sufficient mice to allow 10 to receive each selected dose of acetonitrile solution, and not less than 3 doses are used. Results are given for 8 different thyroid samples together with the total iodine content of each sample. The relation between biological activity and total iodine is somewhat variable and the factors to which the variations may be due are discussed. G. R. K.

***d*-Tubocurarine Chloride; Statistical Examination of Assay on Isolated Rat-Diaphragm.** G. A. Mogey, J. W. Trevan and P. A. Young. (*Analyst*, 1949, **74**, 577.) A Bülbring preparation of the rat diaphragm and phrenic nerve is supended in 100 ml. of oxygenated Ringer-Locke solution at 37°C., and the nerve stimulated by approximately rectangular pulses of direct current, not exceeding 1 m.sec. in duration, passing down the nerve. The assay starts with doses chosen to keep the responses between 20 and 80 per cent. paralysis, each dose being allowed to act for 5 minutes. Three washes with Ringer-Locke solution are used between each pair of doses, taking about 5 minutes altogether. The 4 doses (2 of Standard and 2 of test) are assigned at random to the elements of a 4 × 4 Latin square to determine the order of testing; the analysis of variance determines whether an order effect is significant and can be eliminated. The accuracy obtainable by this method is very high for a biological assay. When the test gives fiducial limits ($P = 0{\cdot}95$) with little more than 1 per cent. either side of the mean, as it frequently does, it is of interest to examine the following possible elements of variance: (1) errors of measurement of dose, (2) errors of measurement of volume of Ringer-Locke solution, (3) inherent variability of response of endplate, and (4) possible errors due to variation in temperature, etc. Then, when the fiducial limits are just over 1·0 per cent., the standard deviation will

be 0·5 per cent. and the variance 0·25 on a percentage scale. This is the mean derived from 16 observations and the variance will be 1·0 for a single observation. Divided equally between the first 3 sources of variability the variance for each will be 0·3 and the standard variation 0·548 per cent. This corresponds to filling the bath to a mark with 100 ml. of Ringer-Locke solution with a standard deviation of 0·548 ml., and measuring 1 ml. to 0·0548 ml. with a "tuberculin" syringe of 4 cm. length for each ml., setting the piston with a standard deviation of about 0·25 mm. S. L. W.

BACTERIOLOGY AND CLINICAL TESTS

Chemicals, Bacterial Contamination of. A. T. Dalsgaad and N. Kjaer. (*Arch. Pharm. Chemi*, 1950, **57**, 57.) Tests were carried out on samples of 30 chemicals, used for injection solutions and eye-drops, with the object of comparing the official test for resistant spores with the results obtained with agar plates and by a filtration method. The greatest degree of contamination was found with glucose, sodium chloride, sodium bicarbonate, magnesium sulphate and sodium sulphate (exsiccated). It was concluded that, in general, the contamination was small, and the proportion of resistant germs especially so. Tests on samples infected by the addition of earth showed that it is necessary to use a fairly large volume of solution for the official boiling test in order that the results may be of practical value, and it is to be supposed that the content of resistant germs (Gram-positive rods) would be too small to be shown by the test. In suitable cases the use of agar plates is of advantage. The filtration method is very delicate, and can only be carried out by using aseptic precautions, since the organisms to be expected may also be present in air. G. M.

Cobalt: Effect on Bacterial Growth. A. L. Schade. (*J. Bact.*, 1949, **58**, 811.) The growth of a wide variety of bacteria of all types, aerobic or anaerobic, Gram-positive or Gram-negative, can be completely inhibited by the addition of cobalt to the culture medium, the proportion needed being from 1 to 100 p.p.m. The factors concerned in determining the effect of cobalt were investigated in a strain of *Proteus vulgaris* isolated from a patient with cystitis. The nature of the medium is of primary importance, the amount of cobalt necessary to prevent growth in a meat extract peptone broth being 100 times that needed in a synthetic medium containing glucose and ammonium sulphate. Of 17 amino acids tested only histidine and cysteine can overcome the inhibitory action of cobalt on growth, the amount required being equivalent to a molar ratio, histidine to cobalt, of at least 2 to 1. Temperature and *p*H have no effect on the activity of cobalt. The concentration of cobalt needed to show an initial effect is the same for *P. vulgaris* as for *Staphylococcus aureus*, but under anaerobic conditions the cobalt concentration needed to inhibit growth completely is 2-3 times that needed under aerobic conditions. Cells of *P. vulgaris* differ in sensitivity to cobalt at different stages of their life cycle. Viability during the resting stage is little affected by 4 hours' exposure to cobalt, while cells in the lag phase are of intermediate sensitivity. The viability after 4 hours' exposure to cobalt in the resting, lag and logarithmic phases of growth is decreased respectively to 75, 40 and 2 per cent. of the initial value. The variations in sensitivity as determined by viability at different stages of the life cycle are closely paralleled by the respiration sensitivity of *P. vulgaris* to cobalt. H. T. B.

[*Continued on page* 470