ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Ergot Alkaloids, Paper Chromatography of. V. E. Tyler and A. E. Schwarting. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 354). Descending paper partition chromatography (22·5 in Whatman No. 1 paper) was used. Filter paper strips were impregnated with propylene glycol, and formamide, and after application of the alkaloids, the wash liquids of benzene, toluene, and xylene, each equilibrated with the stationary-phase liquid, were used to form the chromatograms; separations of the water-insoluble alkaloids were possible, but not adaptable to all of the alkaloids. Hydrophobic filter paper was prepared by dipping strips or sheets of paper in a 5 per cent. v/v solution of Dow-Corning Silicone No. 1107 in heptane, allowing to dry in air, and heating in an oven at 150° C. for 3 minutes. After application of the alkaloids the paper was equilibrated for 12 hours with the vapour of the saturated butanol phase of the wash liquids, the chromatograms were formed with the saturated aqueous phase of the several wash liquids. The aqueous phases of the butanol-acetic acid-water and butanol-*p*H 3·0 buffer systems gave the broader separations; R_F values of the alkaloids obtained with these two systems are reported. The methods fail in the separation of ergotoxine and its isomer; ergotoxine and ergotinine are separable when chromatographed with a butanol *p*H 4·5 buffer system, the R_F of ergotoxine being 0·60 and R_F of ergotinine 0·69. R. E. S.

Veralbidine, A New Alkaloid from *Veratrum album*. A. Stoll and E. Seebeck. (*Science*, 1952, **115**, 678). From the mother liquors obtained in the isolation of protoveratrine, jervine, and rubijervine, a new alkaloid "veralbidine" was separated. The pure substance crystallises from dilute acetone in pentagonal plates, from dilute methanol in prisms, and from ether in fine needles. It melts between 181° and 183° C. and has $[\alpha]_D^{20°\,C.} = -11·7°$ in pyridine and $+5·4°$ in chloroform. In 84 per cent. sulphuric acid, veralbidine gives a colourless solution. It is sparingly soluble in ether, ethanol, and acetone, insoluble in water but readily soluble in chloroform. It is irritating to the nasal mucosa, causing sneezing. The empirical formula is $C_{37}H_{61}O_{12}N$. Veralbidine yields a crystalline thiocyanate $C_{37}H_{61}O_{12}N,HNCS$ which melts at 235° to 236° C. with decomposition and frothing and a crystalline hydrochloride $C_{37}H_{61}O_{12}N,HCl$ readily soluble in ethanol and water and melting at 250° to 251° C. R. E. S.

ANALYTICAL

Alcohols, Colorimetric Determination of. V. W. Reid and R. K. Truelove. (*Analyst*, 1952, **77**, 325). The use of ceric ammonium nitrate as a colorimetric reagent for the quantitative determination of various alcohols in dilute aqueous solutions is described. A standardised reagent is used and after mixing with the sample the colour is measured after exactly 5 minutes as the colours produced with lower alcohols are unstable. Calibration data for methanol, ethanol, *iso*propanol, *n*-butanol, *sec.*-butanol, *tert.*-butanol, monoethylene

glycol and triethylene glycol are given. Tertiary alcohols, in contrast to primary and secondary alcohols, produced a colour of excellent stability almost directly proportional to the concentration. Certain polyhydric alcohols gave stable colours, but others either no coloration or unstable colours. Sucrose gave an excellent calibration curve, whereas dextrose did not. Glycerol produced deep unstable colours in dilute solutions. Certain oxidising agents (such as organic peroxides), reducing agents, sulphate ions and other compounds containing hydroxyl groups interfered; methods for the separation of interfering substances are given. The application of the method to the analysis of effluents and to the determination of trace amounts of alcohol is discussed.

R. E. S.

2:8-Diaminoacridines, Colorimetric Determination of. J. G. Devi and M. L. Khorana. (*Indian J. Pharm.*, 1952, **14**, 43). A method is described for the assay of proflavine sulphate and hemi-sulphate. The substance in solution is diazotised and the diazotised product is coupled with resorcinol in an alkaline solution; the intensity of the colour developed is then compared with a similarly treated standard solution. Although alkaline solutions of resorcinol darkened on standing and had to be freshly prepared, this substance produced a stable intense red colour on diazotization which possessed advantages over the colours yielded by α- and β-naphthols, phoroglucinol, thymol and guaiacol. The colour produced with resorcinol showed a broad spectrum maximum at 544 to 548 mμ and could be measured photoelectrically; the colour intensity obeyed Beer's Law and experiments showed that it was stable up to 3 hours when kept at a low temperature; at room temperature the value for the blank changed quite rapidly. It is claimed that the results indicated the method to be as accurate as the official B.P. method. R. E. S.

Digitalis Glycosides, Chemical Determination of. M. Kâern. (*Dansk Tidsskr. farm.*, 1952, **26**, 89.) A mixture of digitoxin and digitoxigenin can be assayed by the following process. 5 ml. of a chloroform solution, containing 0·25 to 0·5 mg. of digitoxin, is evaporated to dryness and the residue is dried for 30 minutes at 100° C. The residue is dissolved in 5 ml. of ethanol and the digitonin is determined colorimetrically (see below). Another 5 ml. of the original solution is passed through a column of alumina (2 g. in 0·5 cm. diameter tube) and eluted with 3 quantities, each of 5 ml., of chloroform. This solution is assayed for digitoxigenin. The column is then again eluted with 3 quantities, each of 5 ml., of a mixture of 3 parts of chloroform and 1 part of ethanol, to give a solution containing the digitoxin. For the colorimetric assay 5 ml. of a methanol solution is mixed with 5 ml. of Knudson-Dresbach reagent (0·95 g. of picric acid and 5 ml. of 10 per cent. sodium hydroxide solution in 100 ml.). The colour is measured within 15 to 30 minutes in a 1 cm. cell at 492 mμ. The factor for conversion of E to digitoxin (in 10 ml. of reaction mixture) is $1{\cdot}07 \times 10^{-2}$; and for digitoxigenin $2{\cdot}21 \times 10^{-2}$. The method may be applied to digitoxin tablets, but not to tincture of digitalis. G. M.

Galenical Preparations, Identification of, by Paper Chromatography. A. B. Svendsen. (*Dansk. Tidsskr. farm.*, 1952, **26**, 125.) Strip paper chromatography may be applied to the identification of galenicals. Examples are given of the application to morphine preparations, the solvent being prepared by shaking together 10 volumes of butanol, 2 of glacial acetic acid, and 10 of water, and, after separation, using the butanol layer. For development either nitrite reagent or potassium bismuth iodide solution is used. Morphine

solutions may be tested directly, using the equivalent of about 100μg. of the salt. For injection of scopolamine, morphine and ephedrine, 0·01 ml. of the solution is run in the usual way, and developed successively with potassium bismuth iodide (morphine + scopolamine), nitrite (= morphine) and ninhydrin (ephedrine). In the case of tetrapon injection the presence of glycerin interferes with the chromatography, since the glycerin itself accumulates at an R_F of about 0·45 and interferes with the morphine and codeine, although not with narcotine and papaverine. Thus nitrite reagent gives a long streak which has a weaker colour in the middle, where the glycerin is concentrated. In tincture of opium the morphine can easily be detected. For morphine suppositories it is necessary to prepare an extract by shaking with warm water; and for opium suppositories by shaking with water containing 1 per cent. of hydrochloric acid.

G. M.

Histamine in Pharmaceutical Products, Determination by means of 2:4-Dinitrofluorobenzene. F. C. McIntire. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 277.) A test solution is prepared to contain 20 to 100 μg. of histamine base per ml. in 0·004M sodium diethylthiocarbamate buffered with a concentration of 0·1M sodium carbonate and 0·1M sodium bicarbonate. The solvent is distilled water of low copper content. 1 ml. of a 0·6 per cent. v/v solution of 2:4-dinitrofluorobenzene is mixed with 1 ml. of the test solution, allowed to stand for 20 minutes and diluted to 20 ml. with 0·1N hydrochloric acid. The solution is extracted with benzene and the optical density of the aqueous phase measured at 358 mμ, the histamine concentration being calculated from the data for pure histamine. A reaction blank test and 2 different concentrations of a histamine standard should be included in each set of determinations. The method has a high degree of precision and reproducibility. Solutions containing a high proportion of phenol as a preservative should be submitted to a preliminary extraction with benzene or ether to remove most of the phenol which may interfere in the reaction. G. B.

***iso*Nicotinyl Hydrazide and γ-Picoline, Photometric Determination of.** C. W. Ballard and P. G. W. Scott. (*Chem. Ind.*, 1952, 715). The method is based on the fact that a purple colour with 1-chloro-2:4-dinitrobenzene is given by γ-picoline and *iso*nicotinyl hydrazide but not by 2:6-lutidine, α-picoline, β-picoline, pyridine, *iso*nicotinic acid or ethyl *iso*nicotinate. To 5 ml. of a solution in dehydrated ethanol containing up to about 0·1 mg. of γ-picoline or *iso*nicotinyl hydrazide is added, 5 ml. of a 5 per cent. solution of 1-chloro-2:4-dinitrobenzene in dehydrated ethanol and 0·1 g. of borax; after heating in a boiling water bath for 10 minutes, cooling and adding 25 ml. of methanol, the mixture is filtered and the optical density is measured at 560 mμ (γ-picoline) or 530 mμ (*iso*nicotinyl hydrazide). Calibration curves are almost linear and reproducibility is about $\pm$ 2 per cent. for γ-picoline and $\pm$ 3 per cent. for *iso*nicotinyl hydrazide. Up to 0·2 ml. of water may be present in a determination without appreciable effect. The colour fades in intense artificial light or bright daylight but not in weak artificial light or in ultra-violet light. The method can be used for the determination of γ-picoline in mixtures of picolines containing pyridine and for the determination of *iso*nicotinyl hydrazide in pharmaceutical preparations and, possibly, biological fluids. R. E. S.

Oxalates in Fresh Plant Material, Determination of. C. J. L. Baker. (*Analyst*, 1952, **77**, 340). A method is described for determining total oxalates in plants, by extraction with hydrochloric acid, precipitation as calcium oxalate

from the deproteinised extract and subsequent titration with potassium permanganate; removal of proteins is accomplished with a sodium tungstate-phosphoric acid reagent. Water-soluble oxalates are determined similarly on an aqueous extract. In each case the oxalate in the final stages is precipitated with calcium chloride, the precipitate being cooled overnight in the refrigerator, separated by centrifuging and then heated with dilute sulphuric acid before titration with permanganate. The efficiency of the extraction method was examined by studying the variation in oxalate content of the extract with length of time in contact with the solid material; results showed that extraction was complete after 16 hours. The method is designed for fresh green plants only, as oxalate is lost on drying the material. R. E. S.

Pilocarpine, Colorimetric Assay of. J. W. Webb, R. S. Kelley and A. J. McBay. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 278.) The following method is applicable to the determination of pilocarpine and its salts even in low concentrations. It is based on the method of Shupe, but avoids the liberation of the unstable free base during the process. To solutions containing 2 to 6 mg. in 5 ml. of water, a mixture of 1 ml. of acetic acid (20 per cent.), 10 ml. of chloroform, 1 ml. of a 5 per cent. solution of potassium chromate and 2 ml. of a 3 per cent. solution of hydrogen peroxide is added, and the liquid shaken in a separator. The chloroform solution is separated and the aqueous solution extracted successively with 10 and 5 ml. quantities of chloroform, the mixed chloroform solutions being made up to 25 ml., and submitted to a determination of light absorption at 560 mμ with a spectrophotometer. The determination is completed as rapidly as possible to avoid errors due to temperature changes, evaporation and the sensitivity of the colour to light. Beer's law applies at the optimum *p*H, 2·9. Results are compared with those of the titration and the Kjeldahl methods. In the former, the end-point is difficult to determine because of the buffering power of the pilocarpine salts in aqueous solution, and the Kjeldahl method is unreliable since partly decomposed pilocarpine may have the correct nitrogen content. G. B.

Solanaceous Alkaloids, Colorimetric Assays for. A. B. Colby and J. L. Beal. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 351). An examination of two previously described colorimetric assays for solanaceous alkaloids has been made to determine their suitability for routine use. The first was based on the purple colour formed in Vitali's test, the second involved the use of ammonium reineckate to precipitate the alkaloids as reineckates, which were then dissolved in acetone to form a coloured solution. Details of the two assay procedures are given together with the results obtained with stramonium and belladonna. It was concluded that the reineckate assay gave results which were more comparable to the U.S.P. XIV assay than were the results of the Vitali assay; the standard error for the reineckate assay was smaller than for the official assay. The colour developed in the reineckate assay was much more stable than the colour developed in the Vitali assay, although the latter was the more sensitive. The Vitali assay was the more rapid of the two assays studied although both were quicker than the official process and required less material. R. E. S.

Thiourea, Volumetric Estimation of. K. Appa Rao and K. Neelakantam. (*Indian J. Pharm.*, 1952, **14**, 50). The method of Mahr (*Z. Anal. Chem.*, 1939, **117**, 91; 389; *Angew. Chem.*, 1939, **52**, 618) for the estimation of thiourea using bromate was investigated and found to yield erratic results. Slow titrations yielded better results than fast and even then

the errors were of the order of 4 to 8 per cent. on the calculated titre values. The high acid concentration (6·8N) recommended by Mahr in all cases was found to be neither necessary nor desirable. A modified method using potassium permanganate in the place of bromate, and in the presence of potassium iodide as "oxidation buffer" was found to yield better results, the errors ranging from 0 to 1·3 per cent. for amounts of thiourea ranging from 20 to 154 mg. at an initial acid concentration of 6·6N. The results are more easily reproduced and the method is recommended for general use. R. E. S.

Tocopherol, Colorimetric Determination of. C. Domart. (*Ann. pharm. franç.*, 1952, **10**, 199.) The determination depends upon the oxidation of tocopherol in ethanolic solution by ferric chloride. The quantity of ferrous ions produced is estimated from measurements of the stable red colour formed by reaction with α:α′-dipyridyl. The wavelength of maximum absorption is 522 mμ and Beer's law has been found to apply. Ferric chloride also absorbs in the red region of the spectrum, but the excess may be removed by the addition of potassium fluoride which forms a colourless complex provided the *p*H is not less than 3·2. The dipyridyl ferrous colour being stable within the *p*H range 3·5 to 8·5, it is convenient to carry out the determination at about *p*H 4·1. The following procedure is recommended. To an ethanolic solution of tocopherol add 1 ml. of a 0·2 per cent. ethanolic solution of ferric chloride, shake, add 1 ml. of a 0·5 per cent. ethanolic solution of α:α′-dipyridyl, shake and allow to stand for 8 to 10 minutes. Add 2 ml. of a 0·5 per cent. ethanolic solution of potassium fluoride, shake, allow to stand for 2 minutes, add 5 ml. of buffer solution at *p*H 4·1 and dilute to 25 ml. with ethanol. Determine the red colour by comparison photoelectrically with a similar solution prepared without tocopherol (Meunier instrument, filter No. 49). The tocopherol content is read from a standard curve. G. B.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Cardiac Glycosides, Paper Chromatography of. C. H. Hassall and S. L. Martin. (*J. chem. Soc.*, 1951, 2766.) The procedure is described, and the R_F values are recorded for the cardiac glycosides using a variety of solvent mixtures. The results indicate that all the glycosides investigated may be identified by this procedure when suitable solvents are employed. A. H. B.

GUMS AND RESINS

Resins, Paper Chromatography of. J. S. Mills and A. E. A. Werner. (*Nature, Lond.*, 1952, **169**, 1064.) The work deals with the application of paper partition chromatography to the identification of natural resins and the separation of the constituents, with particular reference to dammar and mastic. The separation can readily be achieved in a reversed-phase system using odourless kerosene (boiling range 170° to 200° C.) as the stationary phase on the filter paper and aqueous *iso*propanol saturated with odourless kerosene as the mobile phase. A descending chromatogram is used and details of technique are given; the strips are dried, developed by spraying with a 50 per cent. w/v solution of phenol in carbon tetrachloride and exposing to bromine vapour for a short period. Resin components which give a positive Halphen-Hicks test show as coloured zones varying from pink to violet. Dammar, mastic, sandarac, rosin, elemi and copal, give characteristic chromatograms which may be distinguished by the number, colour and R_F values of the coloured zones. R. E. S.

ORGANIC CHEMISTRY

Œstrone, Purification of. H. Braunsberg. (*Nature, Lond.*, 1952, **169**, 967.) Traces of an impurity are revealed when the *p*-nitrobenzeneazodimethoxyaniline (fast black salt K) derivative of commercial œstrone is submitted to paper chromatography by the method of Helftmann. The impurity, which yields a blue spot of lower R_F value than the pink dye due to the œstrone derivative, may be equilenin which gives a blue spot of similar R_F value. The impurity may be removed by partition chromatography on celite-sodium hydroxide columns.

G. B.

Visnagan, Crystalline. E. Smith, L. A. Pucci and W. G. Bywater. (*Science*, 1952, **115**, 520.) An optically active extract of the seeds of *Ammi visnaga* L. from which khellin and chellolglycoside had been removed was submitted to chromatographic analysis, using optical activity and ultra-violet absorption as a guide to selecting eluted fractions. An amorphous product, $[\alpha]_D + 16°$ was re-chromatographed and crystalline visnagan, obtained from a central fraction by crystallising from methanol at 4° C. on prolonged standing, and recrystallised, had the following characteristics:—m.pt., 86° to 88° C., $[\alpha]_D + 12{\cdot}5°$, molecular weight, 373. Its vasodilatatory effect in isolated rabbit hearts was 8 times as great as that of khellin. A further chromatographic fraction, $[\alpha]_D + 50°$, yielded a crystalline compound of m.pt. 157° to 159° C., $[\alpha]_D + 96°$, and molecular weight 276. The ultra-violet absorption spectrum closely resembled that of dihydrokhellin. This substance appeared to be a dihydrofuranochromone. In isolated rabbit hearts this substance was as potent a vasodilator as khellin.

G. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Noradrenaline and Accessory Chromaffin Tissue. D. M. Shepherd and G. B. West. (*Nature, Lond.*, 1952, **170**, 42.) Following the finding that the collection of chromaffin tissue in babies, known as the organs of Zuckerkandl, contains large amounts of noradrenaline, experiments were conducted (*a*) to confirm that a sympthomimetic substance is present in accessory chromaffin tissue of animals, and (*b*) to identify such a substance by biological and chromatographic methods. The results indicated that large amounts of noradrenaline may be found in the retro-peritoneal tissue of young dogs, rabbits, guinea-pigs and cats, exceeding in some cases the amount found in the suprarenal glands. These additional chromaffin structures must perform some autonomic function early in life, possibly the maintenance of blood pressure. As the animal grows older and the suprarenal medulla matures, so the amine content of this accessory tissue declines. Extracts of the carotid bodies and of the aortic bodies of rabbits, cats and guinea-pigs did not yield any sympathomimetic material; this may be explained by the fact that chromaffin tissue is not necessary for the production of adrenaline and noradrenaline. Precursors of noradrenaline, such as hydroxytyramine and dihydroxyphenylalanine were not detected in any of the extracts.

S. L. W.

BIOCHEMICAL ANALYSIS

Dried Plasma, Water Content of. H. Sager. (*Pharm. Acta Helvet.*, 1952, **27**, 121.) The activity of a comparatively stable product such as diphtheria anti-toxin decreases much more rapidly in the form of a solid containing 5 to

8 per cent. of water than when in solution. Thus the water content of such materials is of great importance for stability. It has been shown that under tropical conditions large quantities of water are able to penetrate through rubber bungs. When removing water by freezing, 3 factors are important in order to attain a low final water content: the thickness of layer of the frozen product must be as uniform as possible; the cooling at the beginning of drying must be sufficient to avoid water inclusions; and de-mixing in the form of separation of pure ice on the walls of the container must be avoided, as this prevents the direct contact of the product with the walls. The minimum residual moisture content depends on the temperature of the condensing surface, the maximum permissible temperature for the product, and the vapour pressure curve of the product. For the determination of water content, 3 methods were compared—the U.S.P. method (phosphorus pentoxide), the Thomann and Kaelin method (distillation with xylene) and the Karl Fischer method. The results obtained diverged considerably. Moreover all these methods have the disadvantage that the contents of the container, after removal of the sample, cannot be used for transfusion. The author is attempting to develop a new method based on the measurement of vapour pressure, which rises very steeply with increase in water content. The starting point is a "physically dry" plasma prepared at a plasma temperature of + 50° C., using a condenser cooled with liquid air. Results of these experiments are to be reported later. G. M.

Hydrazine Derivatives of *iso*Nicotinic Acid in Blood, Determination of. S. H. Rubin, L. Drekter, J. Scheiner and E. de Ritter. (*Dis. Chest.*, 1952, **21**, 439). A colorimetric method is described for the determination of blood plasma levels of *iso*nicotinic acid and its derivatives, the hydrazide and the *iso*propylhydrazide. The substance is converted to *iso*nicotinic acid and determined colorimetrically by the reaction with 10 per cent. cyanogen bromide and ammonia described for nicotinic acid (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 513); the hydrazide derivatives, which yield relatively slight colour *per se*, are converted to *iso*nicotinic acid by treatment of the protein-free plasma with permanganate. A blank determination to correct for non-specific colour can be made by assay of a control sample taken before dosage or the blank can be estimated by the reaction with 3 per cent. cyanogen bromide and metol, in which *iso*nicotinic acid yields no colour. Details of the procedure are given. Results are given of plasma levels over a 24-hour period after a single oral dose for dogs having 3·5, 7, or 14 mg./kg. and for humans receiving up to 3·4 mg./kg. of *iso*nicotinyl hydrazide and *iso*propylhydrazide. Maximum plasma levels were found within one-half to 2 hours after the dose and declined rapidly. No significant amount of either compound was found in the plasma after 24 hours. R. E. S.

***iso*Nicotinyl Hydrazide in Biological Fluids, Estimation of.** J. M. Kelly and R. B. Poet. (*Amer. Rev. Tuberc.*, 1952, **65**, 484). Two methods are described for the estimation of *iso*nicotinyl hydrazide in blood plasma or urine. The substance is extracted from alkalinised plasma or urine into an *iso*amyl alcohol-ether-ammonium sulphate system and subsequently estimated either spectrophotometrically or colorimetrically after extraction into 0·1N hydrochloric acid. The spectrophotometric method using the absorption peak in the ultra-violet at 266 mμ is reliable for concentrations of 5 μg./ml. in

plasma and 20 μg./ml. in urine. The colorimetric method using *p*-dimethylaminobenzaldehyde as the reagent has a lower limit in plasma of 1 μg./ml. and 5 μg./ml. in urine. Details of procedure are given; blank determinations on the biological material are necessary. R. E. S.

Penicillin in Culture Fluids, Determination of. A. Beloff-Chain and F. Dentice D'Accadia. (*Analyst*, 1952, **77**, 423). A rapid iodimetric method of determining penicillin in culture fluids or crude preparations is described. The penicillin is extracted from an aqueous phase with amyl acetate at *p*H 2 and is re-extracted from an aliquot of the amyl acetate solution by a known volume of phosphate buffer at *p*H 7. The penicillin is then assayed by the iodimetric titration method, details of which are given. A blank determination must be made to correct for substances other than penicillin present in the culture fluid that are extracted and that take up iodine under the specified conditions; in addition some of the inactivation products of penicillin are extracted by amyl acetate and take up iodine, and a correction factor depending on the penicillin present in the original broth must be subtracted from the titration value of the blank. Results on culture fluids are given and it is concluded that for all normal routine penicillin fermentation work the rapid assay is sufficiently accurate between the limits of 150 and 1200 I.U./ml.; at concentrations of less than 150 I.U./ml. it is advisable to use the biological assay method; above 1200 I.U./ml. it is necessary to dilute the culture fluid before assay. R. E. S.

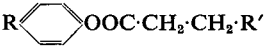
Urinary 17-Ketosteroids, Analysis of. B. M. Bray. (*Analyst*, 1952, **77**, 426). An examination of the methods available for the preparation of crude urine extracts for 17-ketosteroid analysis has been made. 3 methods for isolating the ketonic fraction from a mixture of urinary steroids by means of Girard's reagents were compared with respect to reproducibility, the time required for the formation of the hydrazone and the degree of dryness necessary in the reaction mixture. Condensation with Girard's reagent T for 3 minutes in a bath of boiling water gave an average recovery of 95 per cent. for amounts of dehydro-*iso*androsterone between 0·5 and 2·5 mg. and 94 per cent. for the same steroid added to a urinary extract. Girard's reagent P gave lower results. Drying the urinary extract for 12 to 36 hours *in vacuo* did not improve the recovery. The method of Talbot *et al* (*J. biol. Chem.*, 1940, **132**, 595) was used for the hydrolysis of the hydrazone; for the estimation of the sterone Zimmermann's reaction was used. R. E. S.

Vitamin A, Reproducibility of Geometrical Correction Procedures in the Spectrotrophotometric Estimation of. H. H. Bagnall and F. G. Stock. (*Analyst*, 1952, **77**, 356). Recent assessments of the precision of geometrical correction procedures for the spectrophotometric estimation of vitamin A are discussed. Results are given for correction procedures as applied to halibut liver oils; for the estimation of vitamin A, the method of the B.P. Addendum 1951 was followed except that all three correction equations recommended by Cama (*Biochem. J.*, 1951, **50**, 48) were used with each of three separate weights of oil dissolved in *cyclo*hexane so giving 9 "corrected" values for $E_{1\text{ cm.}}^{1\text{ per cent.}}$ at 327·5 to 328 mμ. The fiducial limits (P = 0·05) of the mean of the 9 values from ± 0·60 to ± 2·33, with average value ± 1·17 (intra-laboratory). Results were then calculated. The P = 0·05 fiducial limits of the mean of 9 values for the corrected $E_{1\text{ cm.}}^{1\text{ per cent.}}$ at 328 mμ, expressed as a percentage of the mean, varies are also given of a small scale inter-laboratory test. R. E. S.

CHEMOTHERAPY

Diphenylmethane and Diphenylamine, Some Alkylamino Derivatives of. G. Benoit R. Delavigne and F. Elipoulo. (*Ann. pharm. franç.*, 1952, **10**, 181.) Diphenylmethane derivatives of the general formula $(C_6H_5)_2{\cdot}HC{\cdot}(CH_2)_n{\cdot}N(C_2H_5)_2$, HCl were prepared where $n = 1$ to 6 and 10. Compound $n = 1$ was prepared by catalytic reduction of diphenylacetonitrile in the presence of Raney nickel, followed by ethylation. Compound $n = 2$ was made by the interaction of diethylaminochloroethane and the sodium derivative of diphenylmethane, and a similar process was applied for compound $n = 3$. Compounds $n = 4$ and 5 could not be obtained in this manner because cyclisation occurred. They were prepared from the phenoxyalkyl bromide by reaction of the magnesium derivative with benzophenone, followed by dehydration of the tertiary alcohol produced and hydrogenation of the double bond. The phenoxy group was removed with hydrogen bromide and the product heated in a sealed tube with diethylamine. A corresponding series of derivatives of diphenylamine of the general formula $(C_6H_5)_2N{\cdot}(CH_2)_n{\cdot}(C_2H_5)_2$ was prepared. The lower members were obtained by condensation of diphenylamine with the appropriate diethylaminoalkyl chloride in the presence of sodamide. For the higher members of the series the phenoxy derivative was heated with hydrochloric acid in a sealed tube and the product treated with diethylamine. The antiacetylcholinic and antihistaminic activities of the diphenylamine derivatives were much less than that of the corresponding members of the diphenylmethane series. G. B.

Phenyl Esters of β-Dialkylaminopropionic Acids as Antispasmodics. T. O. Soine and F. E. DiGangi. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 236.) Derivatives of phenyl propionate of the type



where R = $-NO_2$, $-NH_2$, $-NH{\cdot}CO{\cdot}CH_3$, or $-H$ and R′ = diethylamino, di-*n*-propylamino, di-*n*-butylamino, di-*n*-amylamino, di-*iso*amylamino and piperidino were prepared. *p*-Nitrophenyl β-chloropropionate was made by heating β-chloropropionyl chloride with *p*-nitrophenol and magnesium in benzene, and purified. The β-dialkylamino derivatives were prepared by heating this substance under a reflux condenser with the dialkylamine in ether, filtering, drying the ethereal solution with sodium sulphate, adding a solution of hydrochloric acid in ether and crystallising. Corresponding *p*-amino compounds were prepared by reduction with hydrogen in the presence of palladium on charcoal and ethanolic hydrogen chloride. The *p*-acetylaminophenyl compounds were prepared from *p*-acetylaminophenyl chloropropionate, obtained by catalytic reduction of the nitro compound, and reaction with acetic anhydride. Melting points and analytical data are given. *p*-Aminophenyl β-dialkylaminopropionates showed a moderate antispasmodic activity, and the other compounds are undergoing pharmacological testing. G. B.

Sulphonamides: Relative Potencies and Specificity of. J. Francis. (*Brit. J. Pharmacol.*, 1952, **7**, 189.) The relative potencies of the common sulphonamides and of 4:4′-diaminodiphenyl sulphone were compared *in vitro* against a number of pathogens. Though not a "sulphonamide," the sulphone was included because its action is strongly antagonised by *p*-aminobenzoic acid. The compounds were divided into a non-heterocyclic group (sulphanilamide, sulphaguanidine and sulphone) and a heterocyclic group (sulphapyridine,

sulphamezathine, sulphamerazine, sulphadiazine and sulphathiazole). The sulphone was more active than any other compound against *Str. pneumoniæ* and *Str. pyrogenes*, with the exception of sulphathiazole. Against all other organisms (*Past. septica Bact. coli, Salm. cholera suis, N. gonorrhœa, N. meningitidis*) the non-heterocyclic compounds were less active than the heterocyclic. The latter group were nearly always placed in the same order of activity, sulphapyridine being the least potent and sulphathiazole the most potent; the exceptions were sulphamezathine against *Str. pyrogenes* and sulphadiazine against *N. gonorrhœa*. There was no difference in the action of sulphamerazine, sulphadiazine and sulphathiazole against *Bact. coli*, whereas there was a fivefold difference with some other organisms. In the most satisfactory series of experiments the average sulphanilamide coefficient of the heterocyclic sulphonamides was only 2·3 against streptococci but 139 against *Salm. cholera suis* and 36 against *Bact. coli*. S. L. W.

PHARMACY

GALENICAL PHARMACY

Hyoscyamine, Racemisation of, in Preparation of Dry Extract of Belladonna. L-E. Fryklöf. (*Pharm. Acta Helvet.*, 1952, **27**, 175.) The rotation of the alkaloids in belladonna extract may be determined by extracting an alkaline mixture of the extract and kieselguhr with chloroform, and passing the solution through a column of kieselguhr, which absorbs the alkaloids. They can then be extracted with chloroform saturated with ammonia and, after passing through a layer of alumina, the colourless solution can be used for a determination of the optical rotation, while total alkaloids may be determined by titration after removal of the ammonia. Application of this method has shown that, while slow drying of the extract at 100° C. leads to a considerable amount of racemisation, drying at 60° C. for 24 to 48 hours has no such effect, while 8 hours at 80° C. is also permissible. In this respect there is practically no difference between an extract containing chlorophyll and one freed from it.

G. M.

NOTES AND FORMULÆ

Piperazine Œstrone Sulphate (Sulestrex Piperazine). (*New and Non-official Remedies, J. Amer. med. Ass.*, 1952, **149**, 443.) Piperazine œstrone sulphate is an equimolecular compound of piperazine and œstrone sulphate stabilised by the addition of a small amount of free piperazine. It is a fine, white to creamy-white, odourless, crystalline powder, slightly soluble in water and ethanol. It melts between 185° and 195° C. to a light brown syrup, which solidifies on further heating and finally melts at about 240° to 250° C., with decomposition. When dissolved in water and boiled with a solution of quinone in ethanolic acetic acid, a currant-red colour appears. When heated with a solution of β-naphthol in sulphuric acid, cooled, and diluted with water, an orange-yellow colour appears which becomes red-orange on further heating. When dried at 105° C. for one hour, it loses not more than 1·0 per cent. of its weight; it yields not more than 0·1 per cent. of sulphated ash. It contains œstrone 56·0 to 63·0 per cent., equivalent to 90·4 to 101·7 per cent. of piperazine œstrone sulphate, and nitrogen 6·4 to 7·0 per cent. The œstrone is assayed by heating an aqueous solution with hydrochloric acid, extracting with benzene,

treating the residue from the benzene solution with a solution of phenol in sulphuric acid, and determining the optical absorption at 5220 Å of this solution and of solutions prepared similarly from standard œstrone. The nitrogen is assayed by a semi-micro Kjeldahl method. G. R. K.

PHARMACOGNOSY

***Colchicum autumnale*, Alkaloidal Content of.** F. Šantavý and T. Reichstein. (*Pharm. Acta Helvet.*, 1952, **27**, 71.) In the spring, when the first leaves are showing, old corms of *Colchicum autumnale* contain 3 times as much total alkaloid as in autumn, and 14 days later the amount is still greater. The largest amount is of substance F, followed by colchicine and finally substance G. Some months later the content in the old corms is reduced, as they atrophy considerably and the new ones grow: only small amounts of colchicine-like substances could be isolated. At the time of ripening of the seeds the old corms were so much atrophied that they could not be analysed; the young ones contained the same substances as later, in the autumn. Small amounts of another compound—substance S were found. Ether-soluble alkaloids were present especially in old corms in the spring months. The results show that in Central Europe the harvesting is most profitable during flowering in autumn: although there is a greater percentage of alkaloids in old corms, in spring, they are more difficult to find, and moreover the corms are larger in autumn and the total amount of alkaloid is greater. G. M.

Digitalis, Occurrence of Free Genins in. F. Neuwald. (*Arch. Pharm. Berl.*, 1952, **285**, 22.) Although free genins are often stated to be present in digitalis leaf, the evidence for this is not decisive, and there are no enzymes in the leaf capable of splitting the glycosides of the digitoxin stage into genins and digitoxose. In order to decide this question the genin-glycoside fraction (free from digitoxose) was extracted, and assayed by the modified genin method of the author (depending on the genin content) and by the digitoxose method of Soos. Tests were carried out on 3 samples of the drug which had been stored under different conditions and contained about 10 per cent. of water. The agreement between the 2 methods was very good and indicates that free genins were absent in the 3 samples, within the limits of accuracy of the photometric determination ($\pm$ 2 per cent.). G. M.

Ergot, Alkaloidal Content of Naturally Occurring. L. Fuchs. (*Scientia Pharm.*, 1952, **20**, 1.) Samples of natural (wild) ergot were assayed by the method of Fuchs for water-soluble and insoluble alkaloids. Most of the 67 samples contained less than 100 mg. per cent. of insoluble alkaloids. Some samples from localities where artificial inoculation had been carried out in the same season showed a considerably higher content (up to 252 mg. per cent.) but this appeared to be a secondary infection, since elsewhere in the same region ergot was found which was almost alkaloid-free. There also appear to be other strains of ergot which are distinguished from one another by the content of water-soluble alkaloids. Thus all samples from Lower Austria showed less than 20 mg. per cent. (generally less than 10 mg. per cent.), while most of those from Steiermark contained more than 20 mg. per cent. and up to 39·5 mg. per cent. of water-soluble alkaloids. G. M.

PHARMACOLOGY AND THERAPEUTICS

p-Aminosalicylic Acid, Effect on the Allergic Process. E. R. Trehewie. (*Med. J. Aust.*, 1952, **1**, 638.) Sodium salicylate and acetylsalicylic acid have been previously found by the author to inhibit the release of histamine from the lungs of sensitised guinea-pigs by the sensitising antigen, egg albumen, and experiments were made to discover whether *p*-aminosalicylic acid also had an anti-allergic effect. Guinea-pigs were sensitised with crystalline egg albumen, and the antigen was injected immediately after being dissolved in Tyrode solution, containing the salicylate compound, in the same concentration as in the perfusing fluid. Samples of the perfusion fluid were collected 5 minutes before and 0 to 10 and 10 to 15 minutes after intra-arterial injection of 5 mg. of the egg albumen. The samples were immediately boiled and the histamine determined on the isolated jejunum of the guinea-pig. The results are tabulated and show that *p*-aminosalicylic acid is a powerful inhibitor of histamine release. The author suggests that the anti-allergic activity of *p*-aminosalicylic acid may be of therapeutic importance in tuberculosis. Since completion of this work evidence has been published that in some tuberculous patients antihistaminics cause an extremely rapid and unexpected subsidence of the pathological process while histamine stimulates the growth of tubercle cultures. It is suggested that *p*-aminosalicylic acid may be of value in certain allergies in which antihistamines fail because inhibition of the antigen-antibody response might be expected to block a wider variety of effects. H. T. B.

Basic Ketones and Related Compounds, Pharmacology of. P. B. Marshall, N. Ahmad and R. E. Weston. (*Brit. J. Pharmacol.*, 1952, **7**, 85.) The 5 series of compounds investigated included: β-dialkylaminoketones, bispidines, γ-dialkylaminoketones, γ-dialkylaminobutyramidines and 2-(γ-dialkylaminopropyl)dihydroglyoxalines. Most are spasmolytics having non-specific direct action on plain muscle. 2 compounds $(C_6H_5)_2CH{\cdot}CO{\cdot}CH_2CH_2NR_2$, where $R = CH_3$ and C_2H_5, possessed fairly high activity. Many of the compounds have well-marked local anæsthetic action. Intracutaneously in guinea-pigs, 10 compounds showed greater activity than procaine while two of the β-dialkylaminoketone series proved to be half as active as cinchocaine. One compound, in the bispidine series, containing a β-chloroethyl group attached to nitrogen, showed some protective action against lethal doses of adrenaline in mice. Its potency is about 1/16th that of dibenamine. None of the compounds was found to have any analgesic or neuromuscular blocking action or very marked action on blood pressure, spleen volume and respiration. J. R. F.

Chloramphenicol Administration, Fatal Aplastic Anæmia Following. L. A. Hawkins and H. Lederer (*Brit. med. J.*, 1952, **2**, 423), B. Wolman (*Brit. med. J.*, 1952, **2**, 426), R. K. Smiley, G. E. Cartwright and M. M. Wintrobe (*J. Amer. med. Ass.*, 1952, **149**, 914), and P. Sturgeon (*J. Amer. med. Ass.*, 1952, **149**, 918). 1 adult and 8 child cases of fatal aplastic anæmia following chloramphenicol therapy are described in the 4 papers. Wolman describes 1, Smiley *et al.* 2, Hawkins and Lederer 2, and Sturgeon 4 cases. The British papers report prolonged treatment with large doses (44 days, 28 g. Hawkins and Lederer), the American papers report intermittent and prolonged treatment. Sturgeon reports 1 case where another drug, an antihistaminic, which has been known to produce the disease, was administered for 1 day only, and 2 cases where exposure to organic solvents and dyes occurred. The other authors

state that no drug, known to produce the disease, was given during the antibiotic treatment. Hawkins and Lederer administered adrenocorticotrophic hormone to both of their patients, 1 and 3 days before death. The hormone had no apparent effect, but no conclusions are drawn, as the period of administration was too short. Wolman and the American authors suggest that as the drug has not produced the anæmia in a large number of cases a small number of persons must possess an idiosyncrasy to it. Smiley *et al.* report that both cases treated by them had a history of allergic manifestations. All the authors conclude that as no other drug with known aplastic anæmia-producing properties had been used during treatment, and as chloramphenicol was the common factor, its nitrobenzene radical, which is a known hæmopoietic toxin, is responsible for the disease. Prolonged treatment is not advised and the possibility of idiosyncrasy should be borne in mind. J. R. F.

Choline Group, Studies of the Structure-action of Related Compounds. H. R. Ing, P. Kordik and D. P. H. Tudor Williams. (*Brit. J. Pharmacol.*, 1952, 7, 103.) Several groups of substances all related in structure to acetylcholine have been tested on cat blood pressure, rabbit auricles, guinea-pig ileum, frog heart and rectus abdominis. 3 isomeric keto-amyltrimethylammonium iodides exhibted primarily nicotine-like activity, the 4-keto-compound possessing the greatest and the 2-keto-compound the least activity. Both the 4-keto and 3-keto-compounds were equipotent with acetylcholine in producing contracture of the frog's rectus abdominis. The action of the 2-keto-compound was considerably weaker. All 3 produced muscarine-like effects, the 4-keto-compound being consistently the most potent. These effects were largely due to stimulation of ganglion cells since they were considerably reduced by hexamethonium iodide. A comparison of 3 isomeric ethers:—*O*-ethylcholine, *O*-methylhomocholine and *O*-*n*-propylformocholine, showed that the *O*-ethylcholine has the most potent muscarine-like activity and that the activities of the other two compounds were alike and resembled those of *n*-amyl-trimethylammonium more closely than those of choline ethyl-ether. In a furfuryl series, 5-methylfurfuryltrimethylammonium iodide was found considerably more active in its muscarine-like effects than furmethide. Of other compounds investigated, the homologues, acetoxyethyl dimethyl-*n*-propyl- and acetoxyethyldimethyl-*n*-butyl-ammonium iodides, of acetoxyethyldimethylethylammonium iodide, unlike the latter, exhibited only feeble activities and the activities of the sulphur analogue of acetylcholine (acetoxyethyldimethyl-sulphonium) were consistently less than those recorded for acetylphosphocholine. J. R. F.

Dextran in the Treatment of Blood Loss and Shock. J. S. Wilson, E. H. Estes, Jr., J. T. Doyle, W. L. Bloom and J. V. Warren. (*Amer. J. med. Sci.*, 1952, **223**, 364.) The immediate product of the fermentation of sucrose by *Leuconostoc mesenteroides* is a viscous mass consisting of very large dextran molecules. By partial acid hydrolysis the molecules are broken down into smaller units and the authors used a product, fractionated by means of ethanol, in which the average molecular weight was about 70,000, comparable to that of human albumin. It is not known whether the untoward reactions obtained in man with some varieties of dextran are related to the size of the molecules they contain. The product was administered as a 6 per cent. solution in normal saline solution. A preliminary test was carried out on 5 normal healthy men, the systemic and the pulmonary arterial blood pressures, the cardiac output and the blood volume being determined before the experiment, after withdrawal of 450 to 900 ml. of blood, and again after injecting 500 ml. of dextran solution. In

4 cases cardiac output was again determined 45 to 60 minutes later. The bleeding produced a fall in systemic arterial blood pressure only in the subject from whom 900 ml. of blood was taken. No appreciable alteration in cardiac output occurred in any, and all showed a fall in pulmonary arterial pressure and the blood volume. The dextran injection resulted in a return to or above normal in all the factors investigated, apart from the dilution effect. 52 patients with clinical shock were subsequently treated with 500 ml. of the dextran solution. There was a prompt disappearance of clinical signs of shock in 44; of these, 10 subsequently received a blood transfusion and in no instance did the dextran interfere with the typing or cross-matching of the blood. Satisfactory clinical improvement was obtained in all cases with shock due to blood loss, trauma and dehydration. In patients with cerebral trauma or severe infection the results were poor. In general the results were essentially the same as might have been expected from plasma transfusion, and dextran is regarded as a convenient, reaction-free alternative which offers the advantages of large scale production and stability on storage. H. T. B.

Diaminodiphenylsulphone (Dapsone), Treatment of Leprosy with. E. Muir. (*Trans. roy. Soc. trop. Med.*, 1952, **46**, 113.) Diaminodiphenylsulphone is administered orally, starting with about 100 mg. twice a week, gradually increased to 100 mg., 4 times a week. A marked improvement in the bacteriological index of the patients may be observed, especially when treatment commences early. In more advanced cases, especially when reaction-anæmia complex is present, initial dosage must be carefully controlled, and where there is a serious reaction to the drug, adrenocorticotrophic hormone or vitamin B_{12} may be useful for controlling the condition. A lepra reaction, similar to the ordinary lepra reaction which occurs without treatment may be induced by large doses and this is accompanied by rapid destruction of the bacilli and consequent improvement in the bacteriological index. Thiosemicarbazone, streptomycin and chaulmoogra oil or esters may be used in conjunction with sulphone therapy. The effectiveness of diaminodiphenylsulphone in small doses is due to its almost complete absorption when given by mouth, and the slow rate of its excretion. The effect of the substituted derivatives promin, diasone and sulphetrone is due to the diaminodiphenylsulphone which they liberate before or after absorption and consequently they are uneconomical and less certain in action. Treatment in early and moderately advanced cases needs only a minimum of medical supervision, so that the drug could be widely used in endemic areas. G. B.

2:4-Diaminopteridines and Sulphathiazole, Activity of, Against *Streptococcus fæcalis* and *Staphylococcus aureus*. H. O. J. Collier and P. D. Waterhouse. (*Brit. J. Pharmacol.*, 1952, **7**, 161.) The activities *in vitro* of 27 2:4-diamino-pteridines against 4 strains of *Str. fæcalis* were studied. Greatest activity was shown in the dialkyl compounds with straight or branched chains containing 3 to 6 carbon atoms, and also depended on the substituents being in the 6 and 7 positions. Higher activity was found against strains requiring preformed pteroglutamic acid than against strains able to synthesise this nutrient. The addition of sulphathiazole potentiated the inhibitory effects upon the latter. The inhibitory effect of dibenzylpteridine on *Str. fæcalis* was not antagonised by 5 per cent. human urine. 18 2:4-diaminopteridines were examined *in vitro* against a strain of *Staph. aureus*. The dibenzyl, di*cyclo*hexylmethyl compounds, and the 6:7-dialkyl compounds with unbranched side chains, exhibited greatest activity. The salts of the dibenzyl compound had toxicity similar to

sulphathiazole when administered intraperitoneally to mice. The phosphate was found to act synergistically with sulphathiazole both *in vitro* and in protecting mice against *Staph. aureus* infections. J. R. F.

Hexamethonium and Apresoline, Caution in the Use of. K. S. Grimson, E. S. Orgain, C. R. Rowe and H. A. Sieber. (*J. Amer. med. Ass.*, 1952, **149**, 215.) Hexamethonium (methium) is an orally effective ganglionic blocking agent. Apresoline (1-hydrazinophthalazine hydrochloride) is a weakly adrenolytic and sympatholytic agent with several complex actions. The former may well prove a valuable drug in the treatment of hypertension, while the latter although a less potent antihypertensive agent might aid the treatment of disorders such as toxæmias of pregnancy or early malignant hypertension. Both drugs may produce untoward results and the following precautions should be noted in their use. Therapy should be instituted slowly during observation in a hospital and should be preceeded by thorough physical examination including retinoscopic study to exclude papillœdema with associated encephalopathy and hazard of respiratory arrest from sudden reduction of blood pressure. The heart and kidneys should also be examined since coronory disease makes marked hypotension or tachycardia hazardous, and reduction of pressure can precipitate uræmia. In patients with encephalopathy or damaged myocardium, the drugs should be used cautiously and in small doses. Patients previously treated by sympathectomy usually tolerate smaller doses and should receive very small initial doses. Patients should be warned of untoward effects and told to vary the dose as necessary. Salt-depletion diet with hexamethonium has resulted in low-salt syndrome and barbiturates used with apresoline have caused excessive drowsiness. With either drug, tolerance for ethanol may be decreased. Combined use of hexamethonium and apresoline is under trial, with hexamethonium usually being used first and apresoline being added after a week or more; the procedure is associated with definite hazards. G. R. K.

***iso*Nicotinyl Hydrazide.** R. Knox, K. S. MacLean and J. M. Robson. (*Brit. med. J.*, 1952, **1**, 1081.) The authors give a preliminary account of their two months' experience of the use of *iso*nicotinyl hydrazide in the treatment of human tuberculosis. While confirming that the human tubercle bacillus (strain H37Rv) is sensitive to the drug in concentrations of the order of 1 in 100 million, they find that cultures are not sterilised but become increasingly resistant to increasing concentrations, even without subculturing. A 3 to 4 weeks old culture has shown continuing growth in as much as 8 μg./ml. of the hydrazide. Resistance to as much as 62·5 μg./ml. has been obtained after only three subcultures. This increased tolerance can be delayed or abolished by culturing in the presence of streptomycin. Tests carried out on intracorneal tuberculosis in mice, using bovine and human strains of the infecting organism, showed that if the drug was given in the diet in doses of 12 mg./kg. of body weight per day the infection was apparently controlled completely but if treatment was discontinued after 4 weeks, lesions developed which were indistinguishable from those in untreated animals. Clinical tests on patients with long-standing fibro-caseous disease treated with *iso*nicotinyl hydrazide in comparison with similar patients given lactose showed striking subjective and objective clinical improvement during 6 weeks although the fall in the erythrocyte sedimentation rate was not commensurate with the clinical improvement. The authors suggest that efforts be made to find effective combinations of *iso*nicotinyl hydrazide and other drugs or antibiotics in the hope of delaying the emergence of resistant strains. H. T. B.

Sodium Antimonyltartrate in the Treatment of Schistosomiasis. B. Girgis and A. Magid. (*Trans. R. Soc. trop. Med. Hyg.*, 1952, **46**, 81.) Forty cases of schistosomiasis were treated with varying dosages of sodium antimonyltartrate to determine the optimal dose and duration of treatment. For an intensive course of treatment the optimal dose was found to lie between 12 and 15 mg./kg. of body weight administered in 6 equal injections over a period of 6 successive days. The smaller dose is recommended for patients who may be suffering from moderate degrees of anæmia or other minor ailments. Chronic rheumatic and syphilitic heart disease, emphysema and chronic bronchitis are not absolute contraindications provided the heart lesion is compensated and the injections are given on alternate days while the patient is at rest in bed. S. L. W.

Succinylcholine, a Muscle-relaxant of Short Action. J. G. Bourne, H. O. J. Collier and G. F. Somers. (*Lancet*, 1952, **262**, 1225.) The need for a drug capable of giving in the anæsthetised patient a brief but complete muscular relaxation led to extensive tests of succinylcholine. The authors summarise its chemistry and pharmacology and comment on its freedom from toxicity. The drug was administered without misadventure to 546 patients ranging in age from 3 to 86 years, and it was found to be particularly valuable in short procedures such as intubation, electroconvulsive therapy and orthopædic manipulation. It was also used to supplement relaxation when the effects of a long-acting relaxant were waning. Dosage ranged from 5 to 300 mg. in single injections, while up to 2300 mg. was given by intravenous drip continued for 3 hours. In the majority of cases the duration of the relaxant effect of a single dose did not exceed 8 minutes, and in none did it exceed 15 minutes. Details are given of 4 different ways in which succinylcholine was applied to abdominal surgery, namely as a supplement to curare or gallamine, as a supplement to decamethonium in some old and bronchitic patients, as the sole relaxant administered by frequently repeated injections and as the sole relaxant by continuous intravenous infusion. The drug differs from decamethonium in being destroyed by cholinesterases; neostigmine and other anticholinesterases therefore prolong its action. Although used as an antidote to *d*-tubocurarine and gallamine, neostigmine would be worse than useless for succinylcholine. H. T. B.

Succinylcholine Chloride; Self-Experiments. O. K. Mayrhofer. (*Brit. med. J.*, 1952, **1**, 1332.) Self-experiments were carried out by the author to prove the specific action of succinylcholine chloride on the skeletal muscle of man, its safety as a curarising agent for use in anæsthesia, and its freedom from undesirable side effects. The substance was used in the form of 5-ml. ampoules containing 20 mg./ml. of the dihydrate. 6 tests, spread over several days, showed that the muscular paralysis produced on intravenous injection was regular, quick in onset and short in duration. In 3 experiments the injection was given quickly (within 2 seconds), the dose varying between 0·125 and 0·375 mg./kg. of body weight. In the next 2 tests the drug was injected slowly (within 2 minutes) in doses of 0·25 and 0·5 mg./kg. Finally a continuous drip was set up of a solution containing 2 mg./ml. in normal saline, in an attempt to maintain a constant degree of relaxation and to regulate the depth of muscular paralysis at will. No medication other than oxygen by face mask was given. The action of succinylcholine chloride is due to the same mechanism as that of decamethonium, namely depolarisation of the end-plate region of the skeletal muscles, so that it is not counteracted by anticholinesterases. The compound is destroyed rapidly in the body and no antidote is considered necessary. The paralysis lasts from 1 to several minutes according to the dose, and full respiratory power is recovered within 3 minutes after the first movement of the

diaphragm, even following heavy dosage. No side or after effects were observed. There were no attributable changes in blood pressure, pulse rate or electrocardiographic tracings. Consciousness was not lost. The small margin between the relaxing and the paralysing dose, and the rather painful muscle-twitching at the onset of its action when large doses are given, makes the drug unsuitable for use with analgesics; it should be used only by anæsthetists in conjunction with an anæsthetic machine. The possible uses are thought to be (1) to provide relaxation for abdominal and thoracic surgery; (2) to facilitate endotracheal intubation; (3) to overcome severe laryngeal spasm; and (4) as an adjuvant in electric convulsion therapy. H. T. B.

Succinylcholine, Sensitivity to. F. T. Evans, P. W. S. Gray, H. Lehmann and E. Silk. (*Lancet*, 1952, **262**, 1229.) The response to succinylcholine usually lasts for from 2 to 4 minutes, but it has been noted that an occasional patient fails to recover fully for much longer periods. Having had 2 patients who did not recover for 20 and 21 minutes respectively, the authors thought this alarming reaction might have been related to low cholinesterase levels in the patients' red cells or serum. A study was therefore made of the enzymic hydrolysis of succinylcholine *in vitro*, and the serum-esterase level was measured in 4 people who showed a normal reaction to the drug, and in the 2 patients whose response was prolonged. Details are given of the methods adopted. Blood contains 2 cholinesterases; "pseudo"-cholinesterase, present in the serum is unspecific for acetylcholine, while there is the true acetylcholinesterase in the red cells. It was found that the "pseudo"-cholinesterase level was very low in the 2 patients reacting abnormally. The *in vitro* tests shows that succinylcholine is not broken down by true acetylcholinesterase but that it is metabolised slowly by the "pseudo"-esterase. It is a competitive inhibitor of acetylcholine hydrolysis by both enzymes. It is suggested that succinylcholine should not be administered to patients likely to have a low serum-esterase level, such as may be found in liver disease or severe anæmia and after poisoning with anti-cholinesterase compounds. H. T. B.

Synthetic Sweetening Agents, Chronic Toxicities of. O. G. Fitzhugh, A. A. Nelson and J. P. Frawley. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 583.) Cyclamate sodium (sodium *cyclo*hexyl sulphamate), P 4000 (1-*n*-propionoxy-2-amino-4-nitrobenzene), dulcin (4-ethoxyphenylurea) and saccharin were given to rats in varying amounts for periods up to 2 years. Saccharin and cyclamate sodium were found to have no pathological effect whatever at dose levels of 1 per cent. or less of the diet and to cause only slight toxic effects at 5 per cent.; at this level cyclamate sodium caused diarrhœa. Although it is only one-eighth as sweet as saccharin, cyclamate sodium appears to be safe for use as a saccharin substitute. Dulcin was toxic to rats at dose levels of 0·1 per cent. and above. Adenomata of the liver occurred in 10 out of 20 animals on 1 per cent. and in a few at lower levels down to 0·1 per cent. of the diet. Splenic enlargement and darkening began at the 0·1 per cent. level and increased proportionally with the dosage. Anæmia and decreased growth rate were present at 1 and 0·5 per cent. Because of the extensive damage observed, dulcin cannot be considered safe for food or drug use, even in small quantities. P 4000 was also toxic to rats at dose levels of 0·1 per cent. and above. An unusual pathological change was the presence of large amounts of a melanin-like pigment in the thyroid; at the 1 per cent. level the thyroids were slightly enlarged and almost black. A somewhat increased incidence of focal nephritis was present at 0·1 per cent. and above. Dosage levels of 1 and 0·5 per cent. decreased growth rate; the 1 per cent. level increased the mortality rate.

Although P 4000 has about 10 times the sweetening power of saccharin, the ratio of toxicities is less. Moreover, P 4000 has an undesirable anæsthetic action. It cannot therefore be considered to be a safe or desirable sweetening agent.

G. R. K.

Terramycin Base, Serum Levels after Oral Administration. R. Mason, P. Kice, E. L. Caffery and M. M. Musselman. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 587.) Three groups of 12 patients were given 1, 2 and 3 g. of terramycin base respectively. The drug was given by mouth and the serum levels of terramycin determined 1, 2, 4, 6 and 24 hours later. Similar tests were then carried out with terramycin hydrochloride on the same patients. The patients were arranged in groups according to age and the gastric juice of each was analysed for free hydrochloric acid and *p*H in the fasting state and for free hydrochloric acid after stimulation by histamine, both before administration of the terramycin and before administration of the hydrochloride. The group receiving 1 g. had an average maximum serum level of 0·7 μg./ml. 4 hours after administration of the base and of 0·9 μg./ml. 4 hours after administration of the hydrochloride. For the group receiving 2 g., the maximum levels were 2·4 μg./ml. 4 hours after administration of the base and 1·8 μg./ml. 4 hours after administration of the hydrochloride. The last group showed a maximum of 3·1 μg./ml. 4 hours after the hydrochloride but a maximum of 2·2 μg./ml. 2 hours after the base. In all groups there was a demonstrable level throughout 24 hours. The data accumulated were not significant in regard to the relation of variations in serum level to differences in age or gastric acidity.

G. R. K.

Terramycin, Value of, in Surgical Infections. F. F. Niedner and H. P. Lange. (*Dtsch. med. Wschr.*, 1952, **77**, 242.) On account of the complex flora of surgical infections, and the varying resistance of the strains, it is necessary to use an antibiotic with the widest possible range of activity, and one to which the smallest number of strains are resistant. It has been claimed that terramycin most nearly fulfils these requirements. A report is given of 74 cases treated in this way. *Bronchopneumonia*: results were good: 1 patient died later from post-operative pneumonia, 33 recovered. *Infections of the gastro-intestinal region*: Results were good when the source had been removed by operation. Terramycin treatment alone did not prevent the occurrence of, or cure existing, abscesses, The use of terramycin greatly reduced the time of treatment. *Urinary infections*: These were rapidly cured, but there were recurrences, when there were mechanical causes for the infection, so that these must be removed if a permanent result is to be attained. In the other cases infections were overcome by operation and terramycin treatment. *Gall bladder infections*: The acute phenomena were rapidly relieved by terramycin, but final cure was only attained after a combination of surgical and medical treatment. *Erysipelas and burns* were rapidly healed. The length of treatment was on the average 4 days, with a maximum of 9 days. When treatment was discontinued too early there were recurrences, so that the cost of treatment is actually increased by an attempt to economise on the costly preparation.

G. M.

3:5:3′-L-Triiodothyronine in Human Plasma. J. Gross and R. Pitt-Rivers. (*Lancet*, 1952, **262**, 439.) Investigations of plasma of patients who have received radioactive iodine showed the presence of an iodine-containing substance which behaves, on two dimensional paper chromotograms and a keiselguhr column, in a manner identical with that of 3:5:3′-L-triiodothyronine. It is shown that this compound is not an artefact of analytical procedure or of the destructive effects of radiation. The amino-acid is concluded to be a normal

constituent of the organic iodine fraction of the plasma since it has been found in the plasmas of both euthyroid and hyperthyroid individuals. J. R. F.

3:5:3′-L-Triiodothyronine in Myxœdema. J. Gross, R. Pitt-Rivers and W. R. Trotter. (*Lancet*, 1952, **262**, 1044.) 3:5:3′-L-Triiodothyronine has been shown to be a normal constituent of human plasma and to be several times as active as L-thyroxine in the prevention of goitre in rats. Two human cases of hypothyroidism were therefore treated with the triiodothyronine administered intramuscularly as a solution in 0·0004N sodium hydroxide, sterilised by heating in an autoclave. The concentration is not stated. Chromatographic examination before and after sterilisation confirmed the absence of thyroxine or other organic iodine compound. 1 patient received an initial dose of 20 μg., followed after an interval of 1 day by 20 μg. daily for 9 days, then 40 μg. daily for 5 days and 80 μg. daily for 13 days. Marked improvement resulted. The basal metabolic rate rose from −40 per cent. to −4 per cent., the plasma cholesterol level fell from 390 mg. to 170 mg. per 100 ml. and there was a loss of 9 lb. in weight. The second patient was treated with a daily dose of 40 μg. rising to 80 μg. and comparable improvement resulted. Treatment of both patients was continued with L-thyroxine orally. Experience in these cases indicates that 80 μg. of triiodothyronine intramuscularly produces in man an effect similar to that obtained from 100 to 300 μg. of thyroxine orally. It is thought possible that triiodothyronine is the compound directly responsible for the peripheral action of the thyroid gland. H. T. B.

3:5:3′-L-Triiodothyronine, Physiological Activity of. J. Gross and R. Pitt-Rivers. (*Lancet*, 1952, **262**, 593.) The synthesis of this compound has recently been reported by these authors and its formation during the iodination of 3:5-diiodothyronine has been described by Roche *et al* (*C.R. Acad. Sci., Paris*, 1952, **232**, 997). This paper describes the biological assay of the compound by its effect in preventing goitre in rats treated with thiouracil. Its activity is shown to be about three times that of L-thyroxine. The role of triiodothyronine in thyroid function is not yet known but the possibility exists that it is the form of the thyroid hormone that is active in the tissues. S. L. W.

Tubocurarine and Strychnine; Action on Spinal Reflex Excitability. C. G. Bernard, D. Taverner and L. Widen. (*Brit. J. Pharmacol.*, 1951, **6**, 551). The effects of *d*-tubocurarine and strychnine on the monosynaptic extensor reflex and the polysynaptic reflex evoked from a cutaneous nerve were studied in decapitated, low spinal, deafferented, and anæsthetised cats. *d*-Tubocurarine did not influence the polysynaptic reflex response in any of the preparations tested, but strychnine increased the amplitude of the response in all types of preparations. In low spinal preparations strychnine produced an increase in the amplitude of the monosynaptic reflex response parallel to its effect on the polysynaptic reflex. When the segment giving rise to the reflex under test is in continuity with the upper part of the spinal cord, strychnine produces a decrease in the amplitude of the monosynaptic reflex response, probably due to inhibition of the reflex by an increased continuous activity from upper segmental levels caused by the strychnine. In the same sort of preparation, if tubocurarine is given beforehand, strychnine augments the monosynaptic extension reflex; this favours the view that tubocurarine blocks structures exerting an inhibitory action on this reflex. At the segmental level of the reflexes tested, tubocurarine produces an increase in the amplitude of the monosynaptic reflex response but has no effect on the polysynaptic reflex, whereas strychnine increases the amplitude of both reflexes in parallel. S. L. W.

LETTER TO THE EDITOR

The Melting Point of Amidone Picrolonate

SIR,—In the monograph on amidone hydrochloride, appearing in the Supplement to the British Pharmaceutical Codex 1949, the picrolonate is prepared for purposes of identification and its melting point given as being about 160° C. *New and Nonofficial Remedies* (1951 Edition) gives, under methadone hydrochloride, the melting point of the picrolonate as 160° to 162° C.

It has been found in our laboratories that amidone sometimes gives a picrolonate of m.pt. 178° to 180° C. when prepared according to the B.P.C. monograph. There is little doubt that the salt exists in two forms of which the higher melting is the more stable for, in our experience, it is very difficult to prepare amidone picrolonate of m.pt. 160° C., after the high melting form has been obtained in a laboratory.

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October 9, 1952.

ABSTRACTS (*continued from page* 1087)

BACTERIOLOGY AND CLINICAL TESTS

Antibiotics, Tests for Sterility. D. Videau. (*Ann. pharm. franç.*, 1952, **10**, 204.) Except in the case of penicillin, for which a suitable inactivating agent is available, elimination of the antibiotic is necessary in testing for sterility. In the following method, aureomycin was separated from micro-organisms by centrifuging and washing. 15 g. of aureomycin was dissolved as completely as possible in 50 ml. of sterile water, any undissolved aureomycin being separated by decantation. 5 ml. of the solution was added to each of 10 centrifuge tubes, each containing 1 ml. of a sterile 3 per cent. suspension of kaolin in water. The tubes were centrifuged for 15 minutes the speed being gradually increased to 5000 to 6000 r.p.m. and maintained for 5 minutes. The supernatant liquid was removed with a pipette and bulb and replaced with sterile water, the centrifuging and washing process being repeated twice and the deposit finally suspended in 1·5 ml. of water. To ensure great dilution of any remaining antibiotic the contents of the centrifuge tubes were used to inoculate large volumes of media-tubes containing 50 ml. of meat-liver broth for ærobic and anærobic culture and flasks containing 100 ml. of Sabouraud-Langeron medium. Tubes and flasks were examined for growth after 5-days' incubation at 37° and 10 days' at 25° C. The method was effective in detecting the presence of bacteria, yeasts and fungi in aureomycin, and could be applied to other antibiotics. G. B.