ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Barbiturates, Detection and Identification of. L. Levi and C. E. Hubley. (*Analyt. Chem.*, 1956, **28**, 1591.) Advantage is taken of the formation of the characteristic dark purple-coloured complexes when barbiturates react with aqueous copper sulphate-pyridine solutions. The sensitivity is increased when the copper sulphate-pyridine ratio in the reagent is increased so that as little as 0·1 mg. would form a precipitate on standing. The melting of the complexes is accompanied by decomposition but the melting points are sharp enough to be used as diagnostic features. The compounds have the general formula $(Bb)_2Cu(Py)_2$ where Bb is the barbituric acid ion and Py is pyridine. Infra-red curves of a number of complexes are compared with those of the parent barbiturates, and the curves of both the barbiturates and their complexes show unique features throughout the region 4000 to 650 $cm.^{-1}$, and hence the method affords a high degree of specificity for the detection and characterisation of these drugs.

D. B. C.

Chlortetracycline and Tetracycline, Simultaneous Determination of. J. Doskočil. (*Československ. Farm.*, 1956, **6**, 321.) A sample of a mixture of chlortetracycline and tetracycline is dissolved in water to give a concentration of 1 mg./ml. and this solution is diluted 10-fold with (*a*) 0·2M trisodium phosphate solution, and (*b*) 0·2M phosphate buffer solution having a pH of 6·0. The solutions are set aside for 30 minutes at 20° to 25°; the chlortetracycline in (*a*) decomposes almost completely while the tetracycline remains practically unchanged. Both samples are then boiled with 2N HCl for 5 minutes to convert the antibiotics to their anhydro derivatives, and the extinction of the solutions is measured at 440 mμ. A control experiment is carried out to determine the degree of decomposition of pure tetracycline and chlortetracycline under the same conditions and the amount of each present in the test sample can then be calculated.

E. H.

Neomycins B and C in Neomycin Sulphate, Determination of. A. A. Brooks, A. A. Forist and B. F. Loehr. (*Analyt. Chem.*, 1956, **28**, 1788.) Three methods of simultaneous assay are compared. In the first method, the optical rotation of a solution is measured, an aliquot part is then taken and degraded with sulphuric acid under strictly controlled conditions of time and temperature to split off furfural or a derivative of it the absorption of which is measured. From the known behaviour of pure neomycins B and C under these conditions and their optical rotations, two simultaneous equations may be built up from the data obtained from a mixture, and solved to find the percentage of each in the mixture. Formulae are included. The second method depends upon the variations of the optical rotations of the two isomers with temperature, and consists of measuring the rotation of a solution at two different temperatures. In the third method, the optical rotation is measured as in method I and a neutral equivalent is found on an aliquot of the same solution by mixing with an excess of saturated barium hydroxide solution and back-titrating with standard

sulphuric acid. The total neomycin is calculated from the normality (N) while the percentage of B is read from a plot of $[\alpha]_D^{25}/N$ against percentage B, which was shown to be linear. The quantities required are large for the measurement of optical rotation, viz., 5 g. of neomycin sulphate in 25 ml. of 0·1N sulphuric acid. The first method is preferred and has an accuracy on pure mixtures within about 1 per cent on total neomycin, 3 per cent on neomycin B and up to 10 per cent on neomycin C (when small percentages of C are involved).

D. B. C.

Phenothiazine Derivatives, Polarimetric Determination of. J. Blažek. (*Československ. Farm.*, 1956, **4**, 210.) Phenothiazine derivatives are determined by titration with silicotungstic acid, the end point being determined polarimetrically; in solutions containing a suitable amount of hydrochloric acid the derivatives form an almost insoluble precipitate with this reagent. For the determination of promethazine hydrochloride in tablets, a weighed sample of ground tablets, containing 20 to 50 mg. of promethazine, is placed in a 100-ml. beaker and 1 ml. of 35 per cent solution of HCl is added; the mixture is diluted to 20 ml. with water and titrated against 0·01M silicotungstic acid. A Heyrovsky type polarograph with a galvanometer having a sensitivity of 4×10^{-9} is used to determine the end point; the test-solution is connected to a saturated calomel electrode, forming the anode, by means of a potassium nitrate solution bridge, and a dropping-mercury electrode is used as the cathode. An E.M.F. of 0·65 V is supplied by a 2-volt accumulator with a potential divider. With this E.M.F. an excess of silicotungstic acid gives a diffusion current. Other phenothiazine derivatives are determined in the same way. Results on various proprietary preparations are within ± 4 per cent of those calculated from Kjeldahl nitrogen determinations.

E. H.

Pyrethrins, New Colorimetric Method of Estimation of. H. L. Williams, W. E. Dale and J. P. Sweeney. (*J. Assoc. off. agric. Chem., Wash.*, 1956, **39**, 872.) This method claims greater sensitivity (down to 7 μg. of pyrethrins), greater reproducibility (2 per cent) and greater specificity than methods heretofore used. The colour-developing reagent used was a mixture of 80 per cent by volume of 85 per cent orthophosphoric acid and 20 per cent by volume of reagent-grade ethyl acetate. The standard curve was prepared using a solution containing 25 μg./ml. of pyrethrins in a hydrocarbon fraction b.p. 30 to 60°, prepared from a pyrethrum concentrate analysed by the A.O.A.C. method. Suitable volumes were carefully evaporated to dryness on a water bath, 5 ml. of colour reagent added, and the resulting solutions shaken for 1 minute mechanically, and immersed in a boiling water bath for exactly 3 minutes. After transference to suitably matched test-tubes, the solutions were centrifuged for 15 minutes at a medium speed and the absorbance at 550 mμ read against a blank of 5 ml. of colour reagent. Samples of cotton wool etc. containing pyrethrins were extracted with the hydrocarbon solvent and treated similarly, the blank being prepared by carrying a sample of untreated material, equivalent in weight to that of the sample being tested, through the analytical procedure. When materials such as grains, burlap and cardboard contained substances which suppressed the pyrethrum colour, standards were prepared by the addition of a known amount of pyrethrin standard to extracts of untreated samples. The colour produced in the reaction was stable indefinitely and pyrethrins I and pyrethrins II were found to give colours which had identical absorbtion spectra. Good recoveries of pyrethrins from wool, kraft paper, botany wool, muslin cloth and raisins were obtained, the errors being from about 3 to 6 per cent.

A few substances (e.g. piperonyl butoxide) used as synergists suppressed the colour reaction when present in large concentration compared with that of the pyrethrins. Chromatographic methods of separation enabled good recoveries of pyrethrins to be obtained. D. B. C.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Anticholinesterase Activity of Ethyleneimines and certain other Cytotoxic Agents. K. Bullock. (*Biochem. J.*, 1956, **63**, 484.) A method for the examination of drugs for antiacetylcholinesterase activity at pH 6·3 by the use of insoluble erythrocyte envelopes (stromata) is described. Suspensions of stromata, as a source of acetylcholinesterase, are mixed with inhibitor solutions either with (pH 8·3) or without (pH 6·3) added sodium bicarbonate, maintained at 37° for times varying between 2 and 3 hours, and the residual acetylcholinesterase (AChE) activity determined. *S*-Mustard ($CH_2Cl.CH_2.S.CH_2CH_2Cl$) is shown not to be a potent inhibitor of AChE. The ethyleneimines, TEM, CB 1263, CB 1289 and the nitrogen mustard CB 1348, unlike HN 2, were considerably more active at pH 6·3 than at 7·4 or 8·3. It is pointed out in this connection that cancerous tissues, unlike normal tissue, produce large quantities of lactic acid, and injections of glucose can be used to reduce the pH of such tissues to around 6·3. The anticholinesterase activity of the ethyleneimines is progressive and irreversible. Only very small quantities of TEM are taken up by erythrocyte stromata. J. B. S.

Reduced Diphosphopyridine Nucleotide Derivative, Isolation and Properties of. S. Chaykin, J. O. Meinhart and E. G. Krebs. (*J. biol. Chem.*, 1956, **220**, 811.) A reduced diphosphopyridine nucleotide derivative, DPNH-X, the formation of which from reduced diphosphopyridine nucleotide (DPNH) is catalysed by triose phosphate dehydrogenase, has been isolated and its structure and properties studied. A high concentration of triose phosphate dehydrogenase is used in the formation of the derivative, the reaction being buffered at pH 6·0, rather than at the optimum of 5·0, because of the instability of the product at lower pH. Incubation at 25° was continued until 85 per cent of the ultra-violet absorption at 340° had disappeared. More prolonged incubation gave a product contaminated with an acidic substance. The product was isolated as the barium salt by precipitation with barium bromide and absolute ethanol at −15°. DPNH-X shows an absorption maximum at 265 mμ, and the point of maximum difference between the absorption of DPNH-X and DPN or DPNH is approximately 290 mμ. Analysis of DPNH-X corresponds to a compound with the composition $C_{21}H_{27}O_{14}N_7P_2Ba.4H_2O$ and of the same general structure as DPNH. There is no loss of nitrogen, or addition of phosphate, and all the various moieties of the DPNH structure are retained. On paper electrophoresis at pH 8·0 DPN, DPNH and DPNH-X migrated as single spots 0·70, 2·55 and 2·75 cm. respectively towards the positive electrode, indicating that the net charge on the molecule is the same as that for DPNH. DPNH-X has a spectrum almost identical with that of the primary acid modification product formed in the acid-catalysed reaction of DPNH at pH 4·0, though differences are apparent with the lower peak of the latter at 265 mμ, and an elevated shoulder at 290 to 300 mμ. DPNH-X is actually converted to the primary acid modification product by incubation at pH 3·0 to 4·0, but it has not been detected as an intermediate in the reaction of DPNH with acid.

Catalytic hydrogenations suggest that DPNH-X and the primary acid modification product each contain only one readily reducible double bond, compared with three in DPN, and two in DPNH. J. B. S.

Thiamine (Aneurine), a New Antagonist of. T. L. V. Ulbricht and J. S. Gots. (*Nature Lond.*, 1956, **178**, 913.) 4-Methyl-5-(2-hydroxyethyl)-*N*-(4-amino-2-methyl-thiopyrimidyl-(5)-methyl)-thiazolium chloride hydrochloride has been prepared, and found to be an inhibitory antagonist of thiamine in *E. coli*. The molar ratio of competition (inhibitor/thiamine) was of the order of 100. G. F. S.

BIOCHEMICAL ANALYSIS

Alkaloids in Biological Material, Determination of, by Compound Formation with Indicators. Z. I. El Darawy and S. L. Tompsett. (*Analyst*, 1956, **81**, 601.) A method is described for the determination of alkaloids in urine, plasma and tissues, e.g., liver and muscle, based upon the formation of a compound with an acidic indicator which is soluble in an organic solvent. An aliquot part of the organic solvent containing the base-indicator complex is then taken, and the latter decomposed with an aqueous solution of a strong acid or base. The absorption is then determined with a suitable spectrophotometer against a blank. When the amount of alkaloid is small, concentration is best achieved by passing the urine, diluted plasma, or tissue extract, adjusted to a suitable pH, through a column of Florisil, a synthetic silicate obtainable in various standard particle sizes (60 to 100 mesh used in this work) from the Floridin Co., U.S.A. After elution with a solution of 5 per cent sodium carbonate in 75 per cent ethanol and acidification, the ethanol is removed completely by evaporation to dryness and the assay performed on a solution of the residue, using the bromothymol blue-benzene procedure:—A suitable volume is adjusted to pH 8·0 to 8·5, extracted with benzene, centrifuged, and an aliquot portion of the benzene layer shaken with a buffered (pH 7·4) bromothymol blue solution. After further centrifuging another aliquot of the benzene layer is shaken with a definite quantity of 0·1N sodium hydroxide solution. Readings on the coloured aqueous solution are taken against a blank at 510 mμ. Without preliminary concentration, recoveries of strychnine from about 90 to 100 per cent were obtained from urine and plasma containing 5 μg./ml., and from liver and muscle containing 10 μg./ml. With preliminary concentration, amounts of the order of 0·5 μg./ml. in urine and 2 μg./ml. in plasma and liver could be determined. Further work is described using paper chromatography to increase the specificity of the method, strychnine and brucine being successfully separated and assayed. The recoveries were almost quantitative. D. B. C.

Glutethimide and a Metabolite in Dog Urine, Detection of. H. Sheppard, B. S. D'Asaro and A. J. Plummer. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 681.) The method is based on the reaction of glutethimide or α-phenylglutarimide with hydroxylamine to form a hydroxamic acid derivative, which yields a purple coloured complex with ferric ions. A sample of urine is treated so as to obtain a residue containing the glutarimide derivative. This is dissolved in 1 ml. of dehydrated methanol and 1 ml. of 2M hydroxylamine hydrochloride and 1 ml. of 3·5N sodium hydroxide added. After allowing the solution to stand for 30 minutes, 1 ml. of 3·5N hydrochloric acid and 1 ml. of 0·37M ferric chloride in 0·1N hydrochloric acid are added, and the light absorption is measured in a colorimeter with a suitable filter. Known amounts of glutethimide or α-phenylglutarimide are added to urine samples which are treated in

the same way, a standard curve being prepared from which the assay results are calculated. The conditions specified have been shown experimentally to be the most satisfactory. The colour reaction may also be used to indicate the position of the substances on paper chromatograms. When glutethimide is administered orally to dogs, it is excreted in the urine in the form of α-phenylglutarimide and a conjugated form which yields α-phenylglutarimide on acid hydrolysis. The free α-phenylglutarimide reaches its maximum rate of excretion later than the conjugated form, which may be a precursor of the free form.

G. B.

Oestriol, Oestrone and Oestradiol-17β in Human Urine, Determination of. W. S. Bauld. (*Biochem. J.*, 1956, **63**, 488.) A method is described for the determination of oestriol, oestrone and oestradiol-17β in human urine. The method involves acid hydrolysis of the urine, extraction with ether, separation of the ether extract into oestriol and oestrone-oestradiol fractions by partition between water and benzene. The aqueous extract, after saponification with sodium hydroxide, and extraction with ether, gives impure oestriol. This is chromatographed on a column of Celite using ethylene dichloride as the mobile phase and 70 per cent methanol as the stationary phase to give a purified fraction which can be determined colorimetrically by an improved Kober reaction. The procedure includes a photometric correction for non-oestrogen chromogenic material. The benzene solution, containing oestrone and oestradiol-17β, submitted to column partition chromatography, followed by saponification of the appropriate fractions, yields the two components in fractions, which can also be determined colorimetrically by means of the Kober reaction. Four urine specimens can be analysed in 9 to 10 man hours by this procedure. Results obtained on urine excreted during the menstrual cycle are reproducible and the method appears to be specific for oestrogens. The method is satisfactory for quantities of the order of 5 to 10 μg./day. J. B. S.

CHEMOTHERAPY

***p*-Aminophenoxyalkane Derivatives, Activity of, Against *Schistosoma mansoni*.** A. G. Caldwell and O. D. Standen. (*Brit. J. Pharmacol.*, 1956, **11**, 367.) A study of the schistosomicidal activity of derivatives of *p*-aminophenoxyalkane in mice infected with *S. mansoni* has shown that diphenoxyalkanes with different primary, secondary or tertiary amino groups in the *p*-positions have an activity similar to that of the symmetrical *pp'*-diaminodiphenoxyalkanes. Diphenoxyalkanes, having a *p*-amino group on only one ring, and any of a variety of substituents on the other ring were less active than the diaminodiphenoxyalkanes, but where the other group was acetamido-, acetmethylamido-, ethoxycarbonamido-, ethoxycarbonmethylamido-, or cyano-, there was a high activity. *p*-Aminophenoxyalkoxyalkanes had a moderate activity. *p*-Aminophenol, and a few of its derivatives, which are possible metabolites of these compounds, had no activity. The relationship between structure and activity is briefly discussed.

G. F. S.

Thiacetazone (Amithiozone, Tibione) Analogues. H. C. Caldwell and W. L. Nobles. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 729.) A series of thiosemicarbazones was prepared, including mainly monosubstituted derivatives of benzaldehyde 3-thiosemicarbazone or of acetophenone 3-thiosemicarbazone, and these compounds modified by the introduction of a vinyl group between the aromatic ring and the substituted aldehyde or keto group. The thiosemicarbazones of certain heterocyclic compounds and their vinylogues were also

prepared. The method of preparation of *p*-dimethylaminocinnamaldehyde and of 6-(2-thenyl)-3:5-hexadien-2-one, required as intermediates, is reported. When the thiosemicarbazones were examined for antituberculosis activity *in vitro*, in all cases the vinylogue was found to be at least as active as its parent compound. The thiosemicarbazones were also tested for activity in schistosomiasis, and as amoebicides and carbonic anhydrase inhibitors. 4-(3-Thenyl)-3-buten-2-one semicarbazone showed marked antituberculosis activity, inhibited the growth of *Streptococcus pyogenes* and *Staphylococcus aureus* and showed appreciable amoebicidal activity. G. B.

PHARMACY

Decanol-1 in Soap Solutions, The Solubility of, below the CMC. P. Ekwall and T. Vittasmäki. (*Acta chem. scand.*, 1956, **10**, 1177.) The solubility of decanol in dilute aqueous solutions of sodium caprate, sodium laurate, sodium myristate in concentrations below the critical micelle concentrations (CMC) at 40° has been determined, making use of the fact that the system becomes turbid when a new phase appears. With all three soaps decanol solubility starts to increase in the region of the limiting concentration of the soap. This increase in solubility slows as soap concentration increases, and actually begins to decrease until just below the CMC of the pure soap, when solubility again begins to increase. A second and rapid increase in solubility starts at soap concentrations about 30 per cent lower than the CMC of the pure soap, corresponding to the point at which alcohol-soap micelle formation also commences to increase rapidly. Above this point the increase in solubility is rapid. The minimum points thus give the CMC value for soap solutions containing decanol, and this is supported by the fact that 0·018M and 0·023M laurate solutions begin to solubilise *p*-xylene only after decanol has been added to them. Estimates are derived of the amount of soap bound in the mixed micelles above the critical concentration. Solubility curves show that the maximum ratio of decanol to soap in the mixed micelles remains constant above the CMC.

J. B. S.

Water, Sorption of, by Rubber Closures for Injections; Effect of Inorganic Salts. G. Milosovich and A. M. Mattocks. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 758.) Rubber stoppers of several types were dried to constant weight and placed in vials which were filled with the solution under examination and closed. Air bubbles adhering to the stoppers were removed by giving each vial a sharp tap before placing it in a water bath. After a specified period the stoppers were removed, deprived of surface moisture with a blast of compressed air and weighed. The stoppers were then returned to the vials, fresh solution added and the experiments continued. Inorganic salts were shown to depress the absorption of water by the stoppers. Some variation was observed between individual stoppers of the same type, owing largely to differences in density, and this made interpretation of the results more difficult. The effects of the various ions were compared by an analysis of variance, and it was shown that the results could be explained on the basis of the lowering of the vapour pressure of the solutions relative to water, divalent ions depressing the water absorption more than monovalent ones. The bisulphite ion was an exception, greatly increasing the penetration of water into the rubber. It is suggested that the bisulphite ion or a derivative of it penetrates the rubber and renders it more permeable to water. G. B.

PHARMACOLOGY AND THERAPEUTICS

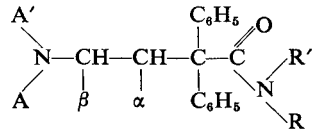
Acetazolamide, an Inhibitor of Carbonic Anhydrase, Effect of, on Gastric Secretion. L. Poller. (*Brit. J. Pharmacol.*, 1956, **11**, 263.) A high concentration of carbonic anhydrase has been demonstrated in the oxyntic cells of the stomach (Davenport, *Amer. J. Physiol.*, 1940, **128**, 725) and the enzyme has been shown to be intimately concerned in gastric acid secretion. The author undertook a study of the effects of acetazolamide on acid secretion to assess whether the inhibitor could be used in the treatment of hyperacidity associated with peptic ulcer. The subjects were twelve normal healthy adults and, after being kept on a fixed weight diet for three days with additional fluid intake restricted to 1500 ml., they were given 250 mg. or 500 mg. acetazolamide by mouth. Gastric, blood and urine samples were collected before and after administration of the drug. The rate of gastric secretion, acid production and rate of pyloric evacuation were all slightly diminished after acetazolamide. Atropine (2 mg. subcutaneously) had a much greater effect under similar conditions. Marked diuresis, increased sodium and potassium excretion and increased urinary pH were seen in all subjects in the first twenty-four hours after acetazolamide administration. It was concluded that, although acetazolamide inhibited gastric acid secretion, its other effects, such as systemic acidosis, would forbid prolonged therapy for this purpose. G. P.

Alphaprodine, Ro-1-7780, a Potent Antagonist of. C. W. White, Jr., R. Megirian and P. S. Marcus. (*Proc. Soc. exp. Biol., N.Y.*, 1956, **92**, 512.) Ro-1-7780 (1 : 3-hydroxy-*N*-propargyl morphinan tartrate) antagonized respiratory depression caused by alphaprodine and morphine in the dog and by alphaprodine in man. In the dog the dose ratio of alphaprodine to antagonist, at which no depression of respiration occurred when the two drugs were given simultaneously, was 75 to 1. When the two drugs were given to man in this dose ratio the antagonism was only partial, some degree of respiratory depression still being experienced. In dogs, Ro-1-7780 given either after or at the same time as alphaprodine gave an initial increase in ventilation rate and/or volume above control values, in spite of the fact that the antagonist given alone had no significant effects. This agrees with similar findings for other opiate antagonists. The narcotic effects of the alphaprodine in man were judged to be unaltered by the antagonist. G. P.

β-Aminoethyl*iso*thiuronium Bromide, The Pharmacology of, in the Cat. V. Di Stefano, D. E. Leary and D. G. Doherty. (*J. Pharmacol.*, 1956, **117**, 425.) In a screening test for drugs affording protection against the lethal effects of radiation the most promising was β-aminoethyl*iso*thiuronium bromide (AET). The pharmacology of this drug was studied further. Low doses (2·5 mg./kg.) caused a fall in blood pressure, bradycardia and apnoea in cats under barbiturate anaesthesia; these effects were all abolished by cutting the vagus nerves, and, except for the apnoea, by atropine. Gut contractions caused by the drug were also abolished by atropine. With larger doses of AET there was a diphasic blood pressure response, the initial fall being followed by a rise. The nature of this rise was obscure as it was still present after the spinal cord had been destroyed in the spinal cat, after bilateral adrenalectomy and after dibenamine or tolazoline. With doses of AET above 10 mg./kg. the contractions of the nictitating membrane of the cat were inhibited, partly by ganglion blockade and partly by a direct action. With these doses there was a slight augmentation of

skeletal muscle contractions. Doses above 25 mg./kg. caused convulsions and death. G. P.

Analgesics, A New Series of Potent. P. A. J. Janssen and J. C. Janssen. (*J. Amer. chem. Soc.*, 1956, **78**, 3862.) A series of over 100 new basic amides of structure I were prepared and some proved to have high analgesic activity in mice, rats, cats, guinea pigs, dogs and man.



(I)

The relation between chemical structure and analgesic activity in this series is briefly outlined. In the α-CH_3 series, one of the optical isomers of each enantiomorphic pair is twice as active as the racemic mixture; the other optical isomer is devoid of significant analgesic activity. The (+)-isomer of I; $\alpha = CH_3$, $\beta = H$, $-N\begin{smallmatrix}A'\\A\end{smallmatrix}$ = morpholino, $-N\begin{smallmatrix}R'\\R\end{smallmatrix}$ = 1-pyrrolidinyl is an analgesic 60 to 100 times more active than pethidine, 10 to 40 times more active than morphine, 5 to 20 times more active than methadone and about 4 times more active than heroin in various experimental conditions. In animals it had a higher oral activity and a better therapeutic ratio than any other analgesic tested. Preliminary experiments with the racemic compound in man indicate an analgesic potency of about 3 times that of morphine; no side effects were observed after subcutaneous injections of up to 12 mg. A. H. B.

Benactyzine, Pharmacology of. F. M. Berger, C. D. Hendley and T. E. Lynes. (*Proc. Soc. exp. Biol., N.Y.*, 1956, **92**, 563.) Benactyzine, an antispasmodic which has been used in the treatment of psychoneuroses, caused hyperexcitability and clonic convulsions in mice after intraperitoneal injection of one-half of an LD50 (155 $\pm$ 9 mg./kg.). In adult Rhesus monkeys intravenous doses ranging from 1 to 6 mg./kg. caused only minor changes in behaviour. After 1 mg./kg. there was mydriasis and some decrease in locomotion, but no demonstrable taming effect. Ataxia and occasional convulsive jerks occurred with 2 mg./kg. and with 6 mg./kg. one monkey went into frank convulsions for about 10 minutes, followed by post-ictal depression lasting one hour. The duration of the anaesthesia with 100 mg./kg. hexobarbitone given intraperitonially to mice was more than doubled when 10 mg./kg. benactyzine was given with the barbiturate. This was markedly greater than the prolonging effect of diphenhydramine. Benactyzine also increased mortality in mice subjected to electroshock seizures. The drug antagonized the actions of acetylcholine, 5-hydroxytryptamine and histamine on guinea pig and rat smooth muscle. In chloralosed cats the pressor effects of adrenaline were potentiated, 1 mg./kg. benactyzine almost doubling the effect of 5 μg./kg. adrenaline. The toxicity of adrenaline in mice was also increased. The knee jerk and flexor reflexes in chloralosed cats were unaffected by 3 mg./kg. benactyzine; increasing the dose to 10 mg./kg. abolished both reflexes and caused respiratory arrest. The effects on the EEG of curarized cats were to block EEG arousal from sensory or thalamic stimulation, while leaving unaffected recruiting responses evolved in the cortex; the EEG records obtained were

indistinguishable from those obtained after suitable doses of atropine under similar conditions. G. P.

Brom-lysergic Acid Diethylamide (BOL), Blockade by, of the Potentiating Action of 5-Hydroxytryptamine and Reserpine on Hexobarbitone Hypnosis. G. C. Salmoiraghi, L. Sollero and I. H. Page. (*J. Pharmacol.*, 1956, **117**, 166.) The hypnotic effect of hexobarbitone in mice is potentiated by 5-hydroxytryptamine (5-HT) and by reserpine; this potentiation is blocked by (+)-lysergic acid diethylamide (LSD). The 2-bromo derivative of LSD, known as BOL, has been shown to inhibit the action of 5-HT on the rat's uterus, but otherwise to differ in its actions from LSD in that it has no hallucinogenic action. It also blocks 5-HT on the guinea pig's ileum, where LSD has no effect. In the present experiments the authors have shown that BOL (10 mg./kg.) antagonized the potentiation of hexobarbitone sleeping time by 5-HT and reserpine in mice and rats. By itself, BOL decreased spontaneous activity in the rats and mice, but did not prolong hexobarbitone hypnosis. These results support the view that LSD does not owe its hallucinogenic actions to blockade of normally occurring 5-HT in the brain. G. P.

Chemical Radio-Sensitizer (Synkavit), Protection by Cysteine Against the Acute Toxicity of. A. F. Phillips and D. B. Cater. (*Brit. J. Pharmacol.*, 1956, **11**, 128.) Toxic doses of the vitamin K substitute, tetrasodium 2-methyl-1:4-naphthohydroquinone diphosphate (Synkavit), caused hyperexcitability and convulsions on intravenous injection into rats. These effects were not due to hypoglycaemia and were presumably on the central nervous system; oxidation-reduction potential measurements in the brain give some support to this view. Death usually occurred within 45 minutes of administration: LD50 for rats was approximately 0·9 mM/kg. Cysteine in doses of 1·3 and 3·9 mM/kg. injected at the same time, or up to three hours before, protected a high proportion of rats against the immediate toxic effects of Synkavit, and increased the average survival times of animals receiving doses of Synkavit from 1 mM to 2 mM/kg. Cysteine by itself had no toxic actions in doses up to 4·7 mM/kg. Oxidation-reduction potentials in the brain rose after cysteine and fell after Synkavit. Glutathione had a similar protective effect, giving an increase in survival time comparable with that of cysteine at the same molar dose. G. P.

Chlorpromazine, Effect of, on Adrenaline Vasoconstriction in Man. J. Ginsburg and R. S. Duff. (*Brit. J. Pharmacol.*, 1956, **11**, 180.) The effects of intra-arterial and of intravenous infusions of chlorpromazine on the blood vessels of the hands of healthy men were assessed by venous occlusion plethysmography. The drug was infused into the brachial artery just above its bifurcation at the elbow, or into a superficial vein of the other arm. The blood flow through the hand increased by an average of about 50 per cent after intra-arterial (1·2 mg.) and by about 400 per cent after intravenous (50 mg.) chlorpromazine. The intravenous dose was calculated so as to give the same local concentration of chlorpromazine. The results indicate the degree of direct vasodilator action (with intra-arterial infusion) and total vasodilator action (intravenous infusion). The proportional reduction in blood flow with intra-arterial adrenaline (0·5 μg./min.) was 75 per cent before and 27 per cent after the infusion of 1·2 mg. chlorpromazine into the brachial artery; however, when the increased control level of flow during chlorpromazine administration, before the adrenaline was given, is considered, the total reduction in flow with intra-arterial adrenaline was not significantly different after chlorpromazine. The average proportional reduction in flow in the hand with intravenous infusion of adrenaline, was

slightly, but significantly, decreased by intra-arterial infusion of 1·2 mg. chlorpromazine. There was no reversal of adrenaline vasoconstriction even after the intravenous infusion of 50 mg. chlorpromazine. The results were discussed in relation to the antagonism between adrenaline and chlorpromazine. G. P.

5-Hydroxytryptamine, Potentiation of, by Phenylethylamine Derivatives with Central-stimulant Actions. J. Delay and J. Thuillier. (*C. R. Acad., Paris*, 1956, **242**, 3138.) Phenylethylamine, amphetamine, dexamphetamine, methylamphetamine and mescaline potentiated the action of 5-hydroxytryptamine (5-HT) on the isolated oestrous uterus of the rat. Two other phenylethylamine derivatives, ephedrine and neosynephrine, had no action on, and antagonized, respectively, the action of 5-HT. The potentiation may result from inhibition of amine oxidase. This may also account for the mental disturbances in man caused by potentiation of 5-HT by mescaline and large doses of central-stimulating amines. (E. Costa, *Proc. Soc. exp. Biol., N.Y.*, 1956, 91, 39.) G. P.

Local Anaesthetics, a Simple New Quantitative Method for Testing. C. Bianchi. (*Brit. J. Pharmacol.*, 1956, **11**, 104.) Two methods for determining local anaesthetic activity are commonly used: inhibition of the corneal reflex by local application of the drug and inhibition of the reaction to pin-prick stimulus of the skin by intracutaneous injection of the drug. The first method measures surface anaesthetic activity, the second also the power of infiltration. A new method for determining nerve trunk anaesthesia has been developed. It depends upon the reaction of a mouse to pressure applied to the tail by a small artery clip with the blades covered with rubber tubing. Mice failing to respond to the stimulus were eliminated from the test and the remainder received subcutaneously 0·1 ml. of a solution of the local anaesthetic, about 1 cm. from the root of the tail. 15 minutes after injection the pain reflex in the tail was again tested and the proportion of animals failing to react, noted. A linear relation was shown to exist between the logarithm of the concentration of anaesthetic and the probit of the proportion of mice showing anaesthesia. By this method the activity of cocaine was nearly half that of cinchocaine, approximately four times that of lidocaine and seven times that of procaine. The anaesthetic activity of all the compounds was of short duration and wore off within 90 minutes of injection. G. P.

Mecamylamine, Ganglion-blocking Properties of. C. A. Stone, M. L. Torchiana, A. Navarro and K. H. Beyer. (*J. Pharmacol.*, 1956, **117**, 169.) The ganglion-blocking properties of mecamylamine (3-methylamino-*iso*camphane hydrochloride) are characterized by a high order of potency, specificity and long duration of action. In the chloralosed cat the ratios of potency of mecamylamine: pentolinium: hexamethonium were:—for blockade of transmission through the superior cervical ganglion, 1:4:1; blockade of the pressor response to nicotine, 4:4:1; and increase in pupillary diameter, 2:4:1. The duration of action of the secondary amine was ten to twenty times longer than that of hexamethonium and three to four times that of pentolinium. In the dog under vinylbarbitone anaesthesia mecamylamine decreased the vasopressor responses to carotid occlusion and to nicotine and the fall in B.P. with stimulation of the peripheral vagus nerve. The effects of injection of acetylcholine were not altered and those to adrenaline, 5-hydroxytryptamine and angiotonin were increased. The drug had no atropine-like, antihistamine, or local anaesthetic properties, but in high doses had a neuromuscular blocking action; the ratio between minimally effective ganglion-blocking doses and skeletal muscle paralyzing doses was of the order of one to one hundred. The

relatively low ratio between the oral and intravenous LD50's, as determined in mice, indicate that mecamylamine was considerably better absorbed after oral administration than were tetraethylammonium, hexamethonium, pentolinium and chlorisondamine, whose ratios were four to six times greater. G. P.

Narcotic Drugs, Possible Mechanism of Tolerance to. J. Axelrod. (*Science*, 1956, **124**, 263.) Several striking similarities have been observed between the receptors for narcotic drugs and the enzymes which *N*-demethylate these drugs. They have in common, substrate specificity, stereospecificity and antagonism by nalorphine. Since changes in enzyme activity during the development of tolerance might reflect similar changes in the receptors, rats were made tolerant to morphine and the changes in enzymic activity of the liver observed. Tolerance was induced by daily intraperitoneal injections of morphine, starting with a dose of 20 mg./kg. and increasing over a period of 35 days to 150 mg./kg. A second group of rats had in addition a daily dose of nalorphine equal to a quarter of that of morphine given. The effects of withdrawal were studied on a third group, in which the dose regimen was the same as the first group, but the drug was abruptly withdrawn for 12 days after tolerance to 150 mg./kg. had been established. 24 hours after the test period the animals were killed and their livers examined for ability to *N*-demethylate morphine, dilaudid, pethidine and cocaine. In the case of the morphine-treated animals a marked decrease in demethylating activity of the livers occurred, both with morphine and dilaudid as substrates; demethylation of pethidine, a drug which exhibits less cross-tolerance to morphine than does dilaudid, was decreased to a less degree and demethylation of cocaine was no different from that of controls. Where the animals received both nalorphine and morphine, the reduction in enzymic demethylation of the three narcotic drugs was significantly less than in those receiving morphine only. The enzyme activity had returned to the control level or above in the animals where the morphine had been withdrawn. In none of the animals were enzymic *O*-demethylation of codeine, hydrolysis of diacetylmorphine or conjugation of morphine affected. From these results it would appear that the continuous interaction of narcotic drugs with enzymes inactivates the enzymes. Similarly, the development of tolerance may be due to an inactivation of receptors. G. P.

Nalorphine, a Potent Analgesic in Man. A. S. Keats and J. Telford. (*J. Pharmacol.*, 1956, **117**, 190.) The analgesic potency of nalorphine was determined in post-operative patients and compared with that of morphine. Morphine and nalorphine were never given to the same patient, thus avoiding any residual antagonistic effects the one might have had on the other. Nalorphine was at least as potent as morphine as an analgesic. Subjective side actions noted included drowsiness, dizziness, coloured dreams, visual hallucinations and disorientation. Respiratory depression caused by nalorphine was equal to that caused by morphine. It has been postulated that the antagonism of narcotic drugs by nalorphine is the result of the substitution of a weak narcotic (nalorphine) for a potent narcotic (e.g., morphine) at cell receptor sites, by competition for these sites. The results obtained with nalorphine cannot support this, since in no respect can it be considered a weak narcotic. G. P.

Rauwolfia Alkaloids, Anticonvulsant Action of Some Anti-epileptic Drugs in Mice Pretreated with. C. Bianchi. (*Brit. J. Pharmacol.*, 1956, **11**, 141.) The anticonvulsant activity of soluble phenytoin, troxidone, phenacemide reserpine and a preparation containing mixed rauwolfia alkaloids, was evaluated in mice against convulsions induced by camphor, leptazol or strychnine. The

anticonvulsant activity of combinations of the anti-epileptic drugs with reserpine or mixed rauwolfia alkaloids was also tested. The convulsant threshold to camphor, strychnine and leptazol was lowered by reserpine and rauwolfia alkaloids. These alkaloids also had the ability to nullify the anticonvulsant activity of phenytoin, troxidone and phenacemide against leptazol-induced convulsions, but had no effect on the activity of phenacemide against camphor or strychnine-induced convulsions. The anticonvulsants decreased the effects of reserpine and rauwolfia alkaloids in lowering the convulsant threshold to doses of camphor, leptazol and strychnine. The convulsions induced in mice by camphor were always clonic under normal conditions, but when reserpine or the rauwolfia alkaloids had been given previously they became tonic; this action was also reduced by the anticonvulsant drugs. As the adrenal ascorbic acid is known to be depleted by reserpine this was investigated as a possible site of the convulsion-facilitating action; adrenalectomized mice, however, did not show any significant change in their susceptibility to the convulsant effect of camphor. G. P.

Reserpine, Release of Blood Platelet 5-Hydroxytryptamine by, and Lack of Effect on Bleeding Time. P. A. Shore, A. Pletscher, E. G. Tomich, R. Kuntzman and B. B. Brodie. (*J. Pharmacol.*, 1956, **117**, 232.) The potent vasoconstrictive action of 5-hydroxytryptamine (5-HT) has led to the postulation that with disruption of platelets during clotting, the released 5-HT might participate in the mechanism of haemostasis. In the present experiments reserpine was shown to release 5-HT from the platelets of rabbits, rats and guinea pigs. The 5-HT content of the platelets was determined by extracting whole blood or a suspension of isolated platelets, first adjusted to pH 10, with butanol, returning the 5-HT to formate buffer at pH 4. The 5-HT content was then estimated in a spectrophotofluorometer by activating at 295 mμ and measuring the resulting fluorescence at 330 mμ. In rabbits injected intravenously with 5 mg./kg. reserpine, the 5-HT content of the blood declined progressively to 50 per cent four hours after injection. At 16 hours the 5-HT level was about 5 per cent of normal, this low level persisting for some 48 hours, normal values being regained only after 6 days. These results are similar to the effects of reserpine on the 5-HT content of the intestinal tract. Similar results were obtained for depletion of platelet 5-HT in rats and guinea pigs. There was no disruption of the platelets to effect the release of 5-HT. Bleeding times were, however, unchanged by the depletion of platelet 5-HT, thus making it unlikely that 5-HT has a role in haemostasis. G. P.

Substance Resembling Kallidin and Bradykinin, a Delayed Slow Contracting Effect of Serum and Plasma Due to the Release of. M. Schachter. (*Brit. J. Pharmacol.*, 1956, **11**, 111.) During experiments on a delayed slow contracting effect of serum and plasma on the isolated guinea pig's ileum, it was found that this slow contraction was not caused by any substance originally present, but had been the result of the release or formation of a substance from the serum or plasma in the organ bath. This substance could be produced by dilution of ox, guinea pig, rat, dog, cat or human serum or plasma. The release of the substance by dilution of ox or guinea pig serum was greatly reduced or abolished by soya bean trypsin inhibitor and by heating serum at 56° for 3 hours before dilution, to destroy kallikreinogen. The smooth-muscle stimulant resembled kallidin and bradykinin in that it contracted, in a characteristic way, the intestine of the guinea pig, cat, and dog and the uterus of the guinea pig, rat and cat; it was also inactivated by serum and by chymotrypsin.

A comparison of the relative ability of various substances which release smooth-muscle stimulants from serum showed that for a particular substance the release was not the same in sera of different species; the results indicate a degree of species specificity of kallikrein and renin for their serum substrates. Rabbit and hen serum failed to release a smooth-muscle stimulant on dilution. Compound 48/80, egg white, and wasp venom did not release kallidin or bradykinin from serum. The hypothesis is advanced that dilution of serum releases kallidin through activation of kallikreinogen. These results are of importance from a practical standpoint, since under the conditions which exist in many biological assays, serum or plasma may be diluted in the procedures and so form a potent smooth-muscle stimulant. G. P.

Tiofenatin, Pharmacology of. S. Ya. Arbuzov. (*Farmakologiya i Toksikologiya*, 1956, **19**, No. 1, 16.) The pharmacological properties of Fenatin (β-phenyl*iso*propylamide of nicotinic acid) and Tiofenatin (β-phenyl*iso*propylamide of thionicotinic acid) are compared. In experiments on cats and rabbits, intravenous doses of Tiofenatin varying from 0·005 to 0·01 g./kg. produced a hypotensive effect much greater in intensity than that obtained with Fenatin. After bilateral vagotomy and denervation of both carotid sinuses this effect was not only maintained but somewhat enhanced and its duration was also increased. Tiofenatin is less toxic than Fenatin; the LD50 of Tiofenatin given subcutaneously to white mice was 2100 mg./kg., which compares with 1200 mg./kg. for Fenatin. Tiofenatin dilates the vessels of the isolated rabbit ear in concentrations of 1 : 100,000 to 1 : 10,000,000; the effect was also observed on the innervated isolated ear when Tiofenatin was given in doses of 0·005 to 0·01 g./kg. In various experiments Tiofenatin showed a powerful hypotensive effect in rabbits and in cats. It also increased the depth of respiration. E. H.

Tubocurarine, The Effect of, on the Neuromuscular Blocks Caused by Di-*iso*propylfluorophosphate (Dyflos) and Acetylcholine. J. A. B. Barstad. (*Arch. Int. Pharmacodyn.*, 1956, **107**, 4.) Acetylcholine (ACh) and dyflos cause reversible neuromuscular blockades in the isolated diaphragm-phrenic nerve preparation of the rat. In the early stages of block with ACh (2 to 4 minutes after addition of the drug) the block was reversed by tubocurarine. Where the ACh had been in contact with the muscle for 15 minutes or more the tubocurarine deepened the block. With moderate amounts of dyflos a similar pattern of reversal and potentiation by tubocurarine could be reproduced. However, where higher concentrations (ca 2 to 5 $\times$ 10^{-4}M) of dyflos were used, the neuromuscular blockade could at all stages of development be increased by tubocurarine. In interpreting these results, where the effect of tubocurarine was to reverse a partial block, this could most readily be explained in terms of a repolarizing effect of the alkaloid on an end-plate membrane depolarized by excess ACh. The additive effect in the later stages of blockade with ACh cannot be understood without simultaneous records of the electrical state of the end-plate membrane. With low concentrations of dyflos the events can be likened to those obtained with ACh, and can be explained by the anticholinesterase effect of the compound, but with higher concentrations another action has to be considered: this may have its site in the presynaptic nerve endings. G. P.