ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Nicotine, Occurence of, with Hyoscine in *Duboisia myoporoides* R. Br. K. L. Hills, W. Bottomley and P. I. Mortimer. (*Nature, Lond.*, 1953, **171**, 435.) This paper reports the finding of hyoscine, nicotine and nornicotine, in the leaves of *D. myoporoides* grown from seeds obtained from New Caledonia. Alkaloidal assays were carried out by the partition chromatographic method. The identities of the alkaloids were confirmed by preparation of the picrate, reineckate or dipicrate and checking the mixed melting point with the respective salt of the genuine alkaloid. This is the first time alkaloids of the propane and pyridine groups have been observed in the same plant, or even in the same species. Hybridisation is considered unlikely. G. F. S.

ANALYTICAL

Ethylenediamine tetra-acetic Acid, Spectrophotometric Titrations with. P. B. Sweetser and C. E. Bricker. (*Analyt. Chem.*, 1953, **25**, 253.) Procedures are given for the use of spectrophotometric end-points for the determination of iron, copper, and nickel with standard sodium ethylenediamine tetra-acetate solutions. For the determination of iron the complex of salicylic acid and ferric ions, maximum absorption at *ca.* 525 mμ, was used as the basis of the end-point. At *p*H approximately 2·4 the ethylenediamine tetra-acetate-iron complex is much stronger than the iron-salicylate complex; in the titration of an iron-salicylic acid solution with sodium ethylenediamine tetra-acetate there is a gradual disappearance of the iron-salicylic acid colour as the end-point is approached. The visual end-point can be detected to within 0·05 to 0·10 ml. and the spectrophotometric end-point at 525 mμ is even sharper. The titration of a copper solution at *p*H 2·4 to 2·8 can also be followed spectrophotometrically at 745 mμ, as the copper-ethylenediamine tetra-acetate complex has, at this wavelength, a molar extinction considerably greater than the copper solution alone. Nickel can be determined similarly except that a wavelength of 1000 mμ is used, and the *p*H of the nickel solution is made approximately 4·0 with a suitable acetate buffer. With the methods given, it is possible without any separation to determine copper in many non-ferrous alloys and iron in ferrous alloys and ores by a single, simple titration. R. E. S.

Fluoride in Natural Waters, Direct Titrimetric Determination of. A. M. Bond and M. M. Murray. (*Biochem. J.*, 1953, **53**, 642.) A volumetric procedure is described in which fluoride is directly titrated in aqueous solution with thorium nitrate using sodium alizarinsulphonate as indicator. The titration is carried out in the presence of acetic acid and at *p*H 3·3. The possibility of interference by SO_4^{--}, PO_4^{---}, CO_3^{--} and HCO_3^- is eliminated by prior treatment of the water with barium chloride. The sensitivity of the titration is unaffected by temperature over the range of 5 to 40° C. The preliminary treatment with barium chloride does not precipitate fluoride and in no way interferes with the subsequent titration, except when the solution contains a high concentration of barium chloride together with more than 15 μg. of fluorine.

Calcium and magnesium in such concentrations as may be expected in very hard water do not interfere with the titration. Aluminium forms a lake with alizarin at the *p*H of the titration and hence interferes with the titration; it is detected by the failure to give the pure yellow colour on addition of 0·05 N hydrochloric acid after filtration. 295 samples of domestic water in Great Britain were examined and only seven were found to contain more than 1 p.p.m. of fluorine. J. B. S.

Glucosamine and Galactosamine, Separation and Determination of. S. Gardell. (*Acta chem. scand.*, 1953, **7**, 207.) Glucosamine and galactosamine are separated on Dowex 50 ion exchange resin using 0·3N hydrochloric acid as solvent, the effluent being collected in fractions of equal volume, which are analysed by the method of Elson and Morgan as modified by Blix (*Acta chem. scand.*, 1948, **2**, 467). Examination of effluent-concentration curves shows that complete separation of the two amino sugars is readily achieved. Recovery is quantitative, the respective sugars being separated as hydrochlorides by concentrating the effluent solution, and crystallised by the addition of methanol and acetone. Glucosamine is the first component to appear in the effluent. J. B. S.

Phosphate Esters, Detection of, on Paper Chromatograms. H. E. Wade and D. M. Morgan. (*Nature, Lond.*, 1953, **171**, 529.) A method is given for the detection of phosphate esters on paper chromatograms depending on the fixation of ferric ions by the esters and the reaction of the free ferric ion with salicylsulphonic acid. If the paper is not strongly buffered it is sprayed with a 0·1 per cent. solution of $FeCl_3,6H_2O$ in ethanol (80 per cent.), dried in air at room temperature and then sprayed with a 1 per cent. solution of salicylsulphonic acid in ethanol (80 per cent.); on drying, the phosphates appear as white spots on a pale mauve background, *o*-phosphate having a band of deeper mauve surrounding it. The colour formation occurs only when the *p*H of the residual moisture in the paper is about 1·5 to 2·5. The method described permits the detection of 1 to 2 μg. of phosphorus, as phosphate ester, distributed over an area of about 1 sq. cm. R. E. S.

Sulphate and Hydrogen Sulphate Ions; Separation from Interfering Substances by Adsorption on Aluminium Oxide, Prior to Sulphate Determination. F. Nydahl and L. A. Gustafsson. (*Acta chem. scand.*, 1953, **7**, 143.) The use of aluminium oxide in the isolation of small amounts of anions from fairly concentrated salt or acid solutions is described. The capacity of the sample of aluminium oxide used was found to be about 0·17 m. mole/ml. for univalent anions and only about 0·08 m. mole/ml. for divalent anions, so that adsorption of sulphate is more effective from acid solutions. Phosphate and sulphate were very much more strongly adsorbed than were the other ions investigated. At *p*H 4 phosphate is more strongly adsorbed than sulphate, but in more strongly acid solutions the order is reversed. Thus separation of these two anions from most of the other common anions can be readily effected. In the separation of sulphate from large quantities of metals these should preferably be present as perchlorates, since the latter is one of the most readily adsorbed anions. Adsorbed sulphate is readily eluted by small volumes of dilute sodium hydroxide solution, sodium carbonate or ammonia. Applications of the method to the isolation of sulphate from pure solutions of acids and salts are described. It can also be used for the determination of sulphate in water or of sulphur in biological materials, dolomite, glass, iron and steel. J. B. S.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Carboxylic, Sulphonic and Anion Exchange Resins, Biochemical Changes in Serum and Fæces During Ingestion of. L. Greenman, W. A. Frey, R. E. Lewis, M. J. Sakol and T. S. Danowski. (*J. Lab. clin. Med.*, 1953, **41**, 236.) Mixtures of acidifying ion exchangers (hydrogen and ammonium forms of the carboxylic or sulphonic type) with alkylene polyamine resin (a strong anion exchanger) and the potassium form of the carboxylic type were tested in an attempt to overcome the disadvantages of the acidifying ion exchangers used alone. Mixtures of the carboxylic and sulphonic resins were no more effective in raising the fæcal output of univalent cations than the carboxylic resin used alone. The admixture of an anion-exchange resin did not consistently raise the fæcal output of chloride, sodium, potassium or nitrogen, but served to decrease the hyperchloræmia and acidosis due to the carboxylic resin. The addition of quantities greater than 8 g. per day of the potassium form of carboxylic resin resulted in partial absorption of potassium into the body and so is contraindicated in renal disease. It is concluded that for the treatment of certain conditions, ion-exchangers of higher efficiency under biological conditions are required. G. B.

Narcotics and Biological Acetylation. W. J. Johnson and J. H. Quastel. (*Nature, Lond.*, 1953, **171**, 602.) Experiments are described which show that low concentrations of narcotics impede the oxidative synthesis of adenosine triphosphate and consequently affect the rates of acetylation processes dependent on the presence of this substance. The effects of narcotics on rates of biological acetylations brought about by various tissue preparations were studied with the conventional Warburg apparatus. Acetylcholine was estimated by measuring the contractions of the dorsal muscle of the leech suspended in an ærated salt and glucose medium; phosphate esters were removed from the samples and assayed by the procedure of Harpur and Quastel; sulphanilamide was estimated by the method of Bratton and Marshall; acetylated sulphanilamide was determined by difference. Addition of pigeon liver extract to rat brain homogenate produces a system which readily acetylates sulphanilamide under ærobic conditions. Acetylation is increased on the addition of pyruvate or acetate to the system, pyruvate being more effective as an acetyl precursor, giving rise to increased oxygen uptake. Addition of chlorbutol (0·004 M) reduces both acetylation and respiration to low levels; adenosine triphosphate diminishes the chlorbutol inhibition of acetylation, though the respiration rate is much less affected. Thus the narcotic inhibition of acetylation results from the suppression of the oxidative synthesis of adenosine triphosphate. Further experiments with pigeon liver extracts confirm that the enzymes involved in sulphanilamide acetylation are not inhibited by narcotics even in relatively high concentrations. Narcotics similarly caused a marked inhibition of respiration and of the acetylation of choline by rat brain mince. The addition of adenosine triphosphate increases the rate of acetylation even in the presence of the narcotic, indicating that the narcotic does not impede its utilisation, but only its synthesis. 5-Allyl-5-*iso*propyl-*N*-methylbarbituric acid (narconumal), similarly, does not effect acetylcholine synthesis by a beef-brain extract undergoing a high rate of glycolysis. J. B. S.

Steroids, Paper Chromatography of. C. D. Kochakian and E. Stidworthy. (*J. biol. Chem.*, 1952, **199**, 607.) A procedure for the separation of C_{19} steroids by paper chromatography is described, using paper impregnated with a 1:1 methanol-propylene glycol, and a mixture (1:1) of benzene and *cyclo*hexane saturated with propylene glycol as the developing solvent. After completion of the run and drying, the paper was exposed to ultra-violet light on a fluorescence scanner and a map of the apparent spots made. The paper was resuspended in front of the electric fan containing a heating unit, sprayed with alkaline *m*-dinitrobenzene reagent, the spots outlined in pencil and the colours noted. The paper was then sprayed on each side with acid 2:4-dinitrophenylhydrazine reagent, which caused the previous colours to disappear as the 2:4-dinitrophenylhydrazones were formed. The presence of non-ketonic steroids was detected by preparing a duplicate paper chromatogram and spraying with phosphomolybdic reagent. 27 steriods were studied, their colours with the reagents recorded, and their mobilities related to the mobility of testosterone. In general, a wide variation in their rates of movement was observed, and the sequence of movement was dihydroxy-, hydroxy-, keto,, mono-substituted- and diketosteroids. The addition of a third group to the molecule retarded the movement, while removal of a group enhanced the movement. Stereoisomers were effectively separated, e.g., testosterone and epitestosterone. A. H. B.

Vitamin A_1, Oppenauer Oxidation of. H. R. Cama, A. C. Field, J. Glover, R. A. Morton and M. K. Salah. (*Biochem. J.*, 1952, **52**, 548.) An Oppenauer oxidation of vitamin A using aluminium alkoxides with diethyl ketone as hydrogen acceptor gave a complex mixture from which a compound $C_{20}H_{26}O$ (isomeric with retinene$_2$) was isolated; it gave an oxime m.pt. 175° to 177°. The compound obtained should be reduced *in vitro* and *in vivo*, but the reduction product was found to differ from vitamin A_2. The mechanism of the reaction is discussed and it was concluded that the Oppenauer oxidation proceeded *via* retinene$_1$, $C_{20}H_{28}O$, which could either be further dehydrogenated to $C_{20}H_{26}O$ or could condense with diethyl ketone to a ketone, $C_{25}H_{36}O$. Vitamin A acetate was not dehydrogenated directly to vitamin A_2. R. E. S.

Vitamin A_2, Spectroscopic Properties of. H. R. Cama and R. A. Morton. (*Analyst*, 1953, **78**, 74.) Detailed absorption intensities of vitamin A_2 alcohol produced by reduction of retinene$_2$ with lithium aluminium hydride, at different wavelengths and for different solvents, have been measured and expressed as fractions of the maximum intensities. Vitamin A_2 shows a main ultra-violet maximum near 351 mμ ($E_{1\text{ cm.}}^{1\text{ per cent.}}$ about 1400) and a secondary peak near 287 mμ ($E_{1\text{ cm.}}^{1\text{ per cent.}}$ about 750). The blue solution (with antimony trichloride reagent) shows $\lambda_{max.}$ at 693 mμ. ($E_{1\text{ cm.}}^{1\text{ per cent.}}$ about 3900). Fish-liver oils in general contain much more vitamin A_1 than A_2, depending on the species, and in cod-liver oils vitamin A_2 may account for about one-seventh of the total vitamin A (molecule for molecule). By determining $E_{1\text{ cm.}}^{1\text{ per cent.}}$ at 693 mμ. (A_2) and at 620 mμ. (A_1) in the antimony trichloride colour test (applied to the unsaponifiable fraction), and the $E_{1\text{ cm.}}^{1\text{ per cent.}}$ at 326 mμ, 351 mμ and 286 mμ in the ultra-violet region, both vitamins can be estimated. The 693 mμ absorption measures vitamin A_2 directly and from it the vitamin A_2 contributions to ultra-violet absorption at 351 mμ and 327 mμ can be calculated. A conversion factor is given for calculating the probable vitamin A_2 contribution to the potency. A cod-liver oil typical of those studied by spectrophotometric methods, corrected for all irrelevant absorption, gave an estimated vitamin potency about 6·5 per cent. lower than the estimate that included the possible vitamin A_2 contribution. R. E. S.

BIOCHEMICAL ANALYSIS

Barbiturates in Body Fluids, Determination of, by Spectrophotometry. J. T. Wright and R. G. S. Johns. (*J. clin. Path.*, 1953, **6**, 78.) The technique described gives a reasonable degree of accuracy with a minimum procedure. Heparinised blood is extracted with chloroform and filtered. The filtrate is shaken with aqueous sodium hydroxide and an aliquot amount added to a borate buffer at *p*H 10. The ultra-violet absorption curve is read over the range 220 to 300 mμ against a blank of sodium hydroxide and borate buffer. The solution is then acidified with hydrochloric acid and the absorption re-determined at the same wavelength. The barbiturate concentration is determined from a given formula. Extraction procedures are also described for urine, cerebrospinal fluid and gastric contents. There was a 60 per cent. recovery of barbitone added to blood samples at concentrations of 20 to 50 μg. per ml. Results are given for 17 clinical cases of barbiturate poisoning.

G. F. S.

Benzidine Reaction, Quantitative Determination of Plasma Hæmoglobin by. M. C. Creditor. (*J. Lab. clin. Med.*, 1953, **41**, 307.) Plasma is diluted to contain less than 10 mg. of hæmoglobin/100 ml. To 0·05 ml. is added 2 ml. of benzidine reagent (2 per cent. in 20 per cent. acetic acid) and, after mixing, 1 ml. of solution of hydrogen peroxide (1·5 per cent.) is added. The mixture is shaken and allowed to stand for 1 hour, diluted with 20 per cent. acetic acid to 100 ml. and allowed to stand for 30 minutes. The optical density at 490 mμ is determined in a suitable photoelectric colorimeter. The colour is stable for 24 hours. Beer's law applies in the range of concentrations 1 to 10 mg./100 ml. but the slope of the line varies slightly with each batch of benzidine reagent; consequently the standard curve must be re-determined with the aid of standard hæmoglobin solutions each time a series of samples is examined. The use of benzidine which has been specially purified is recommended in order to reduce the reagent blank. Incomplete recovery of hæmoglobin from the plasma limits the accuracy of the method. The percentage recovery should be determined experimentally and a correction made.

G. B.

Corticosteroids in Urine, Determination of. S. L. Tompsett. (*J. clin. Path.*, 1953, **6**, 74.) A method is described for the determination in urine of metabolites closely related to corticosterone, metabolites related to cortisone not being included. The method is based on the hydrolysis of the acid-stable conjugates of corticosteroids in urine with hot dilute mineral acid and then subjecting the steroid extract to a modified periodic acid technique and estimating the formaldehyde liberated colorimetrically with chromotropic acid. Desoxycorticosterone and corticosterone added to the urine were recovered quantitatively while cortisone was not recovered. Normal urine was found to contain 4·5 to 7·5 mg. per day of corticosterone-like substances and the values were low in hypo-adrenalism and elevated in hyper-adrenalism. Increased values followed the administration of adrenocorticotrophic hormone.

G. F. S.

Heparin in Blood, Estimation of. M. Bassiouni. (*J. clin. Path.*, 1953, **6**, 39.) A method is described for the extraction and estimation of heparin in 2 ml. of blood. Citrated blood is centrifuged, the plasma is removed and treated with sodium hydroxide and ammonium sulphate and the protein is

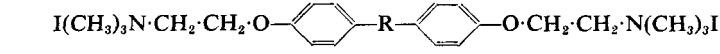
filtered off. Hydrogen sulphide and ammonia are then removed under reduced pressure. The heparin is precipitated by the treatment with a solution of dimethyl thionin (Azure A) for 48 hours at room temperature, and centrifuged. The supernatant liquid is removed, the precipitate is dissolved in acetone and the colour estimated electrocolorimetrically. Concentrations are read from a calibration curve obtained for a range of concentrations of heparin and a blank obtained for the reagents is deducted from these readings. Experiments showed 90 per cent. recovery of heparin added to plasma. Results for estimations of heparin in the blood of 10 healthy individuals are given and the concentrations in the blood obtained in one person after the intravenous injection of 7000 units of heparin. G. F. S.

Œstrogens, Quantitative Separation of, by Paper Partition Chromatography. L. R. Axelrod. (*J. biol. Chem.*, 1953, **201**, 59.) Rapid separation of œstriol, diethylstilbœstrol, 17α-ethinylœstradiol, 17β-œstradiol, 17α-œstradiol and œstrone have been effected on paper using the solvent systems *o*-dichlorobenzene-formamide, methylene chloride-formamide, *cyclo*hexene-formamide. Natural œstrogens are readily separated in a single chromatogram. The methylene chloride-formamide system was used to separate œstriol from highly polar pigments. Chromatograms were run from numerous urine extracts and the eluted œstrogens subsequently compared by comparison of the spectra of the sulphuric acid chromogens. In experiments designed to study the metabolism of 17α-ethinylœstradiol, the separation of 17β-œstradiol from 17α-ethinylœstradiol was best effected in *cyclo*hexene-formamide after preliminary separation in *o*-dichlorobenzene-formamide. The polarity of the œstrogens has a direct influence on their rate of movement, and the spatial configuration of the hydroxyl groups is an additional influence. Spot tests with fuming sulphuric acid, benzoyl chloride-zinc chloride, phenolsulphonate-phosphoric acid, nitrous acid-mercuric nitrate and antimony pentachloride are also described for the identification of the above œstrogens. Measurement of the ultra-violet adsorption of the sulphuric acid chromogens can be applied as a quantitative estimation. Œstriol and 17β-œstradiol were found in bile from an ovariectomised hysterectomised dog after injection of 17β-œstradioldibenzoate; œstrone was absent. Other phenolic and non-phenolic compounds of a steroid nature of which the sulphuric acid chromogen absorption spectra did not correspond to those of known œstrogens, were isolated. It is concluded that the completely hysterectomised dog cannot metabolise 17β-œstradiol to œstrone. J. B. S.

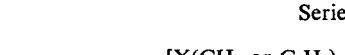
Proteins, Gravimetric Precipitation of, by Trichloroacetic Acid. F. L. Hoch and B. L. Vallee. (*Analyt. Chem.*, 1953, **25**, 317.) The method described uses the specificity of trichloroacetic acid in the precipitation of proteins and allows direct measurement of the weight of dried proteins; drying is performed at a temperature of 110° C. which is sufficient to eliminate water by evaporation and trichloroacetic acid by decomposition. Pure human and bovine serum albumin, pure bovine insulin, and crude bovine pituitary extract were precipitated and the dried precipitate weighed after treatment with trichloroacetic acid. The validity of this procedure is here appraised by a comparison of weights of dried precipitated proteins with weights obtained by direct weighing of pure dry proteins, by spectrophotometric measurements on clear solutions of dissolved proteins, and by nitrogen determinations. Experimental details of the method are given and the protein factors affecting trichloroacetic acid precipitation are discussed. R. E. S.

CHEMOTHERAPY

Curarising Agents, Pharmacological Studies of. S. Levis, S. Preat and J. Dauby. (*Arch. int. Pharmacodyn.*, 1953, **93**, 46.) 31 curarising agents of the 2 types shown below were examined for curarising effect and toxicity. Among the tests used were the sciatic-gastrocnemius and head-drop methods in the rabbit, fall of arterial pressure in the dog, determination of the LD70 dose in mice, isolated phrenic nerve-diaphragm (rat), guinea-pig ileum and rectis abdominis (frog). Some of the substances were 5 to 6 times more



Series I

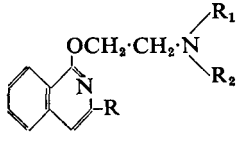


Series II

active than *d*-tubocurarine. A number of compounds were unsuitable for use as curarising agents because of weak action, prolonged periods of induction and activity, or insolubility. 3 of the compounds showed a high activity associated with normal duration of action and the compound R = − CH (C_3H_7*iso*)·CH_2– of series I was submitted for clinical testing because it showed the greatest margin of safety. 2 short-acting compounds were also found suitable for clinical trial, $Cl(CH_3)_3N{\cdot}(CH_2)_5{\cdot}O{\cdot}(CH_2)_5{\cdot}N(CH_3)_3Cl$ being the more active and the corresponding di-iodide having the greater margin of safety.

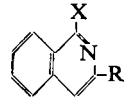
G. B.

***iso*Quinoline Derivatives as Local Anæsthetics.** E. L. Anderson, J. W. Wilson and G. E. Ullyot. (*J. Amer. pharm. Ass. Sci. Ed.*, 1952, **41**, 643.) 22 compounds of series I, 8 of series II and 11 of series III were synthesised and tested as local anæsthetics by the rabbit cornea method. In series I the most active compounds were R = *n*-butyl, *n*-pentyl or *n*-hexyl, and $R_1 = R_2 = -CH_3$ or $-(CH_2)_5-$. The most active compounds of series II had R = *n*-butyl, X = 4-(1-ethyl-2:6-dimethyl) piperidyloxy, 4-(1-methylpiperidyloxy) and 3-(1-methylpiperidyloxy). In series III the compounds R = $-CH_3$, $R_1 = R_2 = R_3 = H$, $R_4 = -OCH_3$, and R = $-C_2H_5$, $R_1 = R_3 = R_4 = H$, $R_2 = -NH_2$ appeared to be the most active.



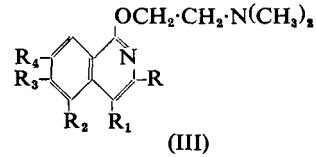
(I)

aminoethoxy*iso*quinolines



(II)

*iso*quinolines



(III)

1-(β-dimethylaminoethoxy) *iso*quinolines

The maximum anæsthetic activity was shown by 1-[3-(1-methylpiperidyloxy)] 3-*n*-butyl*iso*quinoline. *iso*Quinoline derivatives used as intermediates were prepared by methods described in previous work or by a new method of ring closure of phenethyl *iso*cyanates with aluminium chloride. G. B.

PHARMACY

NOTES AND FORMULÆ

Isoniazid. (*New and Nonofficial Remedies, J. Amer. med. Ass.*, 1953, **151**, 740.) Isoniazid is *iso*nicotinyl hydrazide and occurs as a white, odourless, crystalline powder, m.pt. 170° to 173° C., sparingly soluble in ethanol, very slightly soluble in benzene and ether, and freely soluble in water; *p*H of a 1 per cent. solution 5·5 to 6·5. When a solution in methanol is refluxed for 20 minutes with benzaldehyde and a trace of acetic acid, poured over ice, and filtered, the precipitate of white crystals obtained melts at 197° to 200° C., after recrystallisation from dilute ethanol. A 0·001 per cent. solution in 0·1 N hydrochloric acid exhibits an ultra-violet absorption maximum at about 266 mμ ($E^{1\text{ per cent.}}_{1\text{ cm.}}$, about 378) and a minimum at about 234 mμ. Isoniazid loses not more than 0·5 per cent. of its weight when dried at 105° C. for 4 hours and yields about 0·5 per cent. of sulphated ash. It contains 95·0 to 105·0 per cent. of isoniazid when determined by measuring the absorption of a 0·001 per cent. solution in 0·1N hydrochloric acid in a 1-cm. quartz cell at 266 mμ, and 97·5 to 102·5 per cent. when assayed iodimetrically. G. R. K.

Levarterenol Bitartrate (Levophed Bitartrate). (*New and Nonofficial Remedies, J. Amer. med. Ass.*, 1953, **151**, 821.) Levarterenol bitartrate is *l*-α-(aminomethyl)-3 : 4-dihydroxybenzyl alcohol *d*-bitartrate monohydrate, $C_8H_{11}NO_3$, $C_4H_6O_6$,H_2O. It occurs as a white, crystalline, odourless powder, m.pt. 100° to 106° C., freely soluble in water, slightly soluble in ethanol, and insoluble in ether; a 0·1 per cent. solution has *p*H 3·0 to 4·0. It gives an intense green colour with ferric chloride, and a 0·004 per cent. solution in 0·01 N hydrochloric acid exhibits an ultra-violet absorption maximum at about 2790 Å ($E^{1\text{ per cent.}}_{1\text{ cm.}}$, about 84) and a minimum at about 2490 Å. It yields not more than 0·2 per cent. of sulphated ash, and loses not more than 5·8 per cent. of its weight when dried to constant weight in an Abderhalden-pistol dryer at about 77° C. (carbon tetrachloride) for about 8 hours. It contains 95·0 to 105·0 per cent. of levarterenol bitartrate when assayed spectrophotometrically by measuring the absorption of a 0·004 per cent. solution in 0·01 N hydrochloric acid at 2790 Å, using water as a blank. G. R. K.

Polymixin B Sulphate (Aerosporin Sulphate). (*New and Nonofficial Remedies, J. Amer. med. Ass.*, 1952, **150**, 1219.) Polymixin B is a basic polypeptide derived from a strain of *Bacillus polymyxa* (*B. aerosporus* Greer). It contains leucine, threonine, phenylalanine, α,δ-diaminobutyric acid and a fatty acid of empirical formula $C_9H_{18}O_2$. Polymixin B sulphate is stable in the dry state and for extended periods in buffered solutions. It is highly effective *in vitro* against many Gram-negative micro-organisms. Polymixin B sulphate is a white to cream-coloured irregular, scale-like material which decomposes at about 230° C. and is soluble in water and normal saline; a 2 per cent. solution has a *p*H of about 5·7. An aqueous solution gives a blue colour when heated with ninhydrin and pyridine (presence of α-amino acid) and a pinkish-violet colour when made alkaline with sodium hydroxide and treated with a dilute solution of copper sulphate (presence of peptone or proteose), but yields no transitory red colour when treated with an alkaline solution of α-naphthol and a solution of sodium hypochlorite (absence of free protein). Polymixin B sulphate loses not more than 4 per cent. of its weight when dried at 37° C. for 4 hours in a vacuum oven at 5 mm. Hg. or less. It yields not more than 5·0 per cent. of sulphated ash and is assayed microbiologically. G. R. K.

PHARMACOLOGY AND THERAPEUTICS

Adrenaline and Noradrenaline, Influence of Blood Glucose Level on Secretion from the Suprarenal Medulla. H. Dunér. (*Nature, Lond.*, 1953, **171**, 481.) The secretion of adrenaline and noradrenaline in the venous blood from the suprarenal of the anæsthetised cat has been studied after injections of glucose. Adrenaline and noradrenaline were assayed biologically. The average normal secretion of noradrenaline and adrenaline was 75 μg./minute/kg. (16 per cent. of adrenaline). Infusion of glucose 0·1 g./kg. reduced adrenaline secretion to 50 per cent., and 0·8 g./kg. to 7 per cent., of the initial value. Noradrenaline secretion was also decreased but not to the same extent. As the blood sugar decreased adrenaline secretion increased. Denervation reduced the secretion to 25 μg./minute/kg. which was unaffected by glucose. The blood glucose level affects medullary secretion by a nervous mechanism. Cross-circulation studies suggest chemoreceptors located centrally, possibly in the hypothalamus.

G. F. S.

Antihistaminics and Atropine, Action of, in Blocking the Activity of Serotonin on the Guinea-pig Ileum. M. M. Rapport and G. B. Koelle. (*Arch. int. Pharmacodyn.*, 1953, **92**, 464.) Serotonin, the vasoconstrictor substance of mammalian serum is 5-hydroxytryptamine, chemically related to histamine and sympathomimetic amines. Experiments were designed to determine whether the substance acts on histamine sites. Segments of guinea-pig ileum were placed in an isolated organ bath and the concentrations of acetylcholine, histamine and serotonin which produced approximately equal reproducible contractions were determined. The blocking agents diphenhydramine, tripelennamine and atropine were added in turn in gradually increasing concentrations, in each case being allowed to act for 5 minutes before the spasmogenic agent was added. Diphenhydramine had the greatest effect on histamine spasm, less on serotonin and least on acetylcholine, the relative effectiveness being 4 : 2 : 1. It was not possible to block the action of histamine with this agent, without affecting the action of serotonin and acetylcholine. Tripelennamine was more specific for histamine, but the same order of effects was observed. Atropine was most effective against acetylcholine and least against histamine. The importance of controlling the time of contact of tissue with the blocking agent in these experiments is emphasised. It is not claimed that the experiments decide the question of whether serotonin acts on histamine receptors but the implications of the results in terms of histaminic and cholinergic action are discussed.

G. B.

Dicoumarol, Method of Administration. Chr. J. Bjerkelund. (*Lancet*, 1953, **264**, 260.) Two main methods of using dicoumarol are used at present, namely, intermittent dosage and maintenance dosage. Intermittent dosage consists in giving relatively large doses of dicoumarol at intervals of up to 10 to 12 days. Maintenance dosage aims at keeping the prothrombin value most nearly constant in the optimal therapeutic range by giving daily doses in amounts varying according to the patient's tolerance for dicoumarol. The maintenance dosage is the only rational method and is the only practical one when treatment of the ambulant patient has to continue for a long time. For its successful conduct, however, an exact and dependable method of estimating prothrombin is necessary. For this purpose Owren's method (P. A. Owren, *Acta med. scand.*, 1947, Supp. 194; *Scand. J. Clin. lab. Invest.*, 1949, **1**, 81; *ibid*, 1951, **3**, 168 and 201) is preferred to Quick's method and is especially

recommended, and close contact between laboratory, physician and patient is essential. The same initial dosage (250 mg. on the first day and 125 mg. on the second day) may be used for all patients. The effect of this dosage is seen after 2 or 3 days and gives an indication of the patient's tolerance. The dosage should then be adjusted to the shape of the curve of prothrombin values (not the current prothrombin value). Large variations in the dosage from day to day should be avoided as much as possible while trying to reach the correct maintenance dosage. The dosage should be adjusted immediately even to minor fluctuations. An increase or decrease equalling 2 to 4 mg. a day is often enough to provoke a distinct fall or rise in the prothrombin curve after 8 to 14 days. If an immediate anticoagulant effect is desired heparin should be used with dicoumarol until the prothrombin value has reached the therapeutic range. S. L. W.

Guanidine and Streptidine, Influence of, on the Toxicity of Streptomycin. S. Kuna and F. T. Cuchie. (*Arch. int. Pharmacodyn.*, 1953, **92**, 408.) Pure and relatively impure preparations of streptomycin were tested for convulsive action by intracisternal injection into rabbits and for production of vestibular dysfunction by daily parenteral injection into cats. It was shown that the convulsive response is directly proportional to the number of guanido groups injected, whether present in the streptomycin molecule or in the impurities. The vestibular effects of impure samples of streptomycin were greater than those of pure samples but a quantitative relationship could not be demonstrated. Guanidine produced no vestibular effect when administered alone, but enhanced the production of vestibular disturbances by streptomycin. Streptidine produced effects similar to streptomycin but 4 times the stoichiometric amount was required. The injection of guanidine with inositol caused vertigo differing from that caused by streptidine or streptomycin in being transitory instead of permanent. It is concluded that the streptidine moiety in streptomycin is mainly responsible for vestibular dysfunction, but that interactions or inter-relationships occur which account for the differences in toxicity of streptidine, streptomycin and dihydrostreptomycin. G. B.

Hydralazine: Effect on Hypertension. M. A. Khan. (*Brit. med. J.*, 1953, **1**, 27.) Hydralazine (apresoline) is an antihistaminase which has the combined effect of decreasing blood pressure and increasing renal blood flow. In this study the effect of the drug was observed in 12 cases of arterial hypertension. Hydralazine was administered orally, commencing with a test doses of 25 mg., increasing by 25 mg. 8-hourly to levels reaching 400 mg. three times a day. The effect of the drug lasted from 4 to 6 hours, with a maximum at from 1 to 2 hours after administration. Parenteral therapy, with doses of 50 mg. intramuscularly, was tried in 2 cases, but was abandoned because of unpleasant side-effects. The effect of combined oral therapy with hexamethonium bromide and hydralazine was studied in 4 cases. In most of the cases, whether hydralazine was used alone, or in combination with hexamethonium, there was a slight reduction of blood pressure at the beginning of treatment, but this was not maintained later in spite of greatly increased dosage. Side-effects noted in all patients were headache, flushing and sweating of the face, dryness of the mouth, nausea, vomiting, anorexia and epigastric discomfort, sleepiness and lethargy, tachycardia, transient erythematous rash, and giddiness on standing. In most patients the unpleasant side-effects persisted during treatment. S. L. W.

Insulin, Long-acting (Lente Insulin). R. D. Lawrence and W. Oakley. (*Brit. med. J.*, 1953, **1**, 242.) It has been found (Hallas-Møller *et al.*, *Science*, 1952, **116**, 394) that insulin in the presence of very small quantitites of zinc (1 mg./1000 units) is less soluble at the *p*H of the blood than protamine insulin, provided that anions such as phosphate and citrate are not present. If a different buffer from that normally used, namely phosphate, is employed, it is unnecessary to add such materials as protamine or globin to make insulin insoluble at the *p*H of the blood. With the use of acetate buffer these workers were able to prepare a suspension of pure insulin in media at the *p*H of the blood in the presence of very small quantities of zinc. The length of action of such preparations was found to vary with the form of the insulin, whether pure amorphous, amorphous and crystalline, or pure crystalline. The action of amorphous insulin is shorter than that of crystalline, the larger the size of the crystal the greater being the length of activity. Three preparations of insulin were produced and given the names "semi-lente," "lente," and "ultra-lente." These preparations are miscible to produce preparations of intermediate ranges of activity, but they cannot be mixed with the present soluble insulin. Preliminary therapeutic trials carried out on 11 adult diabetics support the claim that it exerts a prolonged hypoglycæmic action, lasting at least 24 hours, which is strong enough to control the blood sugar of moderately severe diabetics throughout the day without causing hypoglycæmia during the night. This was effected by giving a single dose of lente insulin before breakfast equal in amount to the total quantity of insulin required to stabilise the diabetes. It does not appear to produce either local or general reactions or to be more likely to produce hypoglycæmic reactions than the insulin preparations at present in use. S. L. W.

Isoniazid, Effect of, on Carbohydrate Metabolism. G. R. W. N. Luntz and S. G. Smith. (*Brit. med. J.*, 1953, **1**, 296.) Oral glucose tolerance tests were performed on 6 diabetic and 6 non-diabetic tuberculous patients, before starting treatment with isoniazid, and after administration of the drug for 3 days. In addition fasting tolerance tests were done after 6 days' treatment. Isoniazid delayed the peak and fall of the blood-sugar curve in the glucose tolerance test on non-diabetic patients and the same pattern was observed in an exaggerated form in diabetics. The mean average rise over the 3-hour glucose tolerance test was 20 mg./100 ml. for non-diabetics and considerably more for diabetics. Isoniazid also caused a temporary rise in the fasting blood-sugar levels. In the experiments, plasma isoniazid levels ranged from 0·3 to 0·9 mg./100 ml. and it was shown that concentrations of this order had no effect on the blood-sugar estimations. It is concluded that the insulin requirements of diabetic patients receiving isoniazid may be increased. G. B.

Mercuhydrin, Diuretic Effects by Several Routes. R. Marsh, T. Greiner, H. Gold, S. Mathes, F. Palumbo, L. Warshaw and J. Weaver. (*New Engl. J. Med.*, 1952, **247**, 593.) The diuretic effects of mercuhydrin administered by the subcutaneous, intravenous, intramuscular, oral and rectal routes was investigated by a bioassay technique, using 49 ambulant patients with congestive heart failure as subjects and the effect of the intramuscular injection as standard. The measure of response was the loss in body weight 24 hours after the dose. The drug produced substantially the same diuretic effect when administered parenterally, but by the oral route the effect was reduced to 4 per cent. of the standard. The largest dose of the drug administered orally caused gastro-intestinal irritation in 1/3 of the patients, while results from 21 patients given mercuhydrin in suppository form were unsatisfactory, as the suppository was

not retained. An estimate of 7 per cent. of the effect of the standard was made from the data available. The belief that ascorbic acid, when given with the drug orally, enhances the diuretic action and reduces gastrointestinal irritation, was not confirmed. J. R. F.

Racemorphan (DL-Dromoran), Pharmacological Analysis of the Nervous Control of the Respiration by. C. G. Breckenbridge and H. E. Hoff. (*Arch. int. Pharmacodyn.*, 1953, **93**, 1.) Morphine-like drugs depress selectively multiple-neurone relay systems and may be used in investigations of the neural control of respiration. For this purpose racemorphan (DL-dromoran) is preferable to morphine because it does not produce the precipitous fall in blood pressure and rapid death caused by morphine in certain experiments, so that it becomes possible to investigate the whole range of physiological preparations. Racemorphan has a lower effective dose and produces a lesser degree of tachyphylaxis. Since the drug has a greater selectivity of action than morphine, the results more closely parallel those of anatomical transections. In intact dogs, racemorphan induces changes similar to decortication or mid-collicular decerebration, that is, development of the all-or-nothing pattern of breathing with suppression of normal breathing after each deep breath, reduction in rate and amplitude of normal breathing, and panting. In the animals after mid-collicular decerebration, the drug produces effects similar to vagotomy of the preparation, reducing eupnœa and increasing post-sigh suppression of it and replacing it by an accelerated all-or-nothing respiration. In other preparations racemorphan produces the effects of further serial transections of the brain stem, producing Biot's periodic breathing, medullary breathing and apnœa, this last being the primary effect when the vagi and pons are removed. Control of respiration by the reticular system of the brain stem is discussed in the light of these results. G. B.

Suxamethonium (Succinylcholine); Use in Electric Convulsion Therapy. D. J. Adderley and M. Hamilton. (*Brit. med. J.*, 1953, **1**, 195.) The suitability of use of the short-acting relaxant suxamethonium, with particular reference to its effect on blood pressure, was investigated in 12 female patients. Blood pressures were taken immediately before the injection of sodium thiopentone (0·2 g.), one minute after the injection, again after the injection of suxamethonium (1·5 ml. of a 5 per cent. solution) had become effective, and immediately after the convulsion was over. The lungs were inflated 2 or 3 times with oxygen containing 5 per cent. of carbon dioxide, just before a shock of 30 joules was given. It was shown that suxamethonium produces a slight but unimportant rise in blood pressure. If necessary, this can be controlled by the use of a ganglion-blocking agent, e.g., 2 ml. of a 2 per cent. solution of hexamethonium iodide, or 1 ml. of a 10 per cent. solution of tetraethylammonium bromide, injected immediately before the sodium thiopentone. It was concluded that suxamethonium is a safe relaxant for electric convulsion therapy and can be used to abolish the convulsion completely. Even with the doses necessary to do this the patient's recovery from the fit is always rapid. When complete muscular relaxation is advisable suxamethonium can be administered with safety without any elaborate system of dosage. In such cases the presence of a fit can be detected by one of two methods; (*a*) by watching for dilatation of the pupils and, particularly, injection of the conjunctivæ, or (*b*), when a ganglion-blocking effect is employed, a tourniquet or sphygmomanometer cuff is fixed round the opposite arm immediately before the suxamethonium is injected—if this is kept tight enough to compress the artery until the current is passed the effects of the shock will be observed in that arm only. S. L. W.

(ABSTRACTS *continued on p. 479.*)

laboratory supply of cats being checked from time to time with the standard preparation. The assay on guinea-pigs is described separately, and the determination of the lethal dose in anæsthetised pigeons has been added.

The main effect of all these changes is to provide a guide to the most convenient of the well established methods of assay, and to give simple standard procedures for designing and analysing the assays so that their reliability shall be calculable on evidence provided largely by the assays themselves. This is a substantial advance on the previous articles on the same topic and is a reminder that much is lost when variable data are treated with uncritical simplicity and the accuracy of assays is merely specified as good or poor, or by an unvarying figure.

(ABSTRACTS *continued from p. 476.*)

BACTERIOLOGY AND CLINICAL TESTS

Isoniazid-resistant Strains of Tubercle Bacilli, Development and Stability of. M. Barnett, S. R. M. Bushby and D. A. Mitchison. (*Lancet*, 1953, **264**, 314.) Strains of *Mycobacterium tuberculosis* resistant to isoniazid, whether produced *in vitro* or isolated from patients, were found to be unstable both in viability and in their degree of resistance, and two such strains and one resistant variant of H37RV were investigated in mice and guinea-pigs. The animals were infected by intravenous injection of a culture of the organism in a modified Dubos medium. Treatment was started immediately after infection, the isoniazid being given subcutaneously twice daily in doses of 10 mg./kg. of bodyweight, and was continued until the animal died or was killed at the end of the experiment. Previous reports that isoniazid is destroyed in Dubos medium at 37° C. were confirmed, but evidence that continuing growth in the medium was due in part to the development of resistance and not solely to destruction of the drug was provided by the fact that the resistance during the next passage through the medium was increased. Resistant variants of H37RV produced *in vitro* reverted to sensitivity if they had been in contact with the drug during only one subculture. If subcultured more than once in the presence of isoniazid, resistance was retained during several passages through drug-free medium. Viability of resistant variants was poor during the first 3 passages in the presence of isoniazid but thereafter it seemed normal. Repeated passages in the presence of isoniazid failed to produce a strain with an inhibitory end-point higher than 25 to 50 μg./ml. With 10 to 16 resistant strains isolated from patients, the resistance fell substantially during 3 subcultures in the absence of the drug. Mice and guinea-pigs infected with sensitive strains were completely protected by doses of 20 mg./kg. With mice infected by *in vitro* resistant strains, survival time was considerably increased. Guinea-pigs similarly infected were almost completely protected and a chemotherapeutic response to the drug by these animals is likely even when they are infected with a strain which will grow in the presence of 1 μg./ml. H. T. B.