ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Alkaloid from Greek Belladonna Root. E. Steinegger and G. Phokas. (*Pharm., Acta Helvet.*, 1956, **31**, 330.) The alkaloids from the root were separated on a cellulose column by the formic-acetic acid method of the authors. The new alkaloid, "Hellaradine", gave a picrate of m.p. 117 to 118·5°. Pharmacological tests showed a mydriatic action, and a spasmolytic effect slower and weaker than that of atropine. The proportion of alkaloid in the root is about 0·002 per cent. G.M.

ANALYTICAL

Alkaloids, Buffered Chromatography of. A. Bettschart and H. Flück (*Pharm. Acta Helvet.*, 1956, **31**, 260.) For the buffered chromatography of alkaloids certain factors must be taken into consideration. A relatively high concentration of buffer (e.g., M/2) is often objectionable for paper chromatography since, if the components do not have the same solubility, then it is possible for a change in pH to occur when the paper is equilibrated with the solvent. The McIlvaine citrate-phosphate buffer is recommended. The buffer should not be soluble in the mobile phase, especially for column chromatography. Finally, the buffer should not be sensitive to carbon dioxide when used on paper. The authors report a considerable number of trials on the separation of morphine alkaloids, and show that morphine, thebaine, narceine and codeine can be separated on a phosphate-citric acid buffered paper at pH 6·8, while papaverine and narcotine can be separated at pH from 3·8 to 4·1, using ether as solvent. For detection, fluorescence and iodine-potassium iodide reagent are used. The authors give a review and bibliography of the chromatography of alkaloids. G. M.

Alkaloids of Hemlock (*Conium maculatum* L.), Separation, Micro-estimation and Distribution of. B. T. Cromwell. (*Biochem. J.*, 1956, **64**, 259.) Four of the five alkaloids of *Conium maculatum* have been isolated by extraction with ethanol and steam distillation, and separated by chromatography on paper (Whatman 3MM) using a single phase mixture of *tert.*-pentanol—*tert.*-butanol—N HCl. The following R_F values are recorded, coniine (0·74), *N*-methylconiine (0·64), conhydrine (0·50) and γ-coniceine (0·36). The relative amounts of the alkaloids present in the tissues varies with the stage of development and reproduction of the plant. An unknown steam-volatile base (R_F 0·33) was present in small amounts in the leaves and flowers, and in trace amounts in the root tissues of young plants. It was not identical with piperidine (R_F 0·34) and may be pseudoconhydrine. Root tissues of young plants had a low alkaloid content, mainly γ-coniceine. Sap exuding from stumps of decapitated plants also contained no alkaloids, but small quantities of a compound R_F 0·21 and giving a red colour with nitroprusside reagent was present. A method for the microdetermination of γ-coniceine in the presence of the other alkaloids, based on the nitroprusside reaction, is described. A method is also described for the

microdetermination of *N*-methylconiine, coniine and conhydrine after chromatographic separation by a colorimetric method, based on their reaction with bromothymol blue. Very young seedlings in the cotyledon stage show the presence of γ-coniceine, coniine and *N*-methylconine, whilst the two latter alkaloids were absent from older seedlings. γ-Coniceine was also the major alkaloid in the leaves of young plants, but coniine content increased towards the end of the first year. Actively growing plants had a low alkaloid content in their roots, but roots in the resting stage contained more γ-coniceine and coniine. Renewed vegetative growth was accompanied by concentration of much γ-coniceine in the leaves. During the reproductive phase flowers and developing fruits contained mainly coniine, the γ-coniceine content falling rapidly, with *N*-methylconiine as the predominant alkaloid in mature fruits.

J. B. S.

Caffeine, Aspirin and Phenacetin, Separation of, by Paper Electrophoresis. M. Vietti-Michelina. (*Pharm. Acta Helvet.*, 1956, **31**, 347.) A phosphate buffer solution of pH 6·2 is used for the separation. Phenacetin does not change its position after electrophoresis, while both caffeine and aspirin move, the latter at the most rapid rate. The aspirin is recognised by its fluorescence in ultra-violet light; phenacetin by nitration to yellow coloured *o*-nitrophenacetin; and caffeine by exposure to iodine vapour. G. M.

Narceine, Spectrophotometric Determination of. A. H. Witte. (*Pharm. Weekbl.*, 1956, **91**, 588.) To 10 ml. of a solution of narceine in 0·5N hydrochloric acid is added 5 ml. of chlorine water. After 5 minutes, 5 ml. of 10 per cent sodium sulphite solution is added, and the mixture is made up to 25 ml. 5 ml. of this solution is made up to 25 ml. with saturated solution of sodium acetate. After 1 hour the colour is determined at 510 mμ in a 1 cm. cell. A similar colour is given by narcotine, but not by morphine, codeine, thebaine or papaverine, although the latter interfere by combining with the chlorine and giving yellow solutions, and papaverine is precipitated on the addition of sodium acetate. G. M.

***Rauwolfia serpentina* Preparations, Assay of.** D. Banes, J. Wolff, H. O. Fallscheer and J. Carol. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 708.) The following method depends upon an extraction procedure which eliminates interfering alkaloids, followed by treatment with nitrite to produce a fluorescent green colour; it is stated to be more convenient and reproducible than the chromatographic procedure previously described (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 200; abstract, *J. Pharm. Pharmacol.*, 1956, **8**, 1174). An ethanolic extract is prepared in a Soxhlet apparatus, using powdered rauwolfia or tablets, and a quantity of the extract is mixed with sulphuric acid and extracted with trichloroethane to remove reserpinine and methyl reserpate. The weakly basic alkaloids are extracted with chloroform, and the chloroform solution is treated with sodium bicarbonate solution, diluted with ethanol and cautiously evaporated. The residue, redissolved in ethanol is allowed to react with sodium nitrite in the presence of sulphuric acid at 50° to 60° for 20 minutes, after which sulphamic acid is added and the light absorption measured at 390 mμ, against a blank. The quantity of alkaloids of the reserpine-rescinnamine group is calculated as reserpine, by comparison with the light absorption produced in a standard reserpine solution treated with acid nitrite in the same manner. The large blank readings encountered are probably due to substances formed naturally in the plant, by the oxidation of reserpine and rescinnamine. G. B.

Reserpine Preparations, Oxidative Degradation of. D. Banes, J. Wolff, H. O. Fallscheer and J. Carol. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 710.) Reserpine preparations may be subject to decomposition during manufacture and storage, 3-dehydroreserpates being produced by oxidative degradation. Since these substances have a fluorescent green colour, they may give rise to misleading results when the preparations are assayed by the nitrite colorimetric method. It is recommended that a control solution should be prepared and a correction applied for substances absorbing at 390 mμ prior to nitrous acid treatment. A comparison of corrected and uncorrected results indicates that elixirs and injections are susceptible to this type of oxidative degradation, and it also occurs occasionally in tablets. The corrected results of the nitrite method show reasonable agreement with those of the chromatographic assay method. G. B.

Sodium Tetraphenylboron for the Separation and Determination of Drugs with Basic Nitrogen Groups. L. Worrell and W. R. Ebert. (*Drug Standards*, 1956, **24**, 153.) A sample sufficient to react with 2 to 3 ml. of reagent is dissolved in water and the reaction of the solution adjusted to pH 5. A slight excess of a 3 per cent solution of sodium tetraphenylboron is added, while stirring. The solution is allowed to stand until the precipitate has settled, when a further drop of reagent is added to test for completeness of precipitation. The supernatant liquid is removed and the precipitate washed with the aid of an immersion filter. The precipitate is dissolved in acetone and 5 ml. of a saturated solution of mercuric chloride and 25 ml. of 0·02N sodium hydroxide are added and the solution is boiled. After adding 5 ml. of a 20 per cent solution of potassium iodide, the solution is cooled and excess of alkali titrated with a standard acid solution. The method is satisfactory for salts of amphetamine and methylamphetamine, and for cetyldimethylbenzylammonium chloride. Determinations may be performed in the presence of acetylsalicylic acid, caffeine, phenacetin, starch and talc; the method should therefore be useful for estimating amine salts, codeine, etc., in admixture with aspirin, phenacetin and caffeine. The method cannot be used if potassium or ammonium salts are present, because they give rise to insoluble tetraphenylboron derivatives. G. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Histamine, Binding of, in Mammalian Tissues. R. K. Sanyal and G. B. West. (*Nature, Lond.*, 1956, **178**, 1293.) It is well known that tissue mast cells contain heparin and histamine. Experiments have been carried out to study the *in vitro* affinity of heparin and histamine for one another. When the two are mixed in solution and ethanol or acetone added, the precipitated heparin removes about 70 per cent of the histamine if the pH is about 5. This combination is dependent upon pH, since at a neutral or alkaline reaction much less histamine is removed by the heparin. The precipitated heparin-histamine complex can be washed with ethanol or acetone without loss of histamine but the complex is easily rendered soluble by saline, water or weak acid. If adenosine triphosphate is present in the original solution, the uptake of histamine by the heparin is increased to more than 90 per cent. The staining properties and chromatographic behaviour of the complex resemble those of the natural mast cell granules. The formation of this complex is fairly specific for histamine

since adrenaline, noradrenaline and 5-hydroxytryptamine are not removed under similar conditions. Thus the base histamine and the acid heparin can form a complex but so far it has not been possible to release the histamine from the synthetic histamine heparinate without solution of the heparin. M. M.

BIOCHEMICAL ANALYSIS

Fluothane, Estimation of, in Blood. R. R. Goodall. (*Brit. J. Pharmacol.*, 1956, **11**, 409.) Fluothane, ($CF_3CHClBr$) a new anaesthetic, is extracted into light petroleum and aliquots heated in sealed ampoules containing sodium amoxide. The bromide released is determined nephelometrically as silver bromide. For each test, place 4 ml. of light petroleum and 1 ml. of water in a 10 ml. tube. Place 4 ml. of the blood sample under the surface of the petroleum. Extract, centrifuge, and transfer 3·5 ml. aliquots to a 10 ml. ampoule containing 2 ml. of a solution of sodium amoxide (prepared by dissolving 1·1 g. of halide free sodium in 50 ml. of amyl alcohol). Seal the ampoules and heat in a pressure cooker at 15 lb./sq. inch for one and a half hours. Open each ampoule and transfer the contents into a test-tube. Rinse each ampoule with N sulphuric acid followed by sufficient water to make the total volume of the aqueous layer 8 ml. Mix well, allow to settle, remove the lower aqueous layer to a clean tube, add 2 ml. 0·01N silver nitrate, stir and transfer to a dark cupboard for 10 minutes. Read the optical density in a spectrophotometer. Compare with suitable standards, prepared from a standard solution of fluothane in water, treated in a similar way to the blood. Note: an improved method, using reduction by lithium aluminium hydride to release halide from fluothane, is being developed.

G. F. S.

Methanol in Blood and Biological Material, Rapid and Sensitive Test for. O. E. Skaug. (*Scand. J. clin. lab. Invest.*, 1956, **8**, 338.) To 5 ml. of blood (with or without anticoagulant) add 3 to 4 ml. of water and place in a suitable distilling apparatus. Place the flask in a boiling water bath and after 2 minutes collect the condensate. Transfer this with the aid of 0·5 to 1 ml. of water to a test tube and cool in ice. Add 0·1 ml. of 0·1N potassium permanganate followed by 0·5 ml. of sulphuric acid. Mix thoroughly, add 1 ml. of 2 : 7-*naphthalenediol* reagent (0·2 per cent with sodium sulphite 1 per cent), remix and add a further 2 ml. of sulphuric acid. Place the tube in a boiling water bath for 5 minutes. A reddish-violet colour indicates the presence of methanol. When a control experiment is carried out using water in place of blood, it is possible to detect a colour difference when 2·5 p.p.m. of methanol is present in the blood sample. The reaction depends upon the formation of formaldehyde; ethanol, diethyl ether, β-hydroxybutyric acid and acetone do not interfere. Two types of distilling apparatus are described, one for use with acetone and solid carbon dioxide as refrigerant, and the other for use with ice. G. B.

Tetraethyl-lead, Isolation from Liver, after its Inhalation. C. D. Stevens, C. L. Feldhake and R. A. Kehoe. (*J. Pharmacol.*, 1956, **117**, 420.) Tetraethyl-lead (TEL) was isolated from liver tissue of rats which had inhaled TEL vapour. The rats were exposed for four to seven hours to air nearly saturated with TEL (8 to 9 mg. per litre). They were then killed with carbon monoxide and their livers homogenized. The TEL was extracted by pentane, concentrated by low temperature vacuum distillation and identified by infra-red spectra and lead analysis. No homologues containing methyl groups were detected in the concentrates. G. P.

CHEMOTHERAPY

Novobiocin and Vancomycin; Antibacterial Activity. R. W. Fairbrother and B. L. Williams. (*Lancet*, 1956, **271**, 1177.) Tests to determine the sensitivity of the common pathogens to these two antibiotics were carried out by the dried disc technique and by serial dilution. 1350 organisms were tested against vancomycin by the disc method, the results showing that this antibiotic is active against Gram-positive and Gram-negative cocci but has little effect on Gram-negative bacilli. Of 540 strains of *Staph. aureus* tested all proved sensitive, though many of the strains were resistant to penicillin and other established antibiotics. These observations were confirmed in a limited number of tests by serial dilution; *Str. haemolyticus* (group A) proved the most sensitive organism by these tests. 1100 organisms were tested against novobiocin by the disc method, the results indicating considerable activity against Gram-positive cocci but only limited action against Gram-negative organisms. All of 470 strains of *Staph. aureus*, many of which were resistant to penicillin and other antibiotics, proved sensitive, as did strains of *Str. haemolyticus* and pneumococcus. All members of the coliform group proved resistant, and all strains of proteus. Strains of *Haemophilus influenzae* were sensitive; *Neisseria gonorrhoeae* and *Str. faecalis* gave irregular results. *Staph. aureus* proved the most sensitive organism by the serial dilution test. *Str. haemolyticus* proved relatively resistant and *Str. faecalis* even more resistant. The coliforms and pseudomonas showed marked resistance. The results indicate that vancomycin and novobiocin should prove useful additions to the list of routine antibiotics. The range of antibacterial activity of vancomycin is similar to that of penicillin, erythromycin and bacitracin, and it should be valuable in the treatment of infections caused by penicillin-resistant strains of *Staph. aureus*; novobiocin should also be of value in such infections, but carefully controlled clinical trials are necessary for a final assessment. Novobiocin-resistant strains of *Staph. aureus* have already been isolated in U.S.A. and this antibiotic should not be used indiscriminately. S. L. W.

Vancomycin; Laboratory and Clinical Experiences. G. E. Geraci, F. R. Heilman, D. R. Nichols, W. E. Wellman and G. T. Ross. (*Proc. Mayo Clin.*, 1956, **31**, 564.) Vancomycin is a bactericidal antibiotic obtained from *Streptomyces orientalis*. It is amphoteric and acts primarily against Gram-positive bacteria. Bacteria are slow to develop resistance to it. Vancomycin hydrochloride is a white solid, very soluble in water and relatively stable. It has a molecular weight of about 3300. Its activity is little affected by changes in the pH of the medium and by a variety of inorganic salts, amino acids, reducing agents and growth factors in the test medium. It acts only against multiplying bacteria. Injected parenterally into mice it protects them from experimental infections with *Micrococcus pyogenes*, *Streptococcus pyogenes*, *Diplococcus pneumoniae* and *Borrelia novyi*. Most micrococci are killed by 2 to 3 μg./ml. of medium; a concentration of 2·5 μg./ml. completely inhibited 110 of 112 strains of *M. pyogenes* tested. Of 12 strains of *Str. mitis* isolated from the blood of patients previously treated for bacterial endocarditis, half were killed by the same concentration. The therapeutic effectiveness of the drug was most convincingly demonstrated in a case of acute micrococcal endocarditis in a 71-year old man. 0·5 g. of vancomycin was given intravenously every 6 hours for 3 weeks, then every 8 hours for 4 days, and finally every 12 hours for 3 days, making a total of 51 g. in 28 days. The patient, who was almost comatose at

the beginning of treatment, began to improve almost immediately, and within a few hours much of the toxicity had vanished. The temperature was normal within 3 days. The patient has remained well for 5 months. Assays indicated that little or no vancomycin is present in the blood serum and only small amounts in the urine after 0·5 g. orally 6-hourly, but after a single intravenous injection of 0·5 g. an average value of 33 μg./ml. was obtained at 1 minute, and after 24 hours the value was still 0·7 μg./ml. Large quantities were excreted in the urine after intravenous administration, the average concentration after 24 hours being 100 μg./ml. After single and multiple intravenous injections adequate therapeutic levels were found in the pleural, pericardial, ascitic and synovial fluids; only small amounts appeared in the bile. With intravenous injections only small amounts appeared in the stools but with oral administration of 0·5 g. 6-hourly very much larger amounts appeared. Vancomycin does not appear to diffuse through the uninflamed meninges. After multiple intravenous doses of 0·5 g. 6-hourly it appeared to accumulate in the blood serum over the first 2 or 3 days. The only signs of toxicity were an occasional chill, dermatitis, and localised phlebitis. Preliminary clinical trials indicate that vancomycin offers promise in the treatment of micrococcal infections. Except in the treatment of micrococcal ileocolitis, in which it would appear to be the antibiotic of choice, it needs to be given parenterally. Nine detailed case reports are given. S. L. W.

PHARMACY

Cyanocobalamin and its Analogues in Ascorbate Solution, Stability of. H. H. Hutchins, P. J. Cravioto and T. J. Macek. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 806.) When solutions containing 30 μg./ml. of vitamin B_{12} and 1 per cent of ascorbic acid in M acetate buffer, pH 4 were stored at 30° and assayed at intervals, the rate of decomposition of vitamin B_{12} was found to depend upon the proportion of cyanocobalamin present. Solutions prepared from pure cyanocobalamin or a commercial concentrate containing only cyanocobalamin lost about half their potency in 6 days, whereas solutions containing a proportion of other cobalamins decomposed more rapidly. In these experiments, vitamin B_{12} concentrates containing 50 per cent of cyanocobalamin lost about 95 per cent of their activity, and a concentrate containing 20 per cent of cyanocobalamin almost all of its activity in 6 days. Similar solutions prepared from chlorocobalamin, nitrocobalamin and thiocyanatocobalamin lost nearly all their activity on storage for 3 hours at 25°. G. B.

Vitamin B_{12} in B-Complex Injectable Solutions, Stability of. M. Blitz, E. Eigen and E. Gunsberg. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 803.) Solutions containing 5 μg. of vitamin B_{12} per ml. with varying amounts of aneurine and nicotinamide were stored at room temperature and assayed microbiologically at intervals. Solutions containing 50 mg. or more per ml. each of aneurine and nicotinamide lost most of their vitamin B_{12} content on storage for 6 months, whereas those containing smaller amounts of aneurine and nicotinamide were more stable, for example, 25 mg./ml. of aneurine and nicotinamide gave rise to a loss of 30 per cent of vitamin B_{12} in 1 year. Of 6 commercial B complex injections purchased at random 5 were below the stated potency and 2 had lost more than 95 per cent of their original potency. It was shown that a decomposition product of aneurine was responsible for the destruction of vitamin B_{12}. Heating a solution of 50 mg./ml. aneurine and nicotinamide at

100° for 4 hours at pH 4·5 gave a solution which accelerated the decomposition of vitamin B_{12}, as did a solution of aneurine which had been stored for 2 years. Further experiments indicated that it is the thiazole moiety of aneurine which accelerates the decomposition of vitamin B_{12}. G. B.

PHARMACOLOGY AND THERAPEUTICS

***N*-Acetyl-*p*-aminophenol as an Analgesic.** D. R. L. Newton and J. M. Tanner. (*Brit. med. J.*, 1956, **2**, 1096.) *N*-Acetyl-*p*-aminophenol (N.A.P.A.P.) is the non-toxic metabolite of acetanilide and phenacetin through which these drugs appear to exert their antipyretic and analgesic action. A controlled clinical trial of the analgesic effect of N.A.P.A.P. compared with that of Compound Codeine Tablet B.P. was carried out in 42 patients suffering from chronic painful rheumatoid conditions. Each patient received one drug for a period of a week followed by the other drug during the second week, and at the end of a fortnight was questioned concerning his symptoms; no suggestion was made that different preparations were being compared. During the next fortnight a second similar comparison was made. The drugs were given under a randomisation scheme which ensured that at no time did either the patient or the doctor know which drug was being taken. The statistical method known as sequential analysis was used, the principles and advantages of which are described. The analysis showed that Compound Codeine Tablet, 2 tablets 3 times a day, was in general superior to N.A.P.A.P. 1 g. (2 tablets) 3 times a day. At the same time a significant minority of patients considered that for them N.A.P.A.P. was a better analgesic, indicating that there is individual reaction to different types of analgesic. S. L. W.

Antilipaemic Agent without Anticoagulant Action. E. M. M. Besterman and J. Evans. (*Brit. med. J.*, 1957, **1**, 310.) Laminarin sulphates M and N, prepared by the sulphation of laminarin, a polysaccharide from *Laminaria cloustoni*, containing 0·62 and 0·37 sulphate groups per glucose unit, were examined for antilipaemic activity. In contrast to the more highly sulphated laminarins, they had very little anticoagulant activity. 12 patients with ischaemic heart disease were treated with heparin (10,000 units intravenously) and in 11 of these the electrophoretic pattern of the serum lipids was significantly altered. Of these 11 patients, 6 received a single intravenous injection of 100 mg. of laminarin sulphate M and 3 received the same dose of laminarin sulphate N. Samples of venous blood were submitted to paper electrophoresis, each paper being divided, half being stained for proteins and half for lipids. Laminarin sulphate M increased the electrophoretic mobility of lipoproteins and altered their distribution in the same way as heparin, whereas laminarin sulphate N had no demonstrable effect on electrophoretic mobility and distribtion of the lipoproteins. The effect of laminarin sulphate M was observed almost immediately after the intravenous injection of 100 mg. and persisted for 4 to 6 hours; this dose cleared the lipaemic serum of 2 patients who had had a fatty meal, but the serum clearing effect could not be demonstrated *in vitro*. No effect was observed when 100 mg. of laminarin sulphate M was injected intramuscularly. Since laminarin sulphate M has antilipaemic properties similar to heparin, without anticoagulant activity, it would seem to be a suitable substance to use in a long-term investigation of the effect of lipaemia-clearing substances on the course of atherosclerosis in man. G. B.

Barbiturate Poisoning Treated with Amiphenazole and Bemegride. A. Worlock. (*Brit. med. J.*, 1956, **2**, 1099.) Twelve cases of fairly severe barbiturate poisoning were treated with amiphenazole and bemegride. All the patients were unconscious on admission, and all regained consciousness within 24 hours (11 of them within 12 hours). No deaths occurred, there was no incidence of chest infection, and the stay of patients in hospital was reduced dramatically compared with former methods of treatment. On admission the patients were given a stomach wash-out with a dilute solution of sodium bicarbonate, 4 oz. of the solution being left in the stomach after the wash-out. The airway was kept clear and a laryngoscope passed to determine whether laryngeal reflexes were present. A 5 per cent dextrose-saline intravenous drip was given at the rate of 40 drops a minute, continued throughout the treatment. Procaine penicillin, 600,000 units, was given at once and then twice daily for 5 days. Amiphenazole and bemegride were injected intravenously at the rate of 1 ml. of amiphenazole and 10 ml. of bemegride every 3 minutes until a level of light anaesthesia was attained. The strengths of the solutions used were 0·5 per çent solution of bemegride (5 mg. per ml.) and a 1 per cent solution of amiphenazole (10 mg. per ml.), the latter being made up immediately before use. Postural drainage and breathing exercises were carried out daily. Case reports are given. S. L. W.

Bemegride as an Antagonist of Barbiturates. H. H. Frey, E. W. Hushahn and K. Soehring. (*Arzneimitt.-Forsch.*, 1956, **6**, 583.) The stimulating action of bemegride and amiphenazole were compared with that of leptazol, using mice which were under the influence of barbiturates. The first compound was twice as effective as leptazol; the second was inactive. With a combination of 3 parts of bemegride with one part of amiphenazole the effect of the former is increased to 3 times that of leptazol. The action is not that of a specific barbiturate antagonist but rather that of a stimulator with a satisfactory action against various hypnotics. G. M.

Bemegride, New Analeptics and Hypnotics Related to. T. C. Somers. (*Nature, Lond.*, 1956, **178**, 996.) A series of compounds based on the antibarbiturate β-methyl-β-ethylglutarimide (bemegride; Megimide) have been prepared and the results of preliminary pharmacological examinations are reported. The effects of test compounds on the sleeping times (elapsed time between loss and recovery of the righting reflex) induced by pentobarbitone sodium in mice were compared with that of bemegride. The test compounds were administered by intraperitoneal injection in aqueous solution or suspension. β-Methyl-β-ethylglutaramic acid and β-methyl-β-ethylglutaric acid were ineffective as barbiturate antagonists. The *N*-methyl-, *N*-ethyl- and *N*-phenyl-β-methyl-β-ethylglutarimides were also inactive. β-*spirocyclo*Pentaneglutarimide, β-methyl-β-*n*-propylglutarimide and ββ-diethylglutarimides had analeptic activities similar to that of bemegride, the first of these being almost as effective as the parent substance. The methyl *n*-propyl compound is the least toxic. These same compounds and also β-ethyl-, β-methyl-β-*iso*butyl-, β-*spirocyclo*hexane- and β-*spirocyclo*heptane-glutarimides all caused convulsions in mice when administered alone in doses varying from 15 to 50 mg./kg. β-Methyl-β-*n*-butylglutarimide, methyl-*n*-amylglutarimide and methyl *n*-hexylglutarimides have no analeptic activity, but on the other hand exert hypnotic effects in mice, the minimum hypnotic dose being about 150 to 200 mg./kg. Doses of 400 to 500 mg./kg. induced up to 5 hours of sleep. The approximate LD50 is 600 mg./kg., and the hypnotic effect is antagonized by bemegride. J. B. S.

Dextran Sulphate, Anticoagulation Effect and Urinary Excretion of. S. M. Jeavons, K. W. Walton and C. R. Ricketts. (*Brit. med. J.*, 1956, **2**, 1016.) When the effects of single equipotent intravenous doses of heparin and dextran sulphate are compared the latter substance shows a slightly longer anticoagulant activity. One factor which might cause this effect is a difference between the patterns of urinary excretion of the two drugs. Estimates of the urinary output were made in both man and rabbits over a period of 10 to 23 days. These results were related to the clotting time of the blood over that time. It was found that dextran sulphate, when administered intravenously to five patients suffering from thrombo-embolic disease, had a marked cumulative effect when the treatment was prolonged beyond three to five days. As a result, reduction in the dosage and/or lengthening of the spacing of the injections could be made without affecting the efficacy of the dextran. The cumulative effect in man could not be accounted for on the basis of the urinary excretion of a smaller percentage of the injected dose than that of heparin. A dosage schedule of dextran, similar to that used in man, was given to rabbits, but no cumulative effect was produced. It is suggested that in man dextran sulphate differs from heparin in duration of effect because of a slower rate of breakdown in the body and consequent accumulation, probably in the extravascular fluid. M. M.

Dextran Sulphate as an Anticoagulant, and Action in Lowering Serum Cholesterol. H. Cohen and G. R. Tudhope. (*Brit. med. J.*, 1956, **2**, 1023.) The effect on the clotting-time of single and repeated doses of dextran sulphate, given intravenously, and the use of it in the treatment of thrombotic disease are reported. The change in the serum total cholesterol is also measured. It was found that a single dose of 7500 units produced an increase in the clotting-time to more than twice normal for four to six hours. With repeated doses cumulation occurred so that the dose could be reduced progressively. During the treatment there was a fall in the serum cholesterol. It is suggested that dextran is an effective anticoagulant for clinical use and that it provides a possible alternative to the usual combination of heparin and oral anticoagulant. However toxic effects may occur, particularly if the dose is not carefully controlled. M. M.

Digitalis Glycosides and their Metabolites, The Distribution of. B. T. Brown, E. E. Shepheard and S. E. Wright. (*J. Pharmacol.*, 1956, **118**, 39.) An investigation has been made into the presence of cardioactive metabolites of the glycosides digoxin, lanatoside C and digitoxin in the blood and organs of the rat and compared with those found in the urine. The glycosides were given intraperitoneally or intravenously. After killing the rats the tissues were extracted with ethanol, the extract was shaken with carbon tetrachloride which was then rejected and the bulk of the ethanol evaporated off at 30°. The solution remaining was diluted with water and extracted continuously with chloroform for two hours. After evaporation to small bulk the cardioactive constituents were detected by paper chromatography. Cardioactive metabolites, as well as free glycosides, were found to be in the heart, liver, kidney and circulating blood of the rat immediately after the injection of digoxin and lanatoside C, and the metabolites were the same as those in the urine. Digitoxin and its metabolite, previously found in rat urine, were present in rat livers immediately after injection, but only the free glycoside could be detected in blood and kidney. The experiments indicate that although the major portion of the dose of all three glycosides is rapidly removed from the circulating blood, their rate of clearance and eventual disappearance differ. Digitoxin is removed more rapidly than lanatoside C and digoxin slowest of all. The rate of blood

clearance is believed to be conditioned by the relative ability of the liver to combine with each glycoside. Digitoxigenin or digoxigenin could not be detected in any tissue after administration of the glycosides. G. F. S.

1-Dimethylamino-3-cyano-3-phenyl-4-methylhexane HCl (Z-4) and 1-Dimethylamino-2-phenyl-3-methylpentane HCl (Z-134), Analgesic-potentiating and Diuretic Effects of. J. Y. P. Chen. (*J. Pharmacol.*, 1956, **117**, 451.) The above two compounds, which have a structural resemblance to methadone, had a much greater potentiating effect on the analgesic activity of morphine in rats than had chlorpromazine. Intramuscular Z-134 and oral Z-4 caused diuresis in rats which compared favourably with that produced by acetazolamide or mercurhydrin. In non-toxic doses in dogs the two compounds had prolonged respiratory stimulant and intestinal relaxant effects. Moderate local and topical anaesthetic actions were also demonstrated by the guinea pig weal and rabbit corneal methods. The duration of local anaesthesia in the guinea pig with 0·5 per cent solutions of the drugs was of the same order as with 0·5 per cent procaine. Only with higher concentrations did Z-4 produce any local irritation. Acute toxicity studies in mice and rats indicate low toxicity of the two drugs. The tests used for investigating analgesic and diuretic activity in these experiments are simple and give satisfactory results. G. P.

Fluothane, A New Volatile Anaesthetic, Action of. J. Raventós. (*Brit. J. Pharmacol.*, 1956, **11**, 394.) Fluothane ($CF_3CHClBr$) is a volatile, non-explosive inhalation anaesthetic. It is stable in contact with soda lime in a closed-circuit apparatus, but it is not stable in light. It should be stored in amber bottles with thymol as a stabilizer. It is more potent than ether or chloroform in experimental animals. Induction and recovery are both rapid and free from excitement, it produces good muscular relaxation and it does not cause salivation or vomiting. It is best used in a closed-circuit apparatus, as anaesthesia is difficult to control by the open mask method. Both amplitude and frequency of respiration are decreased at the level of surgical anaesthesia. High concentrations stop the respiration, but this apnoea is easily reversible, and the heart continues to beat for some time after respiration has ceased. Inhalation of fluothane causes hypotension roughly proportional to the vapour concentration, which appears to be due to reversible blocking of the sympathetic ganglia. It does not produce cardiac irregularities, but it increases the sensitivity of the heart to adrenaline. Hepatic function is not affected by anaesthesia with fluothane, but there is a mild dilatation of the proximal tubules of the kidney which is not associated with alteration of renal function. G. F. S.

Hemlock Water Dropwort, Pharmacological Studies on. H. F. Grundy and F. Howarth. (*Brit. J. Pharmacol.*, 1956, **11**, 225.) In unanaesthetized rabbits and mice crystalline oenanthotoxin and tinctures of *Oenanthe crocata* caused convulsions resembling those produced by picrotoxin. At the height of the attacks, respiration was impeded and the buccal mucosa was cyanosed. With lethal doses the heart was still beating feebly after respiratory arrest. Death occurred within 30 minutes after 1 mg./kg. of oenanthotoxin and within 60 minutes after 0·5 to 1 ml. of oenanthe tincture. In rabbits and cats the convulsions have their origin in the brain-stem. Oenanthotoxin was six times as toxic as picrotoxin in mice and was a more effective antidote for pentobarbitone poisoning. Respiratory stimulation in the rabbit was also greater with oenanthotoxin. The alkaloid had a biphasic effect on blood pressure in the rabbit: there was a transient fall, due to a direct depression of the myocardium, followed by a sustained rise. The rise was absent in spinal animals. G. P.

Hexamethylene Bis-dialkylsulphonium Salts, Ganglion-blocking Properties of. R. B. Barlow and J. R. Vane. (*Brit. J. Pharmacol.*, 1956, **11**, 198.) The pharmacological properties of tertiary sulphonium salts appear qualitatively similar to those of quaternary ammonium salts. Quantitatively, however, the sulphonium salts are much weaker than their ammonium analogues. This low activity may be due to the sulphonium salts only having three alkyl radicals in the cationic head where the quaternary ammonium has four. To test this hypothesis the ganglion-blocking properties of hexamethylene bis-dimethyl, bis-ethylmethyl and bis-diethyl sulphonium iodides were compared on the superior cervical ganglion of the cat. Activity was greatest in the bis-diethyl compound and least in the bis-dimethyl compound. The tertiary amine analogues, hexamethylene bis-dimethyl, bis-ethylmethyl and bis-diethyl amine dihydrobromides, also had ganglion-blocking properties. Again the bis-diethyl compound was the most active and the bis-dimethyl the least. On a molar basis the bis-diethyl sulphonium compound had about half the activity of hexamethonium on this preparation and over three times the activity of its bis-diethylamine analogue. This strengthens the argument that the low pharmacological activity of the sulphur analogue of acetylcholine, acetoxydimethylsulphonium, could be due to the presence of only two methyl groups at the cationic head. G. P.

5-Hydroxytryptamine, Analysis of the Actions of, on the Isolated Duodenum of the Rat. J. Lévy and E. Michel-Ber. (*C. R. Acad., Paris*, 1956, **242**, 3007.) In concentrations between $2{\cdot}5 \times 10^{-7}$ and 1×10^{-5}, 5-hydroxytryptamine (5-HT) caused contraction of the rat's isolated duodenum. However, in about 5 per cent of the preparations the drug caused a biphasic effect, depressing first before stimulating, an action similar to that of ganglion stimulants. The depressant action was encountered more often (in about 50 per cent) if before addition of 5-HT the duodenum was left in contact with (+)-lysergic acid diethylamide (LSD). Atropine, when used with the LSD, further increased the incidence of the depression to 90 per cent of the muscle preparations. This relaxation caused by 5-HT was blocked by yohimbine, in concentrations which abolished the action of adrenaline, and by high concentrations of nicotine or phenoxycholine, which had no effect on adrenaline. However, the relaxation was unaffected by concentrations of hexamethonium or tetraethylammonium sufficient to block ganglia. The relaxation would appear from the above results to be adrenergic in nature. An excitant action of 5-HT on post-ganglionic adrenergic nerve elements seems probable, either on the axons directly or on receptors in the ganglion cells other than those on which hexamethonium and tetraethylammonium act. G. P.

5-Hydroxytryptamine, and Certain Derivatives of Lysergic Acid, Antagonism Between. E. C. Savini. (*Brit. J. Pharmacol.*, 1956, **11**, 313.) When adrenaline and 5-hydroxytryptamine were injected together into the perfused isolated ear of a rabbit the combined vasoconstrictor activity was somewhat greater than would have been expected from the effects of the two drugs given alone. When 5-HT was combined with noradrenaline, tryptamine or pitressin, potentiation was absent or slight, the effects being simply additive. (+)-Lysergic acid diethylamide (LSD) and ergometrine, in low concentrations (1 μg./litre) both antagonized the vasoconstrictor action of 5-HT, but not that of adrenaline or noradrenaline. In higher concentrations both LSD and ergometrine had a direct vasoconstrictor action. 2-Bromo-(+)-lysergic acid diethylamide (BOL) also blocked in low concentrations the effects of 5-HT but in contrast to LSD and

ergometrine, BOL had no vasoconstrictor action of its own, it antagonized adrenaline and noradrenaline and its action developed quickly and was easily reversible. Also, the antagonism with BOL could be overcome with large doses of 5-HT. G. P.

5-Hydroxytryptamine and its Antagonists, Effects of, on Tidal Air. H. Konzett. (*Brit. J. Pharmacol.*, 1956, **11**, 289.) In anaesthetized or spinal cats and guinea pigs 5-hydroxytryptamine decreased tidal air during artificial respiration at constant stroke volume by a respiratory pump. The action was mainly a direct bronchoconstriction. (+)-Lysergic acid diethylamide, 1-acetyl-(+)-lysergic acid diethylamide and 2-brom-(+)-lysergic acid diethylamide specifically antagonized the bronchoconstrictor action. Atropine and the antihistamine 1-methyl-4-amino-*N'*-phenyl-*N'*-(2'-thenyl)-piperidine (Sandosten) also had some antagonistic effects, but in doses higher than were necessary to block the bronchoconstrictor actions of acetylcholine and histamine, respectively. Ergometrine had practically no blocking effect. G. P.

5-Hydroxytryptamine and Various Antagonists, Some Central Actions of. J. H. Gaddum and M. Vogt. (*Brit. J. Pharmacol.*, 1956, **11**, 175.) The distribution of 5-hydroxytryptamine (5-HT) in the central nervous system and the hallucinogenic actions of a powerful and specific antagonist of 5-HT, (+)-lysergic acid diethylamide (LSD), provoked the hypothesis that the actions of the LSD might be due to antagonism of naturally occurring 5-HT in the brain. To test this, the effects of LSD and other antagonists of 5-HT were studied on the actions of 5-HT injected, through a permanent cannula, into the lateral cerebral ventricle of the cat. The actions of 5-HT given in this way are: the cats become lethargic, hesitant and retiring, muscle tone is reduced and respiration is rapid; these effects last at least 6 hours. The depressant actions were antagonized by LSD, ergometrine, morphine, methadone and amphetamine, but not by 5-benzyloxygramine or methylmedmain. The sedative effects of intraventricular injections of reserpine, which were similar to those of 5-HT, were also antagonized by LSD, morphine and methadone. These antagonisms are probably not related to the specific antagonism between 5-HT and LSD on peripheral tissues. Some, but not all, of the central actions of LSD in man were inhibited by methylamphetamine. G. P.

5-Hydroxytryptamine, Identification of, in the Sting of the Nettle (*Urtica dioica*). H. O. J. Collier and G. B. Chesher. (*Brit. J. Pharmacol.*, 1956, **11**, 186.) In addition to histamine and acetylcholine, nettle stings contain a substance which resembles 5-hydroxytryptamine (5-HT) in the following respects: stimulation of the rat's isolated uterus; lysergic acid diethylamide antagonized the substance and 5-HT to the same extent; the rat's uterus desensitized to 5-HT was also insensitive to the substance; and paper chromatograms of nettle leaf and stem extracts gave a spot with the same colour reaction and R_F value as 5-HT. The estimates of 5-HT content per sting were: on the rat's uterus, 4·86 ± 1·03 ng.; guinea pig's ileum, 4·00 ± 0·38 ng.; rat's colon, 3·43 ± 0·53 ng. If the average sting contains 7 to 9 μg. fluid (Emmelin and Feldberg, *J. Physiol.*, 1947, **106**, 440), then this amount of 5-HT per sting (3·4 to 4·9 ng.) provides a concentration sufficient to cause pain in human skin. 5-HT and the third substance were inactivated at room temperature by nettle sting suspensions; this activity of nettle stings was lost after boiling and was presumably due to an enzyme. G. P.

Marplan; Clinical Studies. W. H. Bachrach. (*J. Lab. clin. Med.*, 1956, **48**, 603.) Marplan is a new atropine-like drug, 1-methyl-3-benziloyl-oxyquinuclidinium bromide. It has been shown by the balloon-kymographic method to inhibit both spontaneous and stimulated motility at all levels of the gut with the exception of post-Dromoran spasm of the choledochal sphincter. The onset of inhibition was immediate when the drug was administered intravenously in a dose of 0·5 to 1 mg., with similar doses the latent period was 5 minutes for the intramuscular and 15 minutes for the subcutaneous route. The inhibition extended for as long as 2½ hours after injection of the drug. The effective oral dose is 20 to 35 mg. Marplan was administered to 88 patients with various digestive disturbances; 62 of these were ulcer patients, 40 of whom had symptoms when treatment was started; 26 of these (65 per cent) obtained complete control of symptoms. The response of the patients with other digestive ailments (functional dyspepsia, functional bowel disturbance, etc.) was relatively poor. The drug was given in an average dose of one 5 mg. tablet orally at appropriate intervals during the day and 2 tablets at bedtime. Neither age nor body weight constitute any guide to the effective dose. The drug is well tolerated and has been given as long as 145 days without requiring an increase of the dose above 10 mg. Troublesome side effects with oral doses were infrequent, and with one exception occurred only in patients who had had similar reactions to other anticholinergic drugs; the principal side-effects were xerostomia, mydriasis and dysuria. In its physiological and clinical properties Marplan has been found comparable to atropine and to several synthetic anticholinergic preparations; while it has no particular advantage over the latter it is at least as good. S. L. W.

Mercury Absorption and Psoriasis. P. M. Inman, B. Gordon and P. Trinder. (*Brit. med. J.*, 1956, **2**, 1202.) The absorption of mercury when used in the form of an ointment containing ammoniated mercury or the yellow oxide in the treatment of psoriasis was studied in 2 groups of 12 patients, mostly women and children. The ointments were applied twice daily for six weeks; occlusive dressings were applied without cleaning or washing off the ointment, and many patients used 1 to 2 lb. of ointment weekly. A surprisingly high proportion became free from the eruption and it was thought the occlusive dressing increased the effectiveness of the treatment although increasing the risk of cutaneous absorption and clinical toxicity. One group was treated with an ointment containing per ounce 10 grains of ammoniated mercury and 30 minims of solution of coal tar in soft paraffin. An ointment containing yellow mercuric oxide 1 part, and prepared coal tar 2 parts, with hydrous wool fat and soft paraffin 10 parts of each was used for the other group. For the first of six weeks the mercury content of a 24-hour sample of urine was determined weekly, and thereafter from time to time in the patients with a high urinary mercury level. Only 2/24 failed to show at some time a mercury level in excess of 80 μg./l., regarded as the upper limit of normality. Absorption showed considerable individual variation and did not always depend on the area of skin covered. Even when 90 per cent of the skin surface was covered the mercury excretion was not as high as in some other cases. 13/24 had a level above 300 μg./l. One patient excreted 3300 μg./l. and 8 excreted more than 1000 μg./l. In no case were there any signs of clinical toxicity although often the level did not revert to normal for many months. In tentative explanation of the absence of such signs in spite of the causative connection of calomel with pink disease, it is suggested that perhaps some mercury compounds are more toxic than others. H. T. B.

Metal Chelates in Gas Gangrene. M. Moskowitz. (*Proc. Soc. exp. Biol. N.Y.*, 1956, **92**, 706.) The calcium chelate of ethylenediaminetetra-acetic acid calcium edetate, which is non-toxic, has been shown to protect mice against a lethal dose of *Cl. perfringens* toxin and guinea pigs against experimental gas gangrene. This may be due to an inhibition of one or more toxins produced by the organism. Mice innoculated intracutaneously with a 2 MLD dose of a toxic culture filtrate of *Cl. perfringens* Type A survived when injected intracutaneously in another area at the same time with a 5 or 2·5 per cent solution of calcium edetate. Guinea pigs were protected against infection with a culture of a human strain of *Cl. perfringens* by a subcutaneous injection of a 5 per cent solution of calcium edetate. G. F. S.

Noradrenaline, Effect of, on Urine and Renal Blood Flow. F. G. W. Marson. (*Brit. J. Pharmacol.*, 1956, **11**, 431.) An investigation has been made in anaesthetised dogs of the reduction in renal blood flow that occurs when noradrenaline is administered in doses which may be used to restore the blood pressure in shock. Urine flow was recorded from the catheterised ureters and blood flow from homotransplanted kidneys. Infusion of noradrenaline caused an abrupt fall in urine flow, proportional to the dose, anuria occurring with doses of 8·7 μg./kg./minute. The blood flow in transplanted kidneys was reduced and 4·6 to 13·8 μg./kg./minute completely arrested it, when the blood pressure was 190 to 250 mm. Following acute haemorrhage, with a fall in blood pressure, both urine and blood flow were reduced and further reduction occurred when the blood pressure was restored with noradrenaline. Renal function rapidly recovered on re-injection of the removed blood. G. F. S.

Oximes and Hydroxamic Acids as Antidotes in Anticholinesterase Poisoning. B. M. Askew. (*Brit. J. Pharmacol.*, 1956, **11**, 417.) Oximes and hydroxamic acids react with and reactivate certain anticholinesterases. In a study of the *in vivo* activity of all available oximes and hydroxamic acids, eight oximes have been found to be the most effective against twice the LD50 of sarin in rats. Four have the same general formula, namely mono*iso*nitrosoacetone (MINA), diacetylmonoxime (DAM), 2-oxo-3-oximinopentane, and 2-methyl-3-oximino-4-oxopentane and a fifth, *iso*nitrosodiethylketone is isomeric with 2-oxo-3-oximinopentane. All five oximes had a similar activity on an equimolar basis In other species DAM, because of its lower toxicity, was more effective than MINA. Both are effective in sarin poisoned rats even when given after the onset of anticholinesterase poisoning. Given before sarin, DAM afforded complete protection for a period of 55 to 95 minutes. G. F. S.

Parathion, Experimental Data on the Therapy of Poisoning by. Yu. C. Kagan. (*Farmakologiya i Toksikologiya*, 1956, **19**, No. 2, 49.) Tropatsin (hydrochloride of the tropine ester of diphenylacetic acid) and Pentafen (hydrochloride of the diethylaminoethyl ester of phenyl*cyclo*pentane carboxylic acid) were more effective than atropine in the treatment of parathion poisoning. Mice were given oral doses of 20 mg./kg. of parathion in 0·2 per cent oily solution; some were then treated by subcutaneous injections of atropine and some by Tropatsin, administered a few minutes after the parathion. After 4 days all the 36 control animals had died, but 10 of the 14 treated with Tropatsin survived; of 18 treated with atropine only one survived. Results obtained in experiments on rats also indicated the superiority of Tropatsin over atropine. Experiments on cats showed that Pentafen was an effective antidote in parathion poisoning. E. H.

APPLIED BACTERIOLOGY

Human Type Tubercle Bacilli, a New Chemical Method to Differentiate from other Mycobacteria. K. Konno. (*Science*, 1956, **124**, 985.) The author describes a simple chemical test by which strains of *Mycobacterium tuberculosis* var. *hominis*, whether virulent or attenuated, may be differentiated from other strains of the species and also other species of the genus *Mycobacterium*. Such a test was desirable because the only methods of differentiation in current use are based on cultural and pathogenic characteristics. Having previously shown a marked difference in nicotinamide production between human type and other mycobacteria (Konno, *Proc. Japan Acad.*, 1953, **29**, 289), this paper deals with a simplified method in which colonies of the organisms are directly tested. A total of 12 well-known strains of human, bovine and avian types were tested, along with 50 strains isolated from tuberculous patients, 10 strains of "atypical acid-fast bacteria," and such non-pathogens as *M. phlei* and *M. ranae*. The organisms were grown on Loewenstein-Jensen medium and allowed to grow for 1 to 3 months. A few colonies were transferred to 1 ml. 4 per cent alcoholic solution of aniline and 1 ml. of 10 per cent aqueous solution of cyanogen bromide was added. A positive test showed an intense yellow colour in the bacterial sediment and, after shaking, in the supernatant liquid. Controls with the medium gave only a slight green colour due to malachite green. A positive reaction was given only by human type strains, irrespective of virulence, no difference being noted between isoniazid sensitive and resistant strains. All other organisms, whether virulent, attenuated or non-pathogenic, gave a negative result. B. A. W.

Extinction Time Estimates, Further Studies on Reproducibility of. A. M. Cook and B. A. Wills. (*J. appl. Bact.*, 1956, **19**, 219.) The authors report variations in extinction times of *Escherichia coli* which result from alterations in the peptone concentration, pH and sterilising treatment of the medium used to detect growth of surviving organisms after exposure to aqueous phenol solutions. The effects of varying the composition and pH of media has received previous study, but the authors consider that these earlier findings were largely invalid because the experimental methods used attributed the within-estimate variability to the factor being studied. Conversely, the method used here enabled this within-estimate variability to be taken into account. A factorial experimental design was used in which each of the factors—peptone concentration, pH and duration of autoclaving—were tested at 2 levels. Experiments were performed on 3 occasions. An estimate of the "mean single survivor time" was obtained from each 15 replicate determination. Analysis of variance of the results showed that all 3 treatments were significant. It also appeared that the effects of increasing the concentration of peptone in the medium became more pronounced as the period of autoclaving was reduced, from which it was concluded that prolonged heating might destroy nutrient material, greater destruction occurring in more concentrated solutions. Provision of rigid conditions for sterilisation of media used in this type of estimation of bactericidal activity is recommended. Finally the authors point out the limitations of previous methods of determining extinction times and conclude that the method which they used provides estimates reproducible within such narrow limits as completely to invalidate all previously described techniques. B. A. W.

***Mycobacterium tuberculosis* in Mouse Tissues, Conversion of Infection to the Latent State by Pyrazinamide and a Companion Drug.** R. M. McCune, R. Tompsett and W. McDermott. (*J. exp. Med.*, 1956, **104**, 763.) Further to a report by McCune and Tompsett (*J. exp. Med.*, **104**, 737) that tubercle bacilli susceptible to various drugs *in vitro* were able to survive long exposure to the drugs when present in certain mouse organs, particularly the spleen, this paper deals with the capacity of pyrazinamide (pyrazine-2-carboxylic acid amide) and a second drug to reduce the numbers of surviving organisms in the mouse to below the level of detectability. The experimental procedure consisted of infecting mice intravenously with strains of *Mycobacterium tuberculosis* var. *hominis*. After suitable intervals from the start of infection the animals were killed. The lungs and spleens were homogenised and cultured quantitatively, colony counts being obtained by inoculation of oleic acid albumin plates. On treatment of the animals with pyrazinamide (2 per cent of daily diet) and isoniazid (0·0125 per cent) together, starting infection and therapy at the same time, no detectable organisms could be found in the lungs after 23 days or in the spleen after 37 days. Most animals receiving either drug alone showed detectable numbers in both organs (especially the spleen) for at least 90 days. For the first 16 days of treatment pyrazinamide appeared to antagonize the action of isoniazid. Animals treated with isoniazid, streptomycin and aminosalicylic acid together showed persistence of organisms in lungs and spleen after 118 days. Pyrazinamide with isoniazid still caused "vanishing" of organisms in the organs when treatment was commenced on the 21st day after infection. Attempts were made to establish presence of the bacteria in tissues which yielded no colonies on culture: increasing the inoculum for the plates to the total tissue homogenate; inoculating into guinea pigs for detection of tuberculin reaction; long-continued incubation of tissue homogenates; prolonged post-treatment observation of the animals. Only the last method detected presence of the organisms: in mice which had been treated with pyrazinamide with isoniazid for 90 days after infection, the organisms were cultured from spleens of 12 and lungs of one out of 30 animals examined after a consecutive 90 day treatment-free interval. The authors consider that the complete disappearance of the tubercle bacilli meets the definition of a truly latent infection—the infection is present but is hidden beyond the limits of diagnostic reach. All but one of the strains of organisms surviving in the animals and detectable in the post-treatment period were susceptible to pyrazinamide *in vitro*. A study was made of the factors essential for uniform disappearance of the organisms. It was found that pyrazinamide must be administered in high daily dosage for at least 8 of the total 12 weeks of treatment and that concurrent or prior treatment with isoniazid, or possibly other antituberculous drugs, was also essential. The authors consider that pyrazinamide susceptibility of *Mycobacterium tuberculosis* is closely related to the capacity of the organism to undergo a type of alteration in response to environmental influences including the presence of other antituberculous drugs.

B. A. W.