ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Alkaloids and Other Basic Drugs, Identification of, by Paper Partition Chromatography. L. R. Goldbaum and L. Kazyak. (*Analyt. Chem.*, 1956, **28**, 1289). A method is presented for the presumptive identification of μg. amounts of alkaloids and other basic drugs by means of the pattern of their R_F values or (better) the ratios of their movement in relation to a known substance (codeine being chosen) at 4 different pH values. Four chromatograms at each pH value are run simultaneously and an assumption as to identity is made by referring to tables recording the behaviour of known compounds (the data for 44 compounds, which are commonly encountered by toxicologists and pharmacologists are included). Confirmation can then be carried out by specific chemical tests if necessary after recovery of the compounds from the paper. D. B. C.

Benzalkonium Chloride, Colorimetric Determination of. K. Yoshimura and M. Morita. (*Pharm. Bull., Japan*, 1955, **3**, 432.) In this assay the benzalkonium chloride (100 to 300 μg./ml.) is precipitated with phosphomolybdic acid in strongly acid solution, the precipitate dissolved in warm acetone and the blue colour produced after the addition of stannous chloride is measured colorimetrically after 80 minutes at 25°. The time and temperature for maximum development are critical. The maximum extinction is at 730 mμ. A blank solution of the reagents is employed. The precipitation reaction is not specific for benzalkonium chloride but the advantage is that it can be determined in small amounts without any special reagent with an error less than 2 per cent.
D. B. C.

Cinchona and Nux Vomica, New Assay of. E. Brochmann-Hanssen. (*J. Amer. pharm. Ass., Sci., Ed.*, 1956, **45**, 74.) The following assay for cinchona and nux vomica depends on extraction of the alkaloids with a cation exchange resin and purification with an anion exchange resin. 0·1 g. of finely powdered cinchona or nux vomica was placed in an extraction tube with 1 ml. of 2·5 N formic acid, 1 g. of activated cation exchange resin (Dowex 50–X2) of high porosity and 25 ml. of boiling water. After shaking for 30 minutes (15 for nux vomica), maintaining the temperature at 80–90°, the extracted drug was removed by back-washing. The resin was washed and the alkaloids eluted with 4 N ammonium hydroxide in methanol (70 per cent). The eluate was purified by passing it through a highly porous strongly basic anion exchange resin (Dowex 1–X1), and the alkaloids determined by ultra-violet spectrophotometry, using as blank, methanolic ammonium hydroxide which had been passed through Dowex 1–X1 resin. The method of extraction of cinchona described above was found to be more efficient than the usual extraction with solvents, giving rise to assay results 10 to 15 per cent higher. The results for nux vomica were also higher than by the official method, in which a loss of strychnine occurs during the oxidation of brucine with nitric acid and sodium nitrite. G. B.

New Indicator for the Titration of Calcium with EDTA. J. Patton and W. Reeder. (*Analyt. Chem.*, 1956, **28**, 1026.) A new indicator for calcium, 2-hydroxy-1-(2-hydroxy-4-sulpho-1-naphthylazo)-3-naphthoic acid, enables it to be accurately estimated in the presence of magnesium using standard disodium edetate. The colour change is from wine red to pure blue. Titration procedures are given for the estimation of calcium and magnesium in water, limestone, salt and boiler scale. The usual ammonia-ammonium chloride buffer is replaced by an odourless monoethanolamine-hydrochloric acid buffer containing complexed magnesium to render the end point sharper. The total calcium and magnesium is estimated by using eriochrome black T as indicator in the presence of the above buffer. Calcium is then estimated using the new indicator in a solution containing 4 ml. of 8 N potassium hydroxide in about 50 ml. In both cases about 30 mg. of each of potassium cyanide and hydroxylamine hydrochloride are added to prevent interference from zinc, copper, cobalt, nickel and manganese. Blank determinations are performed at every stage. The amount of magnesium is calculated from the difference between the two titrations. Interfering ions are discussed. In trial analyses, the maximum variation encountered was 2 p.p.m. as calcium carbonate and 1 p.p.m. as magnesium carbonate, and the results agreed with those obtained by other methods. D. B. C.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

***Digitalis purpurea*, Transformation of Unknown Glycosides into the Known Cardiac Glycosides.** K. B. Jensen. (*Acta pharm. tox., Kbh.*, 1956, **12**, 20.) The glycosides, A_1, A_2, A_3, A_4, A_6, B_2, B_3, B_4, B_6 and B_9 described in the preceding paper (*Acta pharm. tox., Kbh.*, 1956, **12**, 20), were isolated by paper chromatography as previously described. Appropriate cut-out filter strips were treated in M/1 phosphate buffer (pH 5·9) with the enzyme digipurpidase when only three of the glycosides, A, B_3 and A_2, were shown to be hydrolysable. With sodium bicarbonate, B_1 gave digitalinum verum, B_3 gave purpurea glycoside B, B_6 gave strospeside, and B_9 gave gitoxin. Acid hydrolysis was more complex but in every case gave either gitoxigenin or digitoxigenin. J. B. S.

ORGANIC CHEMISTRY

Steroids, Potent Oral Anabolic-Androgenic, Synthesis of. M. E. Herr, J. A. Hogg and R. H. Levin. (*J. Amer. chem. Soc.*, 1956, **78**, 500.) The preparation of three 11-oxygenated C-(19) steroids, 11β: 17β-dihydroxy-17-methyl-4-androsten-3-one 11β : 17β-dihydroxy-9α-fluoro-17-methyl-4-androsten-3-one and 17β-hydroxy-9α-fluoro-17-methyl-4-androstene-3 : 11-dione is briefly described. Their oral anabolic activities in terms of 17-methyltestosterone activity (1·0) are 2·9, 20·0 and 22·0 respectively and their androgenic activities compared with the same standard are 0·9, 9·5 and 8·5. A. H. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

D-Glucosamine, Anaerobic Deamination of, by Bacterial and Brain Extracts. P. Faulkner and J. Quastel. (*Nature, Lond.*, 1956, **177**, 1216.) Experiments have been carried out to investigate the deamination of D-glucosamine. Suitably prepared extracts of *E. coli* and of brain tissue were used. Incubations were carried out in the Warburg manometric apparatus. Estimations of D-glucosamine, ammonia, hexosediphosphate, phosphates and sugar phosphates were

made. Deamination of D-glucosamine and *N*-acetylglucosamine takes place anaerobically in an extract of *E. coli*, the process being dependent on the presence of adenosine triphosphate and magnesium ions. Little or no breakdown of D-glucosamine takes place in the bacterial extract under aerobic conditions. Glucosamine is deaminated at a slower rate than *N*-acetylglucosamine, but when mixtures of the two are incubated with adenosine triphosphate a more than additive rate of ammonia production takes place. Thus it is likely that the substrate for D-glucosamine deamination is D-glucosamine-6-phosphate. A similar conclusion applies for *N*-acetylglucosamine. Dialysed *E. coli* extracts cause phosphorylation of glucose and D-glucosamine in the presence of adenosine triphosphate, the same hexokinase apparently being involved. The phosphorylation of *N*-acetylglucosamine requires a separate kinase. Glucosamine breakdown in an *E. coli* extract is inhibited by the presence of glucose. Adenosine triphosphate cannot be substituted by adenosine-5-phosphate for the anaerobic deamination of D-glucosamine in an *E. coli* extract. However, a mixture of acetyl phosphate and adenylic acid can replace the adenosine triphosphate. The D-glucosamine anaerobic deaminating system in *E. coli* is unaffected by the presence of either diphosphopyridine nucleotide or by L-glutamate or by pyridoxal phosphate. Electrophoresis on filter paper of a mixture of D-glucosamine, a trace of *N*-acetylglucosamine, magnesium ions, adenosine triphosphate and dialysed *E. coli* extract, after anaerobic deamination at 37° has taken place for one hour, shows the presence of D-glucosamine-6-phosphate and hexosediphosphate. It is possible that the hexosediphosphate formed is produced secondarily by phosphorylation of fructose-6-phosphate by excess adenosine triphosphate present. Thus the following reaction in an *E. coli* extract takes place:

$$\text{D-glucosamine} \xrightarrow{\text{ATP}} \text{D-glucosamine-6-phosphate} \rightarrow \text{fructose-6-phosphate} + \text{ammonia} \xrightarrow{\text{ATP}} \text{hexosediphosphate}$$

Small quantities of *N*-acetylglucosamine stimulate the deamination of D-glucosamine. Such an effect cannot be produced by *N*-acetylmethionine, acetylcholine or *N*-acetylglycine. Thus it is suggested that the stimulatory or catalytic action of *N*-acetylglucosamine deamination may be due to an inhibitory effect on the hydrolysis of a phosphate ester formed during the course of the D-glucosamine breakdown. However the phosphate ester of *N*-acetylglucosamine might play a part in the catalytic reaction. D-Glucosamine is deaminated anaerobically by dialysed brain extract in the presence of adenosine triphosphate and magnesium ions. This process however, is not stimulated by the addition of *N*-acetylglucosamine. *N*-acetylglucosamine is not deaminated in brain extracts nor does it undergo phosphorylation in the presence of adenosine triphosphate. The presence of glucose inhibits anaerobic deamination of D-glucosamine in brain extracts, this probably being due to competition between glucose and glucosamine or between their respective esters. The evidence would indicate that D-glucosamine-6-phosphate is the substrate of the anaerobic deaminating enzyme in brain tissue as in *E. coli*, but that the factors in *E. coli*, operating with *N*-acetylglucosamine and which account for the effects of this substance in the bacterial deaminating system, are not present in the brain extract. M. M.

BIOCHEMICAL ANALYSIS

Iron in Serum, Improved Determination of. P. Trinder. (*J. clin. Path.*, 1956, **9**, 170.) Heat 2 ml. of serum, 2·5 ml. of water and 1·5 ml. of 20 per cent

trichloroacetic acid for 10 minutes at 90 to 95°. Cool and centrifuge. To 4 ml. of the supernatant add 0·2 ml. of iron reagent, 0·6 ml. of 40 per cent sodium acetate and 0·4 ml. of 1 : 1 sulphuric acid. Read the optical density of the unknown in an absorptiometer using an Ilford 624 green filter, or in a spectrophotometer at 535 mμ. The iron content is obtained from a standard calibration curve. A blank is prepared by heating 3 ml. of water and 1 ml. of 20 per cent trichloroacetic acid. For the preparation of the iron reagent, add 0·5 ml. chlorsulphonic acid to 100 mg. of 4 : 7 diphenyl 1 : 10 phenanthroline, and boil for 30 seconds. Cool, add 10 ml. of water and heat at 100° until the precipitate is dissolved. Make up to 100 ml. with water and add 1 ml. of thioglycollic acid. The recovery of added ferric iron is quantitative and a moderate haemolysis does not affect the result. G. F. S.

Pyruvate in Plasma, Determination of. K. S. Henley, H. S. Wiggins and H. M. Pollard. (*J. Lab. clin. Med.*, 1956, **47**, 978.) An enzymatic method is described for the determination of pyruvate in plasma using lactic acid dehydrogenase and reduced diphosphopyridine nucletide (DPNH). Heat 1 ml. of plasma from heparinised blood in a boiling water bath for 15 minutes. Add 6 ml. of M/15 phosphate buffer (pH 7·41, prepared by mixing 80·8 ml. of M/15 anhydrous dibasic sodium phosphate solution with 19·2 ml. of M/15 monobasic sodium phosphate solution). Homogenise the mixture in a Potter-Elvehjem homogeniser with a Teflon pestle. Centrifuge and mix 5 ml. of the supernatant with 1 ml. of DPNH solution. Divide the fluid approximately equally between two matched cuvettes. To the first add 0·01 ml. of lactic acid dehydrogenase solution (0·01 ml. of enzyme to 0·5 ml. of 0·225 per cent saline), stir and allow reaction to go to completion (2 to 3 minutes). This is the blank and using it read the optical density of the second cuvette at 340 mμ. This density is an index of the DPNH oxidised and is equivalent to the amount of pyruvate in the solution. If C is the difference in optical density between contents of the two cuvettes and the micromolecular extinction coefficient of DPNH is 2·05 per 3 ml. at 340 mμ then

Micromols. pyruvate/100 ml. $= 100\text{ C} \times 7/1 \times 6/5 \times 1/3 \times 2{\cdot}05 = 137\text{ C}$
or mg. pyruvate/100 ml. $= 88/1000 \times 137\text{ C} = 12{\cdot}1\text{ C}$

The method has a standard error of 3·6 per cent and a normal plasma pyruvate level is 0·69 ± 0·25 mg. per 100 ml. G. F. S.

Pyruvic Acid in Blood, Determination of. S. Segal, A. E. Blair and J. B. Wyngaarden. (*J. Lab. clin. Med.*, 1956, **48**, 137.) An enzymatic spectrophotometric method is described for the estimation of pyruvic acid in whole blood. It is based on the measurement of the oxidation of reduced diphosphopyridine nucleotide (DPNH) by pyruvic acid in the presence of lactic dehydrogenase. Pipette 5 ml. of blood into a rubber stoppered centrifuge tube containing 5 ml. of 7 per cent perchloric acid. Centrifuge twice and place 2 ml. of the clear supernatant into a small beaker in ice. Adjust the pH to 3 or 4 with 5 N potassium hydroxide (about 0·2 ml.). Remove the precipitate of potassium chlorate by centrifuging. Place 1 ml. of the supernatant into a quartz cuvette and add 1 ml. of buffer (phosphate buffer 0·1 M pH 7·0 to 7·4), 0·1 to 0·2 μM. diphosphopyridine nucleotide (about 0·05 to 0·1 ml. of solution prepared by hydrosulphite reduction of DPN) and sufficient water so that after addition of the enzyme the total volume is 3 ml. Read the optical density (OD) at 340 mμ in a spectrophotometer against a water blank. It is initially 0·300 to 0·600 OD. Add lactic dehydrogenase (usually 3 to 5 μg. enzyme protein) such that the reaction, as followed by OD change, is complete in from 4 to 7

minutes. The change usually consists of − 0·40 to − 0·085 OD unit. An enzyme blank is run concomitantly with the substitution of 1 ml. of water for the filtrate. The concentration of pyruvate is calculated from

$$\frac{[(\Delta OD_{340\,m\mu}) - (\Delta OD_{340\,m\mu}\,\text{blank})]}{*\,6{\cdot}22 \times 10^{6}\,\text{cm.}^{2}\,\text{mole}^{-1}} \times \text{mol. wt. pyruvate} \times \text{dilution} \times 100 =$$

mg./100 ml. of blood

where * is the molar extinction coefficient of DPNH.

Recoveries of pyruvate added to whole blood were 97 to 104 per cent of the theoretical. The blood pyruvate level in fasting normal subjects ranged from 0·39 to 0·86 mg. per cent. Wide daily variations occurred. Results are reported in diseases in which elevated pyruvate levels occur, rheumatoid arthritis, liver disease and diabetes etc. G. F. S.

CHEMOTHERAPY

Antibacterial Activity of Dried Australian Plants. N. Atkinson. (*Austral. J. exp. Biol. med. Sci.*, 1956, **34**, 17.) This is an account of tests to detect the presence of antibiotic substances in flowering plants. The general methods used previously had entailed an extraction with water or other solvent and testing of the extracts by the cylinder or cup plate methods against several micro-organisms. The author found that these methods lacked sensitivity as was proved by the results of a new test—called the "direct plate test"—which was applied to 124 specimens of dried flowering plants collected in Australia. The test was carried out by seeding a tryptic digest agar with the test organism and pouring into Petri dishes. A small piece of the required plant part was placed on the agar surface, testing 4 or 5 specimens on each plate. Many of the plants were also powdered and a small heap of the powder placed on the agar. Widths of zones of inhibition were measured after incubation for 18 hours. The 3 test organisms used were specified strains of *Salmonella typhi*, *Staphylococcus aureus* and *Mycobacterium phlei*. Of the 124 plants studied 75 showed some inhibition by at least 1 plant part against at least 1 organism. Many of the reactions were very weak (zone width 0·5–1 mm.), but 30 plants showed fair to good zones of inhibition, best among which were the following which were active against all 3 test organisms: nut kernels of *Cycas circinalis* and *Cycas media* (Cycadaceae), root of *Plumbago zeylanica* (Plumbaginaceae), fruit of *Rhodomyrtus macrocarpa* (Myrtaceae), leaves and branchlets of *Flindersia xanthoxyla* (Rutaceae), and bark of *Lunasia amara* (Rutaceae). The most active family was Rutaceae and the least active was Leguminosae. All specimens were dried and some were about 3 years old. It is suggested that the antibiotics of these plants must be relatively stable substances and should be worth further investigation. B. A. W.

Antibacterial Substances Produced by Flowering Plants. N. Atkinson and H. E. Brice. (*Austral. J. exp. Biol. med. Sci.*, 1956, **33**, 547.) The essential oils from thirty-four Australian plants have been tested for antibacterial activity against *S. typhi*, *Staph. aureus* and *M. phlei* by the Tween agar and Tween broth dilution tests. Only a few of the oils had even a fair activity against *Staph. aureus* and *S. typhi*, but all the oils showed activity against *M. phlei.*, ranging from weak to very good. Oils from numerous members of the Myrtaceae were included, and the best overall activity was shown by oils from *Backhousia augustifolia*, *Backhousia citriodora*, *Leptospermum citratum*, *Leptospermum liversidgei*, *Melaleuca bracteata* and *Dacrydium franklini*. It is considered that Australian essential oils are not strong antibacterial agents. G. F. S.

PHARMACY

Antibacterial Agents in *Pseudomonas aeruginosa* Contaminated Ophthalmic Solutions. S. Riegelman, D. G. Vaughan, Jr. and M. Okumoto. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 93.) The solutions examined were phenylmercuric nitrate up to 0·01 per cent, benzalkonium chloride up to 0·02 per cent, chlorbutol up to 0·8 per cent, phenylethyl alcohol 0·5 and 2 per cent, and polymixin B sulphate 50 to 1000 units/ml. *In vitro* tests were carried out using various dilutions of the antiseptics in conjunction with solutions contaminated with *Ps. aeruginosa*, suitable inactivating agents being used to counteract the effect of the antiseptic during incubation. In addition, *in vivo* tests were carried out by injecting the solutions intracorneally into rabbit eyes. In the intracorneal tests infections were produced after one week's contact with concentrations of benzalkonium chloride and phenylmercuric nitrate shown to be bacteriostatic in the *in vitro* tests. It is concluded that "bacteriostatic" concentrations are not adequate for use in ophthalmic solutions. The best preservative for these preparations appeared to be polymixin B together with a bactericide effective against Gram-positive organisms. G. B.

Emulsions. The Effect of Two-stage High Pressure Homogenisation on the Stability of. J. D. Mullins and C. H. Becker. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 105.) Emulsions were prepared with 25 per cent of arachis oil, cod-liver oil, glyceryl ricinoleate or glyceryl trioleate, using acacia 2·5 per cent or a mixture of polysorbate 80 and sorbitan sesquioleate (Aracel C) as the emulsifying agent, and passing the product twice through a two-stage homogeniser with the first valve at 1500, 2000, 2500 or 3000 lb. wt./sq. in., and the second at 1000 lb. wt./sq. in. Samples of the emulsions were diluted with propylene glycol, placed in a haemocytometer cell and examined microscopically, using a globule size-frequency method of analysis. The specific interfacial area was found to increase directly with the homogenisation pressure, and during storage the emulsions prepared with non-ionic emulsifying agents showed a linear decrease in specific interfacial area. Emulsions prepared with acacia were characterised by a lower specific interfacial area and a greater proportion of relatively large oil globules. Deterioration in acacia emulsions was usually due to coalescence of globules to form larger ones, whereas in the polysorbate 80-sorbitan sesquioleate emulsions, deterioration took the form of separation of free oil rather than coalescence of globules. The viscosity of the emulsions was not affected by changes in the specific interfacial area or by the oil used, although it varied with different emulsifying agents. G. B.

Vitamin B_{12} in Solutions of Ascorbic Acid, Copper-promoted Decomposition of. A. J. Rosenberg. (*J. biol. Chem.*, 1956, **219**, 951.) The decomposition of vitamin B_{12} in solutions containing ascorbic or dehydroascorbic acid is considerably accelerated in the presence of cupric ions, although cupric ions are without effect on vitamin B_{12} alone. One cupric ion appears to be inactivated for each molecule of vitamin B_{12} destroyed. A study of the kinetics of the reaction indicates that the rate-determining step is a unimolecular transformation. It is suggested that a copper ion combines with 1 molecule of ascorbic acid to produce an intermediate product (X) which is then transformed into another product (Y) together with a cupric ion in an inactivated form. The product (Y) reacts with vitamin B_{12} yielding cobalamin-like intermediates which then undergo further decomposition. G. B.

PHARMACOLOGY AND THERAPEUTICS

Anticholinergic Drug (BL700B), Pharmacological Evaluation of. R. D. Judge, R. J. Bolt, B. L. Hirschowitz and H. M. Pollard. (*J. Lab. clin. Med.*, 1956, **47**, 950.) A new anticholinergic drug, αα-diphenyl-γ-dimethylaminobutyramide-ethobromide has been evaluated in humans. The compound was compared orally with atropine in males, all of whom were capable of a continuous secretion of acid gastric juice. Studies were made of the gastric juice obtained by stomach tube and gastric motility recorded from a balloon. Side effects were determined in 5 normal young men placed on a 12·5 mg. dose four times daily. Dosage response data indicated that 10 to 25 mg. orally reduced gastric acidity and secretory volume without significant side effects and 12·5 mg. orally was superior to 0·6 mg. of atropine sulphate orally. No tolerance was observed with 12·5 mg. four times daily for 7 days. G. F. S.

Ganglion-blocking Agents in Hypertension, Two New. S. Locket. (*Brit. med. J.*, 1956, **2**, 116.) This paper is a report on the use over a period of a year and a half of two new ganglion-blocking agents (356c54 and 139c55) in the treatment of 15 severe cases of hypertension. Chemically, 356c54 is *N'N'N''*-trimethyl-*N'*-(6-cyano-6:6-diphenylhexyl)ethylene-1-ammonium-2-morpholinium dichloride, and 139c55 (also called Presidal) is the 5-cyano-5:5-diphenylpentyl homologue. The compounds were given subcutaneously, using a solution containing 20 mg. of the dichloride of either drug per ml. With 356c54 the minimal effective therapeutic dose was 10 mg. and the maximum dose 40 mg.; with 139c55 the minimal dose was 5 mg. and the maximum 27·5 mg. With identical hypotension-producing doses the duration of hypotension, using 139c55, often exceeded 12 hours, and on occasion 24 hours, and was rarely less than 9 hours, while with 356c54 it was rarely less than 7 hours and might exceed 24 hours, though this latter duration occurred much less frequently than with 139c55. The maximum fall in blood pressure was reached 60 to 120 minutes after the onset and persisted for some time before a slow return was made to pre-treatment height. The drugs may also be administered intravenously or by mouth, but in the former case the duration of hypotensive action is much shorter than by subcutaneous injection, and with oral administration, even with doses 15 to 20 times the effective subcutaneous dose, the hypotensive response could not be accurately predicted. No hypertensive symptoms were made worse by the treatment, and where a change occurred it was for the better. Attacks of left ventricular failure stopped when treatment began, breathlessness was relieved and angina of effort ceased to occur. When electrocardiographic changes occurred they were towards normal, and heart size was reduced. In 7 patients the papilloedema which was present subsided. Headaches which were hypertensive in origin were immediately ameliorated, but recurred with inadequate treatment. Side-effects observed included a transient blurring of vision, within a few minutes of injection, dryness of the mouth (more frequent and severe with 356c54 than with 139c55), and mild constipation. Large doses of both drugs could produce postural syncope, and bradycardia was present in most cases. Nausea and vomiting did not occur, and the absence of marked effect upon the small-intestine propulsion was one of the most interesting and useful features of the drugs. The author concludes that the advantages of the compounds, particularly 139c55, in the treatment of hypertension, lie in the more gradual onset of activity, the greater duration of action, the slowing effect on the heart rate, and the relative lack of action on the small intestine. S. L. W.

Spiramycin, Acute Toxicity and Activity of. C. Cosar. (*Thérapie*, 1956, **11**, 324.) Spiramycin, an antibiotic obtained from cultures of *Streptomyces ambofaciens* is not very toxic to mice. By subcutaneous injection the LD50 is about 1·7 g./kg.; 5 g./kg. may be given by mouth. It is very active against peritoneal infections by pneumococci, streptococci and staphylococci, and is effective against strains of *Staphylococcus* which are resistant to other antibiotics such as penicillin and aureomycin. It is more effective against pneumococci than erythromycin or carbomycin. It is not active against *Trypanosoma congolense* and *T. brucei* but shows some activity against *Plasmodium berghei.*

G. B.

Spiramycin, Chronic Toxicity and Effects of Local Administration of. P. Dubost, R. Ducrot and M. Kolsky. (*Thérapie*, 1956, **11**, 329.) The administration of spiramycin to rats and dogs over a period of 4 weeks did not cause any serious untoward effects. Experiments were carried out in which a 10 per cent solution was instilled into the eyes of rabbits, a 2 per cent solution was injected subcutaneously into rats and a 1 per cent solution was injected intramuscularly into rabbits. In all these forms of local administration the drug was well tolerated. G. B.

Tremorine, Tremor produced by. G. M. Everett. (*Nature, Lond.*, 1956, **177**, 1238.) 1:4-Dipyrrolidino-2-butyne (Tremorine) in doses of 5–20 mg./kg. produces tremor, salivation, meiosis, slight muscular weakness and rigidity lasting several hours. The effects are similar in rats, mice, guinea-pigs, cats, dogs and monkeys. In monkeys the picture is very similar to Parkinsonism. The antagonism of these effects is brought about with various drugs, such as atropine and scopolamine, that are used for the treatment of Parkinsonism. In contrast to this, hypnotics, anticonvulsants and ganglionic blocking drugs are ineffective in doses below those causing marked depression. Banthine was found to control the parasympathetic effects of salivation and diarrhoea but had no effect on the tremor; thus demonstrating the distinct peripheral and central actions. Decerebrate animals develop tremor after the drug. It also causes a marked fall in body temperature. The chemical structure producing tremor is very specific within this chemical type. Twenty such analogues show no such action. Thus Tremorine may be a useful tool in the investigation of tremor and the search for more effective agents against Parkinsonism. M. M.

Triiodothyroacetic Acid (Triac): Effect on Blood-Cholesterol Levels. W. R. Trotter. (*Lancet*, 1956, **270**, 885.) Triiodothyroacetic acid (triac) was given to 3 patients with myxoedema and to 18 euthyroid patients. Its relative effects in lowering the blood-cholesterol level and in raising the B.M.R. were compared with those of thyroxine in 1 case of myxoedema; triac appeared to have a greater effect than thyroxine on the blood-cholesterol level. In a similar comparison with triiodothyronine in 2 other cases of myxoedema, the action of the two drugs appeared to be similar but triiodothyronine was about 75 times more effective than triac both in depression of the blood-cholesterol level and elevation of B.M.R. In euthyroid patients triac in a dose of 2–4 mg. daily lowered the blood-chloesterol level by about a quarter; triiodothyronine 0·08 mg. daily had a similar effect. In 3 patients whose dose of triac was increased gradually up to 3 or 4 mg. daily there was no obvious rise in the B.M.R. though the plasma-cholesterol level appeared to fall. Triac reduced the uptake of radioactive iodine in euthyroid patients, the dose required being about 25 times

greater than the triiodothyronine. It cannot be claimed at present that triac has any properties not shared by the other thyroid hormones or that it is likely to be particularly useful in lowering the blood-cholesterol level of euthyroid patients. S. L. W.

Viadril: A New Steroid Anaesthetic. L. H. Lerman. (*Brit. med. J.*, 1956, **2**, 129.) Viadril is a steroid, 21-hydroxypregnane-3:20-dione sodium succinate. It is a non-volatile crystalline solid, freely soluble in water, the solution having a pH of 7·8 to 10·2. Its value as an anaesthetic is that it telescopes the narcotic and relaxant functions and requires only minimal nitrous oxide and oxygen to maintain anaesthesia. The drug is given as a 2·5 per cent solution in normal saline and is injected into the giving-set tubing of a normal-saline drip, just below the inspection chamber. The intravenous injection of Viadril causes loss of consciousness, analgesia, absent corneal reflex, and muscular relaxation, particularly of the larynx. It has no demonstrable hormone effects. No electrocardiographic changes have been observed, no haemolysis, and no depression of respiration. Hypotension commonly occurs but blood pressure is soon restored by small doses of pressor drugs. Nitrous oxide has a marked synergism with Viadril. If necessary, relaxant drugs, pethidine, and the volatile anaesthetic agents can be given at the same time as Viadril, but much smaller doses are needed. Viadril induction is quite different from thiopentone induction. There is no yawning or sighing, no apprehension, and no unpleasant smell or taste. The patient falls asleep quite naturally and there is no stertor. He remains in an apparently normal sleep on return to the ward and can be roused in a short period. At the end of an hour or so he is fully awake, says he feels well and is in no pain, euphoria being a distinct feature of this agent. Vomiting is rare. The disadvantages of Viadril are the slowness of induction and the risk of thrombophlebitis in the injected arm. In the series of 19 cases presented in this paper the average time of operation was 26 minutes and the average dose of Viadril was 824 mg. All except 3 were premedicated with Omnopon and scopolamine. None received pethidine during the operation. Details of the 19 cases are tabulated and 3 case histories given to emphasize special points. S. L. W.

Viadril: An Intravenous Steroid Anaesthetic. A. G. Galley and M. Rooms. (*Lancet*, 1956, **270**, 990.) Viadril is a derivative of pregnanedione (21-hydroxypregnane-3:20-dione, sodium hemisuccinate) which has anaesthetic properties and is sufficiently soluble in water to be given intravenously. Animal experiments indicate that it has a much higher therapeutic index than thiopentone, that it is not carcinogenic and that it shows no signs of hormonal activity, such as salt retention. In contrast to all the ultra-short acting barbiturates, Viadril rapidly suppresses the pharyngeal and laryngeal reflexes, so that even under the lightest narcosis it is difficult to provoke vomiting or laryngospasm. Falls of blood pressure and a rise in pulse-rate are more frequent with Viadril than with thiobarbiturate anaesthesia, but the fall in blood pressure often rectifies itself or can readily be remedied by methylamphetamine. Vomiting is rare after Viadril anaesthesia, and post-operative fatigue is slight or absent, patients often experiencing a sense of well-being during recovery from operation. In concentrations greater than 0·5 per cent Viadril solutions give rise to venous thrombosis and it must therefore be given as an intravenous drip; this may limit the use of the drug for shorter operations, but for long operations where

the intravenous drip is inevitable it may well prove the anaesthetic of choice. A suitable solution may be readily prepared by dissolving the contents of five 500-mg. vials of Viadril in a 540-ml. bottle of intravenous saline; however rapidly it is run in, this solution is trouble-free and causes no venous sclerosis. With a drip-rate of 150 per minute the average patient becomes drowsy within 5 to 7 minutes and in 15 minutes he is deeply asleep, though he reacts to painful stimuli. The usual induction period of 20–25 minutes can be halved as follows: directly the patient falls asleep a mask is held within an inch or so of the face and pure nitrous oxide administered. The mask is gradually lowered until it touches the face, when 20 per cent oxygen is added to the mixture. After 2 minutes 30–50 mg. of pethidine is injected intravenously, followed by a suitable dose of a muscle relaxant. After another minute the mask can be removed, laryngoscopy performed, and an endotracheal tube passed; anaesthesia is maintained with nitrous oxide and oxygen. Doses of Viadril up to 2 g. do not appear to affect either the depth or rate of respiration. From experience gained in the use of Viadril in 100 operations the authors conclude that whether used alone or in place of thiopentone in the hypnotic-analgesic-muscle relaxant sequence, it offers not only ideal operating conditions but ensures a more comfortable recovery period than any other anaesthetic or anaesthetic combination in common use.

S. L. W.

BACTERIOLOGY

***Clostridium perfringens* Type A Toxin, Protection Against, by a Metal-chelating Compound.** M. Moskowitz, M. W. Deverell and R. McKinney. (*Science*, 1956, **123**, 1077.) The α-toxin of *Clostridium perfringens* (*C. welchii*) type A is a lecithinase enzyme known to be an important lethal factor in infections of this organism. The enzyme is activated by calcium and the authors sought to demonstrate whether a lethal outcome of experimental infections could be reduced by removal of Ca^{++} from the area of infection. Intracutaneous injection of lethal doses of organisms mixed with sodium oxalate or citrate into mice gave no decrease in mortality. The use of the metal-chelating agent ethylenediamine tetra-acetic acid (EDTA) as a calcium removing agent gave protection in some but not all cases. Further tests were made in which toxic culture filtrates were used in place of the organisms, when it was found that whereas 2 MLD doses of the toxin killed mice in 10 hours, a mixture of the same dose of toxin with 0·68 per cent solution of EDTA in borate buffer gave complete protection. This protection could not be reversed by addition of excess Ca^{++}, but when Zn^{++}, Co^{++}, or Mn^{++} (which also activate lecithinase) were added to the toxin-EDTA mixture, the toxicity was restored. Tests on the *in vitro* inhibition of lecithinase using the lecithovitellin test gave results similar to those obtained *in vivo*: EDTA inhibited the lecithovitellin reaction; excess of either Zn^{++}, Co^{++} or Mn^{++} reversed the inhibition but excess of Ca^{++} did not. It is suggested that Ca^{++} activates but is not essential for the activity of lecithinase, whereas Zn^{++}, Co^{++} and Mn^{++} are essential for activity, it appearing that these ions are preferentially chelated over Ca^{++}. Further experiments in which toxin and EDTA were separately injected on opposite sides of mice revealed that greater protection was afforded when toxin and EDTA were simultaneously injected, indicating that inhibition of the toxin at the site of injection is involved in the protection. The authors report that EDTA is non-toxic except in high doses which induce hypocalcaemia; but Ca-EDTA can be given in large quantities and has possible uses in gas gangrene therapy.

B. A. W.

Dihydrostreptomycin and Anaerobiosis—Comparison with other Antibiotics and its Selectivity with regard to Obligate Anaerobes. G. M. Williamson and F. White. (*J. gen. Microbiol.*, 1956, **14**, 637.) The authors report on a comparison of the sensitivity to various antibiotics of a number of facultative anaerobic bacteria whilst growing under aerobic and anaerobic conditions. The organisms used were *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae* type B, *Escherichia coli*, *Aerobacter aerogenes*, *Streptococcus pyogenes*, and *S. pneumoniae*. The antibiotics tested were dihydrostreptomycin, penicillin, chloramphenicol, erythromycin, carbomycin, chlortetracycline, oxytetracycline and tetracycline. The tests employed heated blood (10 per cent) agar (pH 7·8). Ditch plates were prepared, the ditches containing either 20 units per ml. penicillin or 20 μg. per ml. of one of the other antibiotics, and the inoculum consisted of one standard loopful of a suitable dilution of a fluid culture of the test organisms. Plates for anaerobic tests were incubated in a McIntosh and Fildes jar for 48 hours. Inhibition of growth was measured as linear distance of complete inhibition of growth extending from the edge of the ditch. It was found that dihydrostreptomycin was unique among the antibiotics tested in giving a greater inhibition of facultative anaerobes when growing aerobically than when growing anaerobically. Dihydrostreptomycin was found to be relatively inactive against *Clostridium welchii* and *C. novyi*, the inhibitory concentration being dependent on the inoculum size. Investigations into the possible use of dihydrostreptomycin as a selective agent for isolation of *C. welchii* revealed that use of the antibiotic (dissolved in cooked meat medium) was less efficient than was heating at 65° for 30 minutes. B. A. W.

Ethylene Oxide, Sporicidal Activity of. J. L. Friedl, L. F. Ortenzio and L. S. Stuart. (*J. Assoc. off. agric. Chem., Wash.*, 1956, **39**, 480.) A study of the sporicidal activity of ethylene oxide when tested against 5 aerobic and 5 anaerobic spore-forming bacterial species is described. The tests were carried out in accordance with the sporicide method of the Association of Official Agricultural Chemists, in which the test organisms are dried on surgical suture loop carriers at room temperature for 24 hours over $CaCl_2$. The contaminated carriers were placed in individual petri dishes with the lids suspended above the bases of the dishes in order to obtain contact between spores and gas. The dishes were stacked in a jar which could be evacuated and then filled with ethylene oxide. Exposure periods of 10, 20, 30 and 60 minutes and of 3, 6 and 18 hours were used. Subcultures were incubated for 7 days at either 37° or 55° depending on the organism. Simultaneous exposures were made on wet drained carriers and on dried carriers in the case of *Cl. sporogenes* and *B. subtilis*, when it was found that dried spores were much more resistant to ethylene oxide than were wet spores. Dried spores of all 10 test organisms were destroyed after an exposure of 18 hours. *B. subtilis* and *Cl. sporogenes* were able to survive a 6 hour exposure; *Cl. tetani*, *Cl. botulinum*, *Cl. lentoputrescens*, *B. stearothermophilus* and *B. coagulans* survived for 60 minutes; *B. anthracis* and *B. globigii* survived exposures of 30 minutes but were killed by an exposure of 60 minutes. Resistances of all the test organisms to constant boiling HCl at 20° were also tested and no direct relation found between these resistances and the resistances to ethylene oxide. The authors conclude that ethylene oxide can be used as a sterilising agent but that prolonged exposures are necessary to ensure destruction of spores of all commonly encountered bacteria. B. A. W.