ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Curare Alkaloids. Purification of *d*-Tubocurarine Chloride and Isolation of *d*-Chondocurarine. J. D. Dutcher. (*J. Amer. chem. Soc.*, 1952, **74**, 2221.) The small but appreciable variations in the physiological potency of commercial *d*-tubocurarine chloride preparations have been found to be due primarily to the presence of additional quaternary alkaloids which accompany *d*-tubocurarine through the isolation procedure. A large sample of commercial *d*-tubocurarine was repeatedly recrystallised from 0·1N hydrochloric acid. After the fifth crystallisation a product of constant solubility properties was obtained, and although the physical properties, such as melting point, specific rotation, ultraviolet absorption, etc., of this product were not detectably different from the original, the physiological potency had now become constant as a value approximately 10 per cent. lower than the original. That the higher potency of the original material was due to the presence of more potent alkaloids was apparent from the increased activity of the molten liquor solids. These molten liquors were shown to contain a highly active related quaternary alkaloid which has been named *d*-chondocurarine. A. H. B.

Ergot Alkaloids, Paper Chromatography of. A. M. Berg. (*Pharm. Weekbl.*, 1952, **87**, 282.) The 3 ergotoxine alkaloids may be separated by paper chromatography. The author used the circular method, with buffered filter paper (Schleicher and Schull 2043B, McIlvaine buffer *p*H 3), the elution medium being benzene containing 10 per cent. of ethanol, saturated with water. The 3 bands obtained were, in order from the centre, ergocornine, ergokryptine and ergocristine. They were identified by comparison with chromatograms of the pure alkaloids. G. M.

ANALYTICAL

Acidimetric Titrations in Non-aqueous Media. C. G. van Arkel and J. Kroonenberg. (*Pharm. Weekbl.*, 1952, **87**, 137.) Many bases which are too weak to be titrated in water may be titrated in non-aqueous solutions, in particular in anhydrous acetic acid. This is prepared by adding to glacial acetic acid an amount of acetic anhydride corresponding to the amount of water present, as determined by titration, and allowing to stand for 24 hours. A standard 0·1N solution of perchloric acid is prepared similarly from 70 per cent. perchloric acid, and a 0·1N solution of sodium acetate is prepared by dissolving 5·300 g. of sodium carbonate (dried at 270° to 300° C.) in anhydrous acetic acid to 1 l. Excess of acetic anhydride is to be avoided, as it interferes in the titration of acetylisable amines. The titration is carried out by dissolving about 0·5 milliequivalents of the substance in 15 ml. of anhydrous acetic acid, adding a drop of indicator solution (crystal violet in acetic acid) and titrating with the perchloric acid till the colour changes to blue-green. This colour change corresponds to the maximum potential jump in potentiometric titration. The perchloric acid is standardised against the sodium acetate solution. For potentiometric titration a glass electrode, type 6B2 and a Ag–AgCl electrode are used, both immersed

directly in the liquid. No salt bridge is required. The potential changes are noted on the *p*H scale. In some cases a sharper colour change is obtained by indirect titration, excess of perchloric acid being added and titrated back with sodium acetate. Applications are as follows: *direct titration*: atropine, codeine, strychnine, narceine, phenazone salicylate, alypine nitrate, strychnine nitrate, codeine phosphate, quinine sulphate or bisulphate, sodium salicylate, sodium acetate, sodium benzoate, sulphanilamide (potentiometric only): *direct or indirect titration*: morphine, quinine, phenazone, dihydro-codeinone bitartrate, atropine sulphate: *indirect titration*: papaverine, colchicine, quinidine, quinine ethylcarbonate, quinine carbonate, pyramidone, theobromine, amphetamine sulphate, calcium gluconate. Quinine and quinidine titrate as di-acid bases. Organic acids present as salts do not titrate, while sulphuric acid behaves as a monobasic acid so that when more than one basic group is present in a sulphate it can be titrated. Hydrochlorides do not appear to give satisfactory results. The limiting factor in the application of this method is the difficulty of dissolving certain compounds in the solvent. Temperature is also important, and a correction may be applied by using the formula:

$$\text{Normality at temperature } T_1 = \frac{N_0}{1 + 0{\cdot}001(T_1 - T_0)}$$

where T_0 is the temperature at which the solution is standardised, T_1 is the temperature during titration and N_0 is the normality at T_0. G. M.

Barbituric Acids, Titration of, in Pyridine. R. Heiz. (*Dansk Tidsskr. Farm.*, 1952, **26**, 69.) All the barbituric acids may be titrated in pyridine solution with sodium methylate, and in practice it is sufficient to use mixed pyridine bases purified from the technical material by standing over solid potassium hydroxide and distilling, the fraction 114° to 155° C. being collected. The titration solution is prepared by dissolving 6 g. of sodium in 100 ml. of methanol and diluting with 150 ml. of methanol and 1500 ml. of benzene. It must be protected from carbon dioxide. Electrometric titrations are carried out with a direct-indicating *p*H meter using an antimony-glass electrode combination. The applicability of the potentiometric and indicator method is shown in the table below.

Substance	Potentiometric titration	Visual titration			Remarks
		Thymol blue	Phenolphthalein	Thymolphthalein	
Diethylbarbituric acid	+	+	+	+	
Ethylallylbarbituric acid	+	+	+	+	Thymol blue to green.
Diallylbarbituric acid	+	+	+	+	Thymol blue to distinct blue.
Ethyl-*N*-butylbarbituric acid	+	+	+		To distinct change.
Allyl-*iso*propylbarbituric acid	+	+	+	+	To distinct change.
Dipropylbarbituric acid	+	+	+		To weak red of phenolphthalein.
Methylphenylbarbituric acid	+				
Ethylphenylbarbituric acid	+		+		
Ethyl*cyclo*hexenylbarbituric acid	+				Not very sharp.
Allylphenylbarbituric acid	+		+		To first colour change.
N-methylethylphenylbarbituric acid.	+	+	+	+	
N-methylethyl*cyclo*hexenylbarbituric acid.	+	+	+	+	
Dimethylhydantoin	+				
Diphenylhydantoin	+	+	+	+	Thymol blue to distinct blue. Often precipitates.

G. M.

Bismuth, Titration of, with Versenate. O. Landgren. (*Svensk farm. Tidskr.*, 1952, **56**, 241.) For metallic bismuth, bismuth nitrate or carbonate, a quantity corresponding to about 0·2 g. of bismuth is dissolved in 10 ml. of 5M nitric acid and diluted to 100 ml.: 10·00 ml. is treated with 25·0 ml. of versenate solution, 4·0 ml. of 2M ammonia, 5·0 ml. of borax buffer and 3 drops of eriochrome black solution, after which the excess of reagent is titrated back with 0·01N magnesium sulphate to the first change from the original violet colour. In the case of bismuth salts of salicylic acid, tribromophenol or β-naphthol the acid component must first be removed by shaking into ether. The solutions required are as follows: eriochrome black—0·5 g. with 4·5 g. of hydroxylamine hydrochloride in methanol to 100 ml.; borax buffer—sodium borate 40 g., sodium hydroxide (50 per cent.) 20 g., water to 1000 ml.; versenate solution—18·60 g. of sodium versenate (sodium ethylenediamine tetra-acetate) in water to 1 l., standardised against 0·1N magnesium sulphate in presence of borax buffer. G. M.

Digitalis, Chemical Assay of. F. Neuwald and A. Diekmann. (*Arch. Pharm. Berl.*, 1952, **285**, 19.) The method of Neuwald (*Arch. Pharm. Berl.* 1950, **283**, 93) is based on the separation and colorimetric determination of the genins of the digitalis glycosides. In a modified form of the method the hydrolysis has been omitted and the glycosides extracted directly with chloroform from the solution after purification with lead acetate. Results from these two methods have been compared with those of the Soos method, which is based on the digitoxose fraction. The results show that the two forms of the genin method give the same average results, but that the scattering is greater with the modified method, while the digitoxose method gives results which are 10 per cent. lower. This is apparently due to differences in the extraction. Moreover, the Soos process has the disadvantage that it takes longer, that up to 5 hours are required for the determination of the maximum extinction, and that the reagent is variable. Thus in practice the original genin method is to be preferred.
G. M.

Gitoxigenin, Fluorimetric Determination of. K. B. Jensen. (*Acta Pharmacol. Toxicol.*, 1952, **8**, 101.) Paper chromatographic separation of the glycosides and aglycones in *Digitalis purpurea* and *Digitalis lanata* yielded the B series (purpureaglycoside B, gitoxin and gitoxigenin) displaying an intense blue fluorescence, and the A series (purpurea glycoside A, digitoxin and digitoxigenin) with a weaker and reddish yellow fluorescence; a fluorimetric method is described for the quantitative determination of gitoxigenin, and thus of gitoxin and purpurea-glucoside B, following paper chromatographic separation of the substances of the B series. A gitoxigenin solution containing 1 to 10 μg. of substance was evaporated on the water bath, cooled, and 10 ml. of a mixture of equal parts of hydrochloric acid and glycerol were added; after shaking, the solution was left for not less than 20 minutes and the fluorescence was then measured. The fluorescence curve was rectilinear for the given range of concentrations. Gitoxin and purpureaglycoside B were determined from the fluorescence of the aglycone, on the basis of equal intensity of fluorescence of equimolecular amounts of the B substances. The fluorescence develops under the dehydrating action of acids at suitable concentrations; the substances of the A series, digitoxigenin, digitoxin, and purpureaglycoside A, are not fluorescent under these conditions. The fluorescence is very stable in daylight, although the intensity decreases rapidly after exposure to ultra-violet rays and with increasing temperature. A reproducibility of ±5 per cent. over the concentration range used is claimed. R. E. S.

Gitoxigenin, Gitoxin, and Purpurea glycoside B, Paper Chromatography of. K. B. Jensen. (*Acta Pharmacol. Toxicol.*, 1952, **8**, 110.) Gitoxigenin, gitoxin, and purpureaglycoside B were separated by one-dimensional filter paper chromatography and determined fluorimetrically, according to the method of Jensen (*Acta Pharmacol. Toxicol.*, 1952, **8**, 101). One-dimensional chromatography was used with a descending mobile phase and solutions of the substances in methanol-chloroform were applied to filter paper sheets, chromatography being performed partly at 22° C. and partly at 17° C; at 22° C. gitoxin and gitoxigenin were found to pass together, but separately from the primary glycoside, while at 17° C. all three substances separated. The chromatogram was developed by spraying with a trichloracetic acid solution, and heating at 100° C. for 2 minutes, the substances giving a blue fluorescence being marked under an ultra-violet lamp. Corresponding paper strips from the non-developed part of the chromatogram were cut out and transferred direct to the fluorescence-producing test solution (equal parts of hydrochloric acid and glycerol), the fluorescence being measured after 30 minutes. A correction for the adsorbent action of the filter paper was made by obtaining a blank reading from the use of a standard substance of the B series. R. E. S.

Iodine, Determination of Organically Bound. B. Zak and A. J. Boyle. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 260.) Chloric acid is preferable to sodium chlorate as a digesting reagent, as it avoids a high concentration of sodium perchlorate in the final solution. The process is rapid and loss of iodine is negligible. Phosphoric acid may be added to form complexes with any iron present which would otherwise tend to cause high results. The following procedure is recommended. Place the sample with 10 to 25 ml. of chloric acid reagent in a 150-ml. beaker and evaporate at a low heat until fumes of perchloric acid are evolved. Cool, dilute to 50 ml., neutralise to phenolphthalein, add phosphoric acid and hydrochloric acid and titrate with sodium thiosulphate using cadmium iodide-linear starch reagent as indicator. Alternatively, dilute the solution after digestion, with 0·2N sodium hydroxide containing 0·15 per cent. of sodium sulphite to remove dissolved oxygen and complete the determination polarographically. The digestion solution may also be assayed spectrophotometrically by adding potassium iodide and measuring the colour of the liberated iodine at 288 or 353 mμ. G. B.

Salicylates, Colorimetric Determination of. R. E. Pankratz and F. J. Bandelin. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 267.) Salicylates may be assayed by measurement of the violet colour obtained by reaction with ferric salts. For the best results the reaction of the solution should be between *p*H 4·0 and 6·0, but the great dilution required generally brings the hydrogen ion concentration within this range without special adjustment. A standard curve may be prepared by treating quantities of a standard solution corresponding to 0·125 to 1·125 mg. of salicylic acid with a 1 per cent. solution of ferric nitrate containing 1 per cent. of HNO_3, diluting to 50 ml. with water and determining the absorption at 525 mμ, with a spectrophotometer. Beer's law applies within this range of concentration. Sodium salicylate solutions of equivalent concentration may be treated in a similar manner and the result calculated from the salicylic acid data. Salicyl-, succinyl- and acetyl-salicylic acids and phenyl and methyl salicylates require hydrolysis with ethanol and potassium hydroxide before carrying out the absorption measurements. The method gives a reproducibility of $\pm 1{\cdot}0$ per cent. and may be applied to the assay of elixir of sodium salicylate, theobromine sodium salicylate tablets and tablets of aspirin, phenacetin and caffeine. G. B.

Strychnine and Brucine, Spectrophotometric Study of. P. Demoen and P. Janssen. (*J. Pharm., Belg.*, 1952, 7, 80.) Absorption curves were determined for strychnine, strychnine nitrate and brucine tetrahydrate. For strychnine, ethanol (95 per cent.) was used as solvent; for strychnine nitrate and brucine, ethanol (95 per cent.) and water gave identical results. The following coefficients were obtained at the various absorption minima and maxima.

Wavelength mμ	$E_{1\text{ cm.}}^{1\text{ per cent.}}$ calculated with reference to the anhydrous alkaloid		
	Strychnine	Strychnine nitrate	Brucine
231	173	166±1	—
240	—	—	130
255	374±1	380±1	—
265	—	—	337±1
285	—	—	150
301	—	—	232±1

Absorption spectra for strychnine and brucine are given, from which it follows that mixtures containing strychnine and brucine may be assayed by absorption measurements at 305 mμ, at which wavelength strychnine exhibits negligible absorption and the quantity of brucine is proportional to the optical density. The total amount of strychnine and brucine is proportional to the optical density at 261 ± 1 mμ, and hence the quantity of strychnine can be calculated. G. B.

Tropine Alkaloids in Tablets, Determination of. K. Jentzsch. (*Scientia Pharm.*, 1952, **20**, 6.) 1 tablet is weighed and powdered, the weight of powder obtained being determined to check any loss. The powder is placed in a small percolator (6 to 7 mm. diameter) and percolated with 1 per cent. aqueous solution of tartaric acid until extraction is complete, testing with Mayer's reagent. The percolate is allowed to drip directly into a small separating funnel, and extracted with 3 quantities, each of 5 ml., of ether; the ethereal extracts are rejected. After the addition of ammonia, the alkaloids are shaken into 5 ml. of chloroform, the chloroformic extract being filtered through a little anhydrous sodium sulphate and then evaporated by repeated brief dipping in a water bath. The extraction is repeated 4 times or until all the alkaloid is extracted. The residue is dried at 103 to 105° C. and dissolved in chloroform so that the solution contains 60 to 100 mg. of base per ml. 1 ml. of this solution is evaporated to dryness, the residue is dried at 105° C. and treated with 7 drops of a reagent, prepared by dissolving 1 g. of pure *p*-dimethylaminobenzaldehyde, added in small portions, in 9·0 g. of 88 per cent. (w/w) sulphuric acid, cooled in ice. After standing for 2 minutes, the mixture is heated for 180 seconds in the water bath and then cooled for 15 seconds in ice and water. 5·0 ml. of acetic anhydride is added from a pipette in a rapid stream, and after 15 seconds the tube is taken out of the ice and water and allowed to stand for 1 hour. The extinction is then determined at 515 mμ against a blank treated in a similar manner. It is important that the strength of the sulphuric acid should be exactly 88 per cent. by weight, as a variation of only 1 per cent. alters the extinction by 10 per cent., while the purity of the reagent is also of great importance. The method works equally well with atropine, methylatropine, hyoscyamine and tropic acid, but with scopolamine the absorption maximum is displaced towards the short wave region, i.e., is at about 502 mμ. In this latter case the standardisation should be done with scopolamine at 502 mμ, but for other or mixed alkaloids standardisation is done with atropine at 515 mμ. G. M.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Terramycin and Aureomycin Hydrochloride, Isomorphism of. R. Pepinsky and T. Watnabe. (*Science*, 1952, **115**, 541.) Single crystal X-ray patterns for aureomycin and terramycin hydrochlorides show striking similarity, indicative of isomorphism. It appears from this and published analytical information that aureomycin differs from terramycin only in containing a chlorine atom in place of a hydroxyl group. Comparative crystallographic data are given. 3-dimensional X-ray scattering data have been collected, probable co-ordinates for the chlorine in aureomycin which replaces the hydroxyl in terramycin have been established and further elucidation of the structure is in progress. G. B.

Vitamin B_{12} in Liver Preparations, Stability of. B. Noer. (*Dansk Tidsskr. Farm.*, 1952, **26**, 47.) The stability of vitamin B_{12} in a purified liver extract was determined by heating at various temperatures. At the most favourable *p*H, 5·5 to 7, an initial content of 6·40 μg./ml. was reduced to about 4·8 by 30 minutes at 100° C., and to 2·0 after 20 minutes at 120° C. Outside this range the loss was considerably greater, especially on the alkaline side. The destruction is a mono-molecular process, and the constant is, at 100° C., $1{\cdot}0 \times 10^{-2}$; at 63° C., $1{\cdot}2 \times 10^{-4}$: and at 39° C., $0{\cdot}6 \times 10^{-6}$. Similar results were obtained for liver extracts to which were added vitamin B factors. In this case a *p*H of 5·0 was taken for the tests, since this is a suitable compromise between the optimum for aneurine (3·0), and that for lactoflavine and panthothenic acid (7·0). The addition of these vitamins produces an improvement in the stability of vitamin B_{12}. The redox potential is lower in liver extract than in extract to which B vitamins have been added, indicating that reduction is one of the factors involved in the destruction of vitamin B_{12}. G. M.

BIOCHEMICAL ANALYSIS

Adrenaline-like Substances in Blood, Estimation of. H. Weil-Malherbe and A. D. Bone. (*Biochem. J.*, 1952, **51**, 311.) A fluorimetric method for the estimation of adrenaline-like substances in blood is outlined. It consists of the following steps: (*a*) filtration of plasma-buffer mixture (*p*H 8·4) through acid-washed alumina and elution of the adsorbed amines by dilute acetic acid; (*b*) heating of the eluate at 50° C. with a mixture of ethylenediamine and ethylenediamine dihydrochloride; (*c*) extraction of a stable fluorescent condensation product with *iso*butanol; (*d*) measurement of fluorescence. The method has the advantage, compared with previous methods, that adrenochrome, a labile oxidation product of adrenaline formed as an intermediary, is trapped in the nascent state and quantitatively converted into a stable condensation product. Both adrenaline and noradrenaline were quantitatively recovered by the process at concentrations equal to or greater than 1 μg./l. The mean concentration observed in human venous blood under normal conditions was about 3 μg./l. Since the fluorescence formed from noradrenaline is 1/5 of that produced by adrenaline, figures for adrenaline concentrations have to be multiplied by 5 to convert them to noradrenaline concentrations. A study of the specificity of the method and of the action of amine oxidase led to the conclusion that the reactive material in blood consists entirely of amines derived from catechol. Whether this is adrenaline, noradrenaline or a mixture of both, remains to be further investigated. R. E. S.

Bromides in Blood and Spinal Fluid, Determination of. W. D. Paul, R. W. Knouse, and J. I. Routh. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 205.) A modification of the procedure for the colour reaction between gold chloride and bromides is described. The method necessitates only half as much plasma, serum, spinal fluid, and reagents as previous procedures, avoids centrifuging, uses all of the filtrate to obtain maximal colour intensity, and is generally less time-consuming. It retains the same degree of accuracy as previous methods; a spectrophotometer is used to measure the gold bromide colour. By using a 0·35 per cent. sodium chloride solution, and a wash solution containing 0·35 per cent. of sodium chloride and 5 per cent. of trichloroacetic acid, superimposable standard curves can be prepared for bromide levels in various body fluids.

R. E. S.

Fungistatic Agents, A Quantitative Method for the *in vitro* Assay of. F. Blank. (*Canad. J. med. Sci.*, 1952, **30**, 113.) Plates of a suitable medium containing peptone, maltose, yeast extract, bovine serum and agar, are prepared. Suspensions of thallospores and mycelium are made in distilled water to which has been added 1 or 2 drops of Tween 80/200 ml. For *Ctenomyces interdigitalis*, 5 ml. is added to a test-tube containing a 3-weeks culture on solid medium, the tube is rolled between the hands and the suspension decanted. For *Trichophyton* and *Sabouraudites* spp. and *Epidermophyton floccosum* the surface of the culture is scraped with a hooked needle. Cultures of *Candida* sp. in liquid media are used. Strips of filter paper are soaked in 0·1M and 0·02M solutions of the chemicals under test dissolved in methyl cellosolve, dioxan or ethanol, and dried. Control strips are prepared with the pure solvent. For ointments, a thin layer is spread on a strip of thin cardboard. Each strip is laid on the surface of the medium in a Petri dish and incubated at 37° C. for 16 hours. Streaks of the various inocula are drawn from the edge of the medium to the strip and the whole incubated at 30° C. For *Candida* sp. zones of inhibition are measured after 24 to 48 hours, and for *Ctenomyces* and *Trichophyton rubrum*, at the 3rd, 4th, and 5th days. *T. Schönleini*, *T. violaceum*, *T. glabrum* and *T. concentricum* are not suitable as test organisms. Fungistatic power is greatly affected by the presence of serum, which must therefore be included in the test medium to prevent misleading results. G. B.

Protamine Zinc Insulin, Estimation of Protamine and Insulin in. F. A. Robinson and K. L. A. Fehr. (*Biochem. J.*, 1952, **51**, 298.) It was found that protamine and insulin could be separated by paper chromatography using an upper phase obtained by equilibrating a mixture of *n*-butanol and glacial acetic acid (3:1 by volume) with an equal volume of water. The strips were developed in a descending manner producing 2 well-defined bands with R_F values of 0 and 0·43 respectively. The concentration of protamine and of various proteins in solution could be estimated on filter paper by retention analysis with a suitable dye, e.g., erythrosine. The area of the unstained wedge formed above the protein spot was proportional to the amount of protein present; the amount of protamine in protamine zinc insulin could be estimated after removal of insulin by paper chromatography. The insulin concentration of protamine zinc insulin could be estimated, after separating the insulin by paper chromatography, by staining with bromocresol green solution, eluting the insulin-dye complex and comparing the colour of the eluate with that given by a known amount of insulin treated in the same manner. Results are given for the composition of suspensions of protamine zinc insulin and on the distribution of the components between the precipitate and the supernatant liquor.

R. E. S.

CHEMOTHERAPY

Esters of Basic Bicyclic Alcohols as Antispasmodics. L. H. Sternbach and S. Kaiser. (*J. Amer. chem. Soc.*, 1952, **74**, 2219.) The basic bicyclic alcohols 2-benzyl-3-quinuclidinol, 1-azabicyclo [3:2:1]-6-octanol, 1-azabicyclo [3:3:1]-4-nonanol, 1-azabicyclo [3:3:1]-2-methyl-4-nonanal and octahydro-1-byrrocolinol were esterified with diphenylacetic acid to produce compounds with possible spasmolytic activity. Several esters of 3-quinuclidinol were prepared including the diphenylacetate, benzilate and fluorene-9-carboxylate. The spasmolytic activities of the various esters were determined on the isolated rabbit intestine by measuring the relaxation produced by the drug against a spasm induced by acetylcholine bromide, and these results are tabulated. The esters of 3-quinuclidinol possess a much higher anti-acetylcholine activity than the analogous esters derived from diethylaminoethanol and other generally used basic alcohols, the most potent compounds being the benzolate, fluorene-9-carboxylate and diphenylacetate. It is of interest that the *lævo* isomer of 3-diphenylacetylquinuclidine had twice the activity of atropine while the *dextro* isomer was almost inactive, but the toxicity of the two isomers was equal. A. H. B.

Polymyxins, Chemotherapy and Pharmacology of. G. Brownlee, S. R. M. Bushby and E. I. Short. (*Brit. J. Pharmacol.*, 1952, **17**, 170.) Polymixins A, B, C, D and E have been shown to have similar antibacterial spectra, their efficiency depending upon the size and phase of the inoculum. Comparisons of the chemotherapy and pharmacology of polymixins A, B and C were made. A was found to be slightly more active than B and E in mice against *S. typhosa* and *H. pertussis*. A sharp fall in the counts of viable fæcal aerobes was effected by oral administration. They were not absorbed from the alimentary canal except in the newborn animal. By parenteral injections, variable blood levels persisting from 3 to 6 hours were obtained in animals and man. Higher blood levels could be obtained with repeated dosage. After single doses only a fraction of the polymixin was detectable in the urine. Polymixin E was detectable in the cerebrospinal fluid of rabbits up to 24 hours after intracisternal injections. The LD50's of A, B and E in mice were found to be about the same by the intravenous as by the intraperitoneal route. An antidiuretic effect was observed with large doses of all three in rats. Polymixins B and E had less nephrotoxic action than A, little evidence of injury was observed with E except in prolonged experiments with large doses in dogs. E was also found to cause less local reaction at the site of injection than B. Results of the chronic toxicity of E in rabbits and dogs are recorded. J. R. F.

PHARMACY

DISPENSING

Calcium Lævulinate, Injection of. F. Ernerfeldt and E. Sandell. (*Pharm. Acta Helvet.*, 1952, **27**, 48.) After autoclaving a 10 per cent. solution of calcium lævulinate there is a slight turbidity which consists of calcium carbonate. Attempts were made to prevent this by excluding atmospheric carbon dioxide, by the addition of a small amount of hydrochloric acid, or by the use of ethylenediamine tetra-acetic acid, but were unsuccessful. Apparently the formation of calcium carbonate is associated with a slight decomposition of the lævulinic acid resulting from oxidation during the heating. By the addition of 0·1 g. of

ascorbic acid per l. it is possible to prepare ampoules in which there is no deposition. The same result may be attained by the use of 0·1 g. of hydroxylamine, although this addition is toxic. G. M.

NOTES AND FORMULÆ

Thiamylal Sodium (Surital Sodium). (*New and Nonofficial Remedies, J. Amer. med. Ass.*, 1952, **149**, 369.) Thiamylal sodium is sodium 5-allyl-5-(1-methylbutyl)-2-thiobarbiturate and is supplied mixed with sodium carbonate. The mixture occurs as odourless, pale yellow, hygroscopic, agglutinated masses of crystals, freely soluble in water; *p*H of a 2·5 per cent. solution, about 10·8. The acidification of an aqueous solution precipitates thiamylal, which after drying at 60° for 1 hour, melts at 130° to 134° and complies with the following tests: when heated with sodium carbonate, the fumes evolved turned moistened red litmus paper blue (presence of nitrogen); when treated with sodium hydroxide and treated with sodium nitroprusside a red colour develops (test for substituted thiobarbituric acids and distinction from thiobarbituric and barbituric acids); when shaken with carbon tetrachloride and filtered, the filtrate decolorises a solution of bromine in carbon tetrachloride. Thiamylal sodium loses not more than 1 per cent. of its weight when dried in vacuo at 56° for 18 hours; a 2 per cent. solution is bright yellow and free of haze, floaters, and other foreign particles. It contains 94·0 to 96·0 per cent. of thiamylal sodium, and is assayed by extracting an acidified solution with chloroform, removing the chloroform, drying the residue at 60° for an hour, and weighing. The specification also includes requirements for the thiamylal used in the preparation of thiamylal sodium. Thiamylal sodium is an ultra-short acting barbiturate.

G. R. K.

PHARMACOGNOSY

***Chenopodium ambrosioides* L., Ascaridole in.** E. Wegner. (*Pharm. Zentralh.*, 1952, **91**, 43.) The presence of ascaridole in *Chenopodium ambrosioides* L. has been disputed, and it has been supposed that it is only present in *C. ambrosioides* var. *anthelminthicum* Gray. Two new approximately quantitative methods for its detection are based on the loss of weight on shaking with ferrous sulphate solution (formation of ascaridole glycol) and the production of propane on heating with titanous chloride. The ascaridole was also identified by the formation of ascaridole glycol monobenzoate. The presence of considerable quantities of ascaridole in the fruit of *C. ambrosioides* was confirmed, although the quantity was less than that in the variety *anthelminthicum*. G. M.

Digitalis Extraction Studies. R. E. Hopponen and O. Gisvold. (*J. Amer. pharm. Ass. Sci. Ed.*, 1952, **41**, 146.) Fresh leaves of *Digitalis lanata* were frozen by packing in solid carbon dioxide and ground to a powder of leaves and solid carbon dioxide which could be stored until required. After removal of the carbon dioxide the powder was extracted with warm water to form a 2 per cent. extract, calculated on the dry weight of the leaves. This solution was extracted with methyl *iso*butyl ketone and the extract concentrated at 60° C. and evaporated at room temperature to give a crystalline material which after further purification melted at 198° to 200° C. The crystalline structure, the absence of sugars other than digitoxose, and the presence of acetic acid and digoxin on de-esterification suggested that this substance was α-acetyldigoxin, although the melting point and specific rotation were not in agreement with

those previously reported. A lower-melting substance was obtained from the methyl *iso*butyl ketone mother liquors and on purification appeared to be β-acetyldigoxin, although the melting point did not agree with that in the literature. A small amount of gitoxigenin-containing substance was detected by the red colour in the Keller test. G. B.

Indian Strophanthus, *Strophanthus wightianus* Wall. K. L. Handa and I. C. Chopra. (*Indian J. med. Res.*, 1951, **39**, 403.) The seeds of this Indian species of strophanthus, which grows wild in Malabar, have been investigated. Cardiac glycosides in 1·9 per cent. yield were extracted and provisionally named strophanthin-w. This glycosidal mixture, as well as the seeds and their tincture, can be distinguished from the seeds and corresponding preparations of *S. kombé* by qualitative chemical tests. Pharmacological study of strophanthin-w shows that it possesses cardiotonic properties and is more potent than strophanthin-k. The tincture is also more potent than that prepared from *S. kombé* seeds. J. W. F.

PHARMACOLOGY AND THERAPEUTICS

1-Amino-and 2-amino-octane, Respiratory Stimulant Action of. D. E. Hutcheon and L. McCullough. (*Brit. J. Pharmacol.*, 1952, **7**, 42.) In experiments on rabbits and cats in which the breathing had been depressed by pentobarbitone sodium, the respiratory rate was increased by intravenous injections of the amines (2·0 to 8·0 mg.) and amphetamine (1·5 to 6·0 mg.). The respiratory stimulant effect of the 1-amino-octane was present after denervation of the carotid body. Both amines increased the respiration rate of rabbits in which breathing was depressed by morphine sulphate. Their action is considered to be direct stimulation of the respiratory centre under the influence of medullary depressant drugs. Both had less stimulant activity than amphetamine on the isolated rabbit heart but the coronary outflow was increased with 1-amino-octane. Although the amines are less toxic than amphetamine the therapeutic ratio is still in favour of amphetamine as a respiratory stimulant. J. R. F.

Aureomycin, Toxicity of, to Guinea-pigs. P. Roine and T. Ettala. (*Nature, Lond.*, 1952, **169**, 1014.) During an investigation of the synthesis of vitamins in the intestines of guinea-pigs and rats it was found that the same proportional dose of aureomycin which had a growth-promoting effect on rats appeared to be toxic to guinea-pigs. Experiments were conducted by feeding a group of 9 guinea-pigs with aureomycin hydrochloride at the level of 100 mg./kg. of food. All began to lose weight on the second day. 6 died in 10 days while of the remaining 3, 2 died in 5 to 6 weeks. In 10 control animals not receiving aureomycin, no trouble occurred. Further experiments in which the animals were given 1 mg. of aureomycin subcutaneously per day had similar results, death occurring in 10 days. Food and water consumption of all guinea-pigs receiving aureomycin fell markedly soon after treatment began. It has been shown that aureomycin appears to be toxic to mice, lambs, steers and dogs, but no reasons have been established. The assumption is that harmful effects on the intestinal flora play an important part, but this seems unlikely in the case of guinea-pigs in view of the rapidity of the effect. The suggestion that the antibiotic owes its toxicity to its effect in inhibiting aerobic phosphorylation is thought to merit further consideration. H. T. B.

Choline Esters and Ethers, Relationships between Structure and Nicotine-like Stimulant Activity in. P. Hey. (*Brit. J. Pharmacol.*, 1952, **7**, 117.) A hypothesis is proposed relating certain aspects of chemical structure to nicotine-like stimulant actions in choline derivatives. This states that increased activity is associated with a reduction of electron density of the "ether" oxygen atom of choline ethers and esters. A brief review of the literature is made and much evidence is produced in support. 21 compounds are used in a pharmacological study to subscribe further to this evidence. Investigations of the effects of modifications to the cationic head, to the chain structure of the molecule and of nuclear substituents are made. 3 compounds, the *m*-chlorophenyl, *m*-bromophenyl and 3:5-dibromophenyl ethers of choline, have twice or three times the activity of choline phenyl ether, the most powerful nicotine-like stimulant drug hitherto described, which itself has about twice the molar activity of nicotine.

J. R. F.

Cough-suppressing Drugs, Assessment of. B. R. Hillis. (*Lancet*, 1952, **262**, 1230.) Although the so-called reflex expectorants are widely used there is no evidence that they increase the flow of secretion from the bronchial mucosa. In troublesome cough reliance is placed on opiates and synthetic analgesics which depress the cough centre in the medulla. There is no doubt about their value but all produce side-effects such as nausea and drowsiness and there is no objective evidence of their relative value. An investigation was therefore carried out to determine the relative merits of the cough suppressants in common use. A long nasopharyngeal sprayer with a small adjustable nozzle was bent so that it could be inserted over the root of the tongue into the lower pharynx, and an extensive series of tests lasting over a year was carried out on the same individual, aged 42, who could tolerate the sprayer in his unanæsthetised pharynx for several hours. The larynx was then sprayed with irritant solutions to provoke coughing. Peppermint water and ether were found most convenient for this purpose. The efficiency of cough suppressants in common use was then determined by ascertaining the number of insufflations required to produce coughing before and after a dose of the drug under test. Control experiments were made throughout by using the identical methods after administration of an inert substance, namely physiological saline solution. The results are shown graphically and are analysed statistically. Morphine, diamorphine and amidone were found to be potent cough-suppressants. No evidence was obtained that codeine had any effect other than the psychological factor. An unexpected finding was the great importance of the psychological factor although the investigator took care to give no indication as to the expected potency of any preparation being given. This lends support to the view that the placebo has considerable importance in therapeutics.

H. T. B.

Gold Sodium Thiosulphate, Influence of Drugs on the Acute Toxicity of. W. R. Byrum and J. L. Lichtin. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 105.) Doses of gold sodium thiosulphate were administered intravenously to mice, and intraperitoneally to rats, in quantities sufficient to cause a high percentage mortality. Antidotes were administered 20 minutes before the gold compound, in quantities previously found to be well tolerated. The following, listed in order of decreasing effectiveness, lowered the acute toxicity:—for rats, vitamin B complex, adrenal cortex extract, inositol, caffeine and sodium benzoate and mercurophylline, and for mice, vitamin B complex, theobromine and sodium acetate, adrenal cortex extract, caffeine and sodium benzoate and mercurophylline. Ammonium chloride, biotin and choline chloride were ineffective.

G. B.

Urethanes with Anæsthetic Properties. R. Hazard, J. Cheymol, P. Chabrier, Y. Gay and P. Muller. (*Thèrapie*, 1951, **6**, 375.) Four derivatives of urethane were compared with procaine. Compounds (1) and (2) were prepared by heating glycol carbonate with the appropriate amine, compound (3) was prepared by condensing (2) with maleic anhydride and forming the sodium derivative and (4) by reduction of (3) with hydrogen and Raney nickel. The solubility of (2) in water is only 0·5 per cent., and (3) and (4) are more soluble derivatives.

Compound	Toxicity LD50 in mice mg. per 100 g.	Local anæsthetic activity Rabbit cornea
Procaine	7·5	1
(1) β-hydroxyethyl benzylcarbamate (242 H.C.)	30	1
(2) β-hydroxyethyl phenyl*iso*propylcarbamate (244 H.C.)	10	2
(3) Sodium derivative of maleic ester of (2)	4	0·5
(4) Sodium derivative of succinic ester of (2)	3·5	1

Compounds (1) and (2) had a depressant action on the central nervous system of rats, whereas comparable doses of the other compounds did not. All the urethane derivatives had a slightly less hypotensive effect than procaine on the arterial pressure of the dog. It is suggested that compound (1) which is as active as procaine, more stable, less hypotensive and has one fourth the toxicity be tested clinically. G. B.

Veratrum Alkaloids, Relative Hypotensive Activity of. G. L. Maison, E. Gotz and J. W. Stutzman. (*J. Pharmacol.*, 1951, **103**, 74.) Determination of the relative depressor potency of 15 substances derived from veratrum plants was made by comparison of hypotensive activity against a standard alkaloidal extract, veriloid. The derivatives were assayed for their equi-hypotensive dose at 32 per cent. fall of mean arterial pressure in anæsthetised dogs, the drugs being given by intravenous infusion of 10 minutes duration. In terms of the standard reference powder (veriloid) taken as 1 the following potencies were found: germitrine 11, neogermitrine 8·7, germerine 5·3, protoveratrine 4·7, germidine 2·4, veratridine 0·5, veratrine 0·3, veradine 0·18, veratramine 0·03. Germine, rubijervine, jervine, isorubijervine were so weak that accurate determination of potency was not possible. Dose-response curves suggest identity of mechanism of hypotensive action of protoveratrine, of veratridine and of mixtures of ester alkaloids. S. L. W.

Veratrum Derivatives, Emetic Properties of. E. D. Swiss. (*J. Pharmacol.*, 1952, **104**, 76.) The emetic dose, ED50 was determined in dogs. Using veriloid, an alkaloidal mixture of constant potency, and a large number of purified alkaloids of *Veratrum viride*, it was shown that the ratio of emetic to hypotensive doses was about the same for all the alkaloids, and that an assay based upon emetic activity would give a satisfactory assessment of the hypotensive potency. There was no significant difference in ED50 by oral and intravenous routes. Denervation of the gastrointestinal tract did not alter the intravenous emetic dose, so that intestinal irritation can be neglected as a cause of emesis. The larger oral emetic dose after gastrointestinal denervation may be due to decreased activity with poorer absorption. It is suggested that the alkaloids act on some specialised structure such as the vomiting centre in the medulla. Some drugs, such as hyoscine, atropine, ephedrine, etamon, dramamine and banthine were tested but no drug was found which might be of clinical use in alleviating the emesis. G. B.