ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

p-Aminosalicylic Acid, Determination of Small Quantities of. Yu. M. Ostrovskiĭ. (*Aptechnoe Delo*, 1955, **4**, No. 6, 10.) When *p*-aminosalicylic acid (PAS) is oxidised with potassium ferricyanide in alkaline solution, an intense orange-brown colour is produced; the reaction can be used to detect 0·01 mg. of PAS in 1 ml. of solution. For the quantitative determination of PAS, 2 ml. of a 0·005 to 0·05 per cent. solution is treated with 0·6 ml. of a freshly prepared solution of potassium ferricyanide (prepared by mixing 15 ml. of 33 per cent. sodium hydroxide solution with 35 ml. of 2 per cent. potassium ferricyanide solution). After 15 minutes the colour is compared with standards prepared with known amounts of PAS, or the density is measured in an absorptiometer and the PAS content of the sample is calculated with the aid of a calibration curve. The solution is stable for 15 minutes; when ascorbic acid is added the stability is increased but the sensitivity of the reaction is reduced. E. H.

Cycloserine, Colorimetric Determination of. L. R. Jones *Analyt. Chem.*, 1956, **28**, 39.) Cycloserine was found to react with sodium nitritopentacyanoferroate in a slightly acidic medium to give an intense blue complex suitable for quantitative measurement at 625 mμ. The colour developed rapidly and was stable for several hours, deviating slightly from Beer's law but being reproducible in the range of 5 to 200 μg. of cycloserine. Several variables were investigated for their influence on the colour formation including the absorption curve, stability and intensity of colour, time and temperature of reaction, stability and concentration of sodium nitritopentacyanoferroate, acid-base concentration, and interference from other compounds. The reagent must be prepared freshly for each set of determinations, two stock solutions of 4·0N sodium hydroxide and 4 per cent. aqueous sodium nitroprusside being prepared, mixed, and used within 15 minutes for each analysis of cycloserine. The blue complex showed a maximum absorption at 625 mμ, the highest precision and reproducibility being obtained at a temperature of $25° \pm 1°$ C. Cooling samples below 15° C. resulted in slow colour formation; heating samples above 50° C., completely destroyed the colour. Interference was found only with derivatives of cycloserine which still retain the basic ring structure, although low recoveries were obtained on urines containing added cycloserine. R. E. S.

Digitalis Glycosides in Chemical Assay. F. H. L. van Os and D. H. E. Tattje. (*Pharm. Weekbl.*, 1955, **90**, 901.) Among recently discovered glycosides of digitalis, strospeside (gitoxigenin + digitalose) is important since it is stated to correspond to about one half of the digitoxin content, or one tenth of the total glycosides. Chemical assay of digitalis by the Baljet reaction (sodium picrate) determines this glycoside, but not the inactive digipurpurin. On the other hand methods of assay based on digitoxose will determine digipurpurin, and therefore give misleading results. The compounds which react to the Baljet reaction are digitoxin, gitoxin, gitaloxin, strospeside, gitorin, with the minor constituents odoroside H, diginin and digifolein. Thus by this method there is some promise of an ultimate agreement between chemical and biological assays. G. M.

Vitamin B_{12} in Liver Injection, A Chemical Method for. P. J. VanMelle. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 26.) Samples of injectable liver extracts containing 120–250 μg. of vitamin B_{12} were treated with potassium cyanide at pH 7·5 to convert all cobalamins to cyanocobalamin. The solutions were mixed with citrate buffer, pH 4 and passed through columns of Amberlite XE97 (carboxylic cation-exchanger) in hydrogen form and of suitable particle size to maintain a sufficient rate of flow. Impurities were washed from the column with 0·1 N hydrochloric acid, acetone (85 per cent.) and again with 0·1 N hydrochloric acid. Cyanocobalamin was eluted from the column with dioxan (60 per cent. in water) containing sufficient hydrochloric acid to make it 0·1 N. Two equal 4-ml. quantities of the eluate of each column were taken, one being treated with potassium cyanide (10 per cent.) and the other with alkaline buffer to obtain a similar pH. The solutions were allowed to stand for 20–30 minutes and the difference in absorbancy measured at 578 mμ. The quantity of cyanocobalamin in each sample was calculated from the datum E (1 per cent. 1 cm.) difference = 60 for cyanocobalamin. Results obtained were in agreement with those of the microbiological assay using *Lactobacillus leichmannii*, but the chemical method was more precise. Preparations containing more than 100 mg. of solids per μg. of vitamin B_{12} required preliminary purification before the method could be applied. G. B.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Diginatin, a New Cardioactive Glycoside from *Digitalis lanata*. J. E. Murphy. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 719.) Diginatin was separated from other glycosides of *Digitalis lanata* by partition chromatography on a Celite column. A mixture of 2 parts of water and 1 part of formamide was used as the stationary phase, and chloroform saturated with the stationary phase was employed as the mobile solvent. Digitoxin, gitoxin and digoxin were obtained in the early fractions and diginatin in the later fractions. The glycoside was further purified by chromatography and crystallised from ethanol. It was soluble in ethanol and in dioxan, and slightly soluble in acetone. Solubility in water was about 1 in 1000 and in chloroform about 1 in 2000. On hydrolysis an aglucone ($C_{23}H_{34}O_6$) was obtained together with 3 molecules of digitoxose. The aglucone appeared to be isomeric with digoxigenin and gitoxigenin, except that it had one extra hydroxyl group. G. B.

Lanatoside ABC, Separation and Determination of Individual Lanatosides and Desacetyllanatosides in. O. Hrdý, Z. Jung and A. Šlouf (*Českoslov. Farm.*, 1955, **4**, 395.) The lanatosides and desacetyllanatosides in lanatoside ABC and commercial lanatoside C are determined by descending paper chromatography. For the separation of lanatoside A, Whatman No. 1 paper is used. The paper is impregnated with formamide and the chromatogram is developed with formamide-saturated chloroform as the mobile phase. Lanatoside B, desacetyllanatoside A, lanatoside C and the desacetyllanatosides B and C are separated on Whatman No. 4 paper, similarly impregnated, a mixture (8:2) of chloroform and ethyl acetate saturated with formamide being used as mobile phase. For evaluation, the spots are cut out and treated with xanthhydrol reagent (0·01 per cent. of xanthhydrol and 1 per cent. of concentrated hydrochloric acid in glacial acetic acid); the extinction of the solution obtained is measured in a Pulfrich photometer with a S 53 filter. A determination requires 100 μg. of sample and the precision is $\pm$ 5 per cent. Results obtained on various commercial preparations are given. E. H.

ORGANIC CHEMISTRY

Hypertension Peptide, Composition of. W. S. Peart. (*Nature, Lond.*, 1956, **177**, 132.) Quantitative study on the composition of the homogeneous pressor peptide (hypertensin or angiotonin) made by the action of rabbit renin on ox serum has shown the empirical structure to be: one residue—leucine, phenyl alanine, tyrosine, proline, aspartic acid and arginine: two residues—valine and histidine. This gives a minimum molecular weight of 1445. A. H. B.

TOXICOLOGY

Barbiturate Poisoning, Acute. P. M. G. Broughton, G. Higgins and J. R. P. O'Brien. (*Lancet*, 1956, **270**, 180.) Blood barbiturate levels have been determined, by a spectrophotometric method, in thirty-six instances of acute barbiturate poisoning. For each barbiturate the blood-level appeared to be related to the degree of consciousness. Blood-levels of the long-acting drugs were higher, and the rates of fall slower, than those of the short-acting ones. Patients who had taken short-acting barbiturates usually recovered more rapidly. Picrotoxin did not influence the rate of fall of the blood-barbiturate levels, but there was a return to consciousness at slightly higher blood-levels. Analysis of gastric contents and washings in six cases showed less than 4 per cent. of the dose to be left in the stomach. Urine analysis showed no correlation between urine levels and the degree of unconsciousness. It is concluded that blood-levels of 9 mg. per 100 ml. of long-acting, 6 mg. of intermediate and 4 mg. of short-acting barbiturates indicate severe poisoning. G. F. S.

Barbiturates, Toxicological Detection of, by Paper Chromatography. M. Ledvina, B. Chundela, B. Večerek and K. Kácl. (*Českoslov. Farm.*, 1955, **4**, 386.) For the detection of barbiturates in urine, a 2 to 10-ml. sample is acidified and extracted with ether; the ether extract is evaporated to dryness and a solution of the residue in ethanol is deposited on Whatman No. 1 paper. The chromatogram is developed with a mixture (2:1) of *n*-pentanol and 20 per cent. ammonia solution. With this solvent extraneous materials present in the ether extract of the sample do not interfere. The descending technique is mainly used. The spots are detected by spraying the paper with 0·5N sodium hydroxide solution and photographing the chromatogram in ultra-violet light at 254 mμ; the light source is a mercury lamp screened with a Jena UG5 and a chlorine filter. The sensitivity of the method is 10 μg. for barbitone, phenobarbitone and phenylmethylbarbituric acid, and 20 μg. for cyclobarbitone, allobarbitone, amylobarbitone, hexobarbitone, 5-allyl-5′-*iso*propyl- and 1-methyl-5-*iso*propyl-5′-bromallylbarbituric acid. E. H.

PLANT ANALYSIS

η-Tocopherol (7-Methyltocol): a New Tocopherol in Rice. J. Green and S. Marcinkiewicz. (*Nature. Lond.*, 1956, **177**, 86.) The lipid fraction was extracted from ground whole Japanese rice with ether. After the usual purification by alkaline saponification and treatment with floridin earth, the vitamin E complex was analysed by two-dimensional chromatography. The papers, after spraying with ferric chloride-dipyridyl reagent in ethanol, showed three well-defined spots in the α, ϵ and ζ-positions; γ and δ-tocopherols were absent. The spot on the ϵ-position, on spraying with diazotised *o*-dianisidine in alkaline solution, showed a strong positive reaction, given only with tocopherols

having a free non-methylated 5-position (the known ε-tocopherol does not react with this reagent). The new spot was shown to be produced by 7-methyltocol (named η-tocopherol) because 1 mg. of the substance was isolated and methylated with formaldehyde, hydrochloric acid and stannous chloride to yield a product which upon examination by two-dimensional paper chromatography gave two new spots identified by R and R_F values and chemical tests as α and ζ-tocopherols. η-Tocopherol has an absorption maximum at 293 mμ [E (1 per cent., 1 cm.), 78], gives a yellow nitroso derivative with nitrous acid, and upon oxidation with nitric acid gives a red *o*-quinone with absorption maximum at 477 mμ [E (1 per cent., 1 cm.), 56]. Rice contains α- ζ- and η-tocopherols in the proportion 47:26:27. A. H. B.

***Artemisia absinthium*, Bitter Principles of.** G. Schenck and N. E. Schuster. (*Arch. Pharm. Berl.*, 1956, **289**, 1.) The substance known as absinthin is actually a mixture of four compounds, which may be separated by chromatography on alumina. These show the following characters:

	Artamarin	Artamarinin	Artamaridin	Artamaridinin
M.pt. °C.	95–96	72	Oil	82
Ultra-violet maximum—mμ	275–280	268–273	275–280	no maximum
Maximum/minimum.. ..	1·27	1·02	2·12	—
Bitter value	850	4450	6800	125000
Ultra-violet fluorescence ..	strong blue	bright yellow	yellow	pale blue

The Table below gives the amount of the different compounds extracted from the drug

Material	Solvent	Bitter substance	Per cent. of dry material
Commercial tincture ..		Artamaridin Artamarinin	0·15 0·02
Sun-dried herb	Ethanol (60 per cent.)	Artamaridin Artamarinin	0·31 0·025
Commercial drug ..	Ethanol (60 per cent.)	Artamaridin Artamarinin	0·15 0·018
Fresh herb	Ethanol (60 per cent.)	Artamaridin Artamarinin	0·15 0·01
Commercial herb ..	Water	Artamarin Artamaridin Artamaridinin Artamarinin	0·025 0·014 0·025 0·08
Fresh herb	Water	Artamarin Artamaridin Artamaridinin Artamarinin	0·023 0·007 0·018 0·06

Other results show that on drying the amount of bitter substances increases. The bitter value of the drug runs parallel with the artamarin content. G. M.

BIOCHEMISTRY

BIOCHEMICAL ANALYSIS

Azovan Blue, Determination of, in Blood and Tissues. D. F. Clausen and N. Lifson. (*Proc. Soc. exp. Biol. N.Y.*, 1956, **91**, 11.) A 5 g. sample of the dye-containing tissue is homogenised in 100 ml. of a concentrated solution of urea (equal parts of urea and water). The homogenate is poured into a flask

and cooled in a cold water bath for five minutes. Two hundred ml. of acetone AR is added and the mixture shaken. Ten ml. of Somogyi reagent I (10 per cent. zinc sulphate 7 H_2O) is added, shaken and followed by 10 ml. of Somogyi reagent II (0·5 N sodium hydroxide). The mixture is shaken for ten minutes and then filtered through a Whatman No. 42 filter paper. The optical density of the filtrate is measured in a colorimeter at 620 mμ. Comparisons are made between samples of tissue plus unknown dye, tissue plus known dye and tissue without dye. For blood use 0·5 to 2 ml. of whole blood, 2 ml. of urea solution, 8 ml. of acetone and 0·5 ml. of each of the Somogyi reagents. The supernatant solution after centrifuging is taken for colorimetry. The method has been used to study the fate of the dye after intravenous injection into rats.

G. F. S.

Plasma and Red Blood Cell Cholinesterase Activity, Determination of. K. B. Augustinsson. (*Scand. J. clin. lab. Invest.*, 1955, 7, 284.) A method for the routine analysis of whole blood cholinesterases, using thiocholine esters, is described. 0·05 ml. of whole blood is dried on a filter paper at room temperature. The blood spot is cut from the paper and placed in small pieces in a 10 ml. Erlenmeyer flask. For the plasma butyrylcholinesterase assay 1·5 ml. of barbital buffer (sodium barbitone 4·1236 g., potassium acid phosphate 0·5446 g., potassium chloride 44·73 g. and magnesium chloride 0·2665 g. in 1 litre of water) is added; and for the erythrocyte acetylcholinesterase assay 1·4 ml. of barbital buffer and 0·1 ml. of a solution of 10-(α-diethylamino-propionyl)-phenothiazine ($3{\cdot}3 \times 10^{-3}$ M). The mixture is shaken for 15 minutes at 25° C. in a water thermostat and then 0·5 ml. of the substrate solution is added (butyrylthiocholine iodide or acetylthiocholine iodide 0·1 M solutions diluted with 4 parts of barbital buffer before use). The reaction mixtures are shaken at 25° C. for 30 minutes when the reaction is stopped by the addition of 4 ml. of 10 per cent. trichloroacetic acid. The mixtures are filtered and the precipitate washed with 4 ml. of 2 per cent. trichloroacetic acid To the filtrates add 1·0 ml. of 0·01 N potassium iodate, 2 drops of 5 per cent. potassium iodide and 3 drops of 0·5 per cent. starch solution and titrate with 0·005 N sodium thiosulphate. If A = ml. of thiosulphate required to reduce excess iodine then μmoles of substrate hydrolysed = (m.equiv. KIO_3 $-$m.equiv. $Na_2S_2O_3$) 1000 = $(1{\cdot}00 \times 0{\cdot}01 - A \times 0{\cdot}005)$ 1000. The results agree with standard manometric procedures and show an accuracy of $\pm$ 3 to 5 per cent.

G. F. S.

PHARMACY

NOTES AND FORMULÆ

Carbopol 934, Pharmaceutical Uses of. B. Misek, J. Powers, J. Ruggiero and D. Skauen. (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 56.) Carbopol 934 is a high polymer containing a large proportion of carboxyl groups. A 0·5 per cent. solution, neutralised to about pH 7 by the addition of sodium hydroxide or carbonate is a satisfactory suspending agent for pine oil, coal tar solution, ichthammol and precipitated sulphur. Coarse emulsions may be prepared by using the 0·5 per cent. neutralised solution, or by mixing the oil with the powder and triturating with water, added gradually. They are improved by passing through a homogeniser. Liquid paraffin emulsions are improved by the inclusion of 0·5 per cent. of Tween 40. Carbopol 934 is not a satisfactory suspending agent for benzoic, salicylic and acetylsalicylic acids, zinc oxide, bismuth carbonate, calamine and substances which yield polyvalent metallic ions.

G. B.

Poliomyelitis Vaccine, Preservation of, with Stabilised Thiomersal. E. O. Davisson, H. M. Powell, J. O. MacFarlane, R. Hodgson, R. L. Stone and C. G. Culbertson. (*J. Lab. clin. Med.*, 1956, **47**, 8.) The presence of thiomersal as a preservative in some poliomyelitis virus vaccines has caused a deterioration in antigenicity, particularly when subjected to increased temperatures during storage. This has now been found to be due to an instability of thiomersal in the presence of traces of cupric ions, the degradation products destroying the antigenicity. The addition of the trisodium salt of ethylenediaminetetra-acetic acid (EDTA), which chelates the cupric ions, stabilises the thiomersal, while the antibacterial and antifungal activities of the thiomersal are not diminished. Trivalent poliomyelitis virus vaccine produced in monkey kidney tissue culture may be satisfactorily preserved with thiomersal in the presence of EDTA. EDTA does not prevent the loss of potency of a vaccine which has excess formaldehyde and thiomersal presumably due to a sensitisation of the virus particle to formaldehyde by the very low concentrations of ethyl mercuric ions formed in the dissociation of the mercurial. It is necessary therefore to neutralise excess formaldehyde in the poliomyelitis vaccine with sodium bisulphite. G. F. S.

Pyrogen Tests, Effects of Drugs on. H. H. Frey, G. Holtz and K. Soehring. (*Arch. pharm. Berl.*, 1956, **289**, 29.) The ordinary pyrogen test cannot be used indiscriminately for testing all injection solutions, since certain drugs are able to suppress the pyrogen reaction. To assume that this will also occur with the patient is hardly justified, in the absence of further information. When testing such solutions, e.g., rutin, the effect on the pyrogen sensitivity should be checked. A possible alternative is to use the leucocyte reaction of Todd and others for determining freedom from pyrogens. In testing drugs for their effect on the pyrogenic reaction, Pyrifer may be used with advantage, as its administration at three day intervals does not give rise to any tolerance. G. M.

PHARMACOLOGY AND THERAPEUTICS

Cardiac Glycosides, Determination of the Duration of Action of. M. Rand and A. Stafford. (*Nature, Lond.*, 1956, **177**, 278.) Cardiac glycosides markedly potentiate the action of injected adenosine, in causing a transient period of partial heart block in the guinea-pig (Rand, and others, *J. Pharmacol.*, 1955, **114**, 119). A method for determining the duration of action of cardiac glycosides is now described. Injections of adenosine are made at regular intervals, and then a dose of the cardiac glycoside is given. With a single injection of digoxin the effect persists for longer than 3 hours, but with digoxigenin the effect is only transient. The rate of elimination of digoxigenin is approximately 1 to 1·5 mg./kg./hr. The rate of elimination of other cardiac glycosides is being studied. G. F. S.

Chloroethylamine in the Treatment of Hodgkin's Disease. L. F. Larionov. (*Brit. med. J.*, 1956, **1**, 252.) A new chloroethylamine, namely, 2-chloropropyl-i-(2-chloroethyl) amine hydrochloride (Novoembichin) has been widely used since 1952 in the U.S.S.R. and has now replaced mustine. It has a stronger effect than mustine on hæmopoiesis and a milder effect on the bone marrow. It also has a milder side-effect on the gastro-intestinal tract. It is given by intravenous injection three times a week, from 8 to 16 injections being necessary.

For the treatment of lymphoid leukæmia a dose of 8 mg. is used and for myelogenous leukæmia 10 mg. Injections are continued until the leucocyte count falls to 2500–3000/c.mm. If this fails to produce complete regression of lymph nodes an additional course of treatment is given 6 weeks later. About 300 patients have been treated with the drug over the last 4 years. Immediate positive results were obtained in nearly all cases. These included a decrease in size of the affected nodes or their complete regression, disappearance or amelioration of general symptoms, such as fever and pruritus, and partial or complete recovery of working capacity. The worst results were obtained in patients in the later stages of the disease, particularly where there was involvement of the retroperitoneal nodes. In spite of good immediate results, relapses occurred in many cases. It is important to repeat the treatment at the onset of relapse when symptoms first appear; if treatment is delayed the next stage of the illness sets in. Provided that the treatment is given in the early stages, positive remote results, namely, preservation of life and working capacity for more than 5 years from the beginning of treatment, may be obtained in 50 per cent. of cases. The immediate and late results of the treatment of early cases are at least as good as those of X-ray therapy. Useful results may be obtained, however, by combining the two treatments as follows. (1) an initial course of chemotherapy is given, followed after an interval of 6 to 8 weeks by X-irradiation of nodes which have not completely regressed; or (2) the two methods of treatment are applied alternately in subsequent relapses. More recently, a new drug which is effective orally has been developed; this is 2:6-dioxy-4-methyl-5-(2-chloroethyl)aminopyrimidine (Dopan). This is given in a dose of 8 to 10 mg. twice weekly, and clinical trials indicate that the immediate results are as good as those with the intravenous drug. S. L. W.

Levallorphan and Alphaprodine in Anæsthesia. F. F. Foldes, E. Lipschitz, G. M. Weber and M. Swerdlow. (*J. Amer. med. Ass.*, 1956, **160**, 168.) In a new technique the short-acting analgesic, alphaprodine, was used in combination with the narcotic antagonist, levallorphan, for the supplementation of nitrous oxide-oxygen-thiopentone sodium anæsthesia. The results in 852 patients controlled with the new technique were compared with those in 756 patients in whom nitrous oxide-oxygen-thiopentone sodium was supplemented by alphaprodine alone. The dose of levallorphan was 0·02 mg./kg. of body weight and that of alphaprodine 1 mg./kg. except in older, debilitated patients to whom one-half or two-thirds of these doses were given. The drugs were injected individually before administration of the anæsthetic, the injection of levallorphan preceding that of alphaprodine by 3 to 6 minutes. If the depth of anæsthesia was inadequate at the start of surgery, additional 5 to 20 mg. doses of alphaprodine were given, 2 to 3 minutes apart, until the desired level of anæsthesia was obtained. When this could not be accomplished without depression of the respiratory rate below 12 respirations per minute additional doses of 25 to 50 mg. of thiopentone were given 2 or 3 minutes apart. When the duration of surgery exceeded 2 to 3 hours, or when the patient's spontaneous respiratory activity was inadequate at termination of anæsthesia, one or more additional 0·4 to 0·6 mg. doses of levallorphan were administered. The administration of additional 5 to 20 mg. doses of alphaprodine was governed by the signs of lightening of anæsthesia, the average interval between supplementary doses being from 8 to 20 minutes. The mg./minute requirements of thiopentone were significantly decreased and the mg./minute requirements of alphaprodine significantly increased in the series of patients who received alphaprodine and levallorphan compared to the patients in the control

series. In 92 (27 per cent.) of 344 patients on whom extraperitoneal surgery was performed satisfactory operating conditions could be obtained by alphaprodine and levallorphan without the use of thiopentone. In both series the mg./minute thiopentone and alphaprodine requirements were inversely proportional to the duration of anæsthesia. Levallorphan antagonised the respiratory depressant effects of alphaprodine to a considerable degree without causing any apparent diminution of analgesic activity. No major post-anæsthetic complications were encountered. The incidence of minor post-operative complications was not markedly different in the two series. (The original paper should be consulted for full details of the technique). S. L. W.

2-Methyl Hydrocortisones, A New Series of Steroids. G. W. Liddle and J. E. Richard. (*Science*, 1956, **123**, 324.) An initial study is reported of 2-methylhydrocortisone and 2-methyl-9-α-fluorohydrocortisone in comparison with hydrocortisone and 9-α-fluorohydrocortisone in normal human subjects, in patients with Addison's disease and in adrenalectomised dogs. In humans, oral administration of single doses of methyl-9-α-fluorohydrocortisone (0·025 to 1·0 mg.); 9-α-fluorohydrocortisone (0·2 to 1·0 mg.); methyl hydrocortisone (10 to 400 mg.) and hydrocortisone (100 mg.) caused sodium retention and potassium loss. Assays in dogs showed the 2-methyl compounds to be many times more potent than their non methylated analogues and methyl fluorohydrocortisone to be more potent that aldosterone in sodium retention. The steroids increased absorption of sodium by the renal tubules. The decrease in circulating eosinophils as an index of "glucocorticoid" activity in both man and dog, showed the methylated steroids to be only slightly more potent than the non methylated compounds during the first four hours of treatment, but to have much longer durations of action. It is suggested that the 2-methyl group alters the susceptibility of the steroids to enzymatic attack. After administration of the methyl fluorohydrocortisone, only 5 per cent. of the dose could be accounted for as 17:21-dihydroxy-20-ketosteroids in the urine compared with 30 per cent. for the unmethylated compound. G. F. S.

Pethidine; Controlled Analgesia with Continuous Drip. H. M. Ausherman, W. K. Nowill and C. R. Stephen. (*J. Amer. med. Ass.*, 1956, **160**, 175.) This is a report on 1000 anæsthetic administrations. In 800 the anæsthetic was thiopentone sodium, nitrous oxide-oxygen and pethidine drip, and in 200, serving as controls, thiopentone sodium and nitrous oxide-oxygen only. In all patients thiopentone sodium in a 2 or 2·5 per cent. solution was used for induction and employed intermittently during maintenance. Nitrous oxide-oxygen in a 75:25 ratio was given by semi-closed circle absorption or non-rebreathing techniques, and pethidine hydrochloride (0·5 mg./ml. in 5 per cent. dextrose in water) was dripped continually or intermittently by vein as required. The principal types of operation undertaken were plastic, orthopædic and other extra-abdominal procedures not requiring deep anæsthesia. In the total patients operated on, the thiopentone required was 675 mg./hour in those receiving thiopentone and nitrous oxide alone, whereas only 350 mg./hour was required in those receiving the pethidine drip. In the pethidine series therefore 48·1 per cent. thiopentone was required. In patients whose operative procedures exceeded 4 hours the respective thiopentone requirements per hour were 350 mg. and 247 mg., or a decrease in thiopentone requirement of 29·4 per cent. In those undergoing plastic procedures the figures were 596 mg. and 380 mg./hour respectively, or a decrease in thiopentone requirement of 36·2 per cent. An average of 72·3 mg./hour of pethidine was required as reinforcement to the analgesia provided by nitrous oxide. The authors conclude

that the continuous drip method of administration of pethidine gives a smoother anæsthesia, with less danger of severe respiratory depression and hypotension than is obtained by single injections of pethidine, and that the technique is a valuable addition to thiopentone sodium—nitrous oxide—oxygen anæsthesia. Details are given of investigations to determine the effect of the technique on tidal volume, minute volume of respiration, oxygen utilisation and alterations in pH in carbon dioxide tension in the arterial blood. S. L. W.

Reserpine in Delirium Tremens. M. Avol and P. J. Vogel. (*J. Amer. med. Ass.*, 1955, **159**, 1516.) Twenty-three patients suffering from delirum tremens were treated by intramuscular or intravenous injections of reserpine. The patients were free of their symptoms of acute hallucinations in an average of 18 hours; most of them were free of symptoms within 24 hours. The shortest time noted was 9 hours (in 2 patients) and the longest 48 hours (in 2 patients). The drug was given in a dose of 2·5 mg. intravenously or intramuscularly followed by a similar dose in 3 or 4 hours if the patients were still agitated. Ordinarily the patients would begin to quieten down in 6 or 8 hours. Many patients were given a dose of paraldehyde (10 ml.) initially in order to keep them controlled for the first few hours. All patients were fully awake or only slightly drowsy the following day. Despite the relatively large doses used no ill-effects were noted in this series. Hypertensive cardiovascular disease *per se* is not a contraindication to the use of the drug, though the drug should be used with caution in these instances. The use of reserpine greatly shortened the time required to free these patients of their post-alcoholic withdrawal symptoms and made them much easier to nurse.

S. L. W.

Zoxazolamine, Clinical Experience with. W. Amols. (*J. Amer. med. Ass.*, 1956, **160**, 742). Electromyography was used to provide a crude but objective index of the effect of relaxant drugs and was found to be a useful supplement to clinical evaluation. Arbitrary values are assigned to certain parts of the tracing according to the amount of electrical activity recorded when the limb is in a position requiring no voluntary effort, and the extent of muscular relaxation obtained by a drug and its duration are shown by plotting degrees of spontaneous relaxation against time. Premedication with chlorpromazine was found to potentiate the effect of mephenesin and it was therefore tried in conjunction with zoxazolamine (Flexin), a muscle relaxant believed to act in the same way as mephenesin by depressing polysynaptic pathways in the central nervous system. Zoxazolamine alone, in doses of 500 to 1500 mg. by mouth four times a day, gives only slight relaxation of spastic muscles. After premedication with chlorpromazine in doses of 10 to 50 mg. by mouth a significant degree of relaxation is obtained with 500 mg. of zoxazolamine four times daily in patients with spastic extremities. No effect was observed in patients with decerebrate rigidity, torticollis, or disease of the basal ganglions. Two patients with grand mal seizures whose anticonvulsant therapy was supplemented by zoxazolamine were made worse. 13 out of 16 patients with spastic extremities secondary to spinal cord disease showed a definite reduction in muscle tone. The amount of relaxation varied greatly in different patients and in the same patient at different times, but the duration of the effect was relatively constant at 6 hours. All patients whose spastic extremities were affected favourably by the treatment complained of greater weakness in the extremities and most of those showing the best response gave up the treatment for this reason. The principal role of muscle-relaxant therapy is as an adjunct to nursing care and physical therapy, to alleviate the discomfort and inconvenience of the spasticity. H. T. B.

BACTERIOLOGY

Tetrazolium Reduction as a means of Differentiating *Streptococcus fæcalis* from *Streptococcus fæcium*. E. M. Barnes. (*J. gen. Microbiol.*, 1956, **14**, 57.) The author reports studies on the reduction of 2:3:5-triphenyltetrazolium chloride (tetrazolium) in a glucose nutrient medium by the Lancefield group D streptococci. Tetrazolium is colourless in the oxidised form and is reduced to the insoluble red triphenylformazan. It was found that ability to reduce this compound served to distinguish strains of *Streptococcus fæcalis* from strains of *Str. fæcium* (Orla-Jensen). Differences in tetrazolium reduction agreed with other tests used to separate the species. Tetrazolium reduction in a 24 hour growth of the test organism in tetrazolium-glucose medium was determined by extracting the formazan with *n*-butanol and estimating colorimetrically. Percentage reduction was calculated by reference to a standard curve, which was obtained from complete reduction of solutions of known tetrazolium content on treatment with ascorbic acid in alkaline solution. 68 strains of *Str. fæcium* and 34 strains of *Str. fæcalis* were tested in the medium (pH 6·8–7·0). Only 6 strains of *S. fæcium* gave over 50 per cent. reduction of tetrazolium, whereas only 2 strains of *Str. fæcalis* gave less than 50 per cent. reduction. Thus the difference in reducing powers of the two species was characteristic. Maximum differentiation is achieved if the test is carried out at pH 6·0–6·2, but the formation of a colourless reduction product in addition to the formazan under these conditions make the use of a less acid medium necessary for quantitative determinations. No differentiation could be achieved in a tryptic digest medium containing tetrazolium. Rapid qualitative methods for differentiating the two species, using either solid or liquid media (pH 6·0–6·1) are described. The author considers that within the Lancefield group D, *Str. fæcium* appears to occupy an intermediate position between *Str. fæcalis* on the one hand and *Str. durans* and *Str. bovis* on the other. B. A. W.

Viable Bacteria in a Culture, Method for Determining the Proportion of. E. O. Powell. (*J. gen. Microbiol.*, 1956, **14**, 153). A rapid method of determining proportions of viable organisms by counts on cellophane graticules is described. The author gives details of a special engraving tool, in which a pile of razor blades, conveniently 11 in number, are held in a block. Discs of cellophane are pressed twice against the blade edges, one impression being made at right angles to the other. A graticule can be made in about 1 minute, including punching out the disc. Suitable cellophane for use is specified and methods of cleaning are described. In making a count, the disc is placed on the surface of a nutrient agar plate, preferably previously dried, and the centre of the disc inoculated with one loopful of a suitable dilution of the culture. A stream of air is blown over the surface to hasten drying, and then the numbers of single organisms and of groups in each square is noted. After 2–6 hours incubation, the number of groups in each square is again counted, the difference in the group count being taken as the number of viable organisms among the single organisms originally counted. Although only a limited range of organisms had been used with this method, the results were found reproducible and agreed generally with those obtained by conventional procedures. The chief advantages are speed, directness, absence of lengthy dilutions, avoidance of errors due to clumping and ability to count organisms which give spreading or diffuse colonies. The principal disadvantages appear to be limited statistical accuracy, apparent non-viability of organisms with a long lag phase and personal difficulties in assessment. B. A. W.