ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Acetophenone and Lobeline, Determination of. K. Matérn and G. Schill. (*Svensk farm. Tidskr.*, 1950, **54**, 445, 462.) Acetophenone is determined photometrically through the dinitrophenylhydrazone. Details are as follows. 5 ml. of a solution containing 0·015 to 0·040 mg. of acetophenone is treated with 5 ml. of 0·1 per cent. solution of 2-4-dinitrophenylhydrazine in 2N hydrochloric acid, and allowed to stand for 2 hours; 10 ml. of carbon tetrachloride is added, and the mixture is shaken strongly for 10 minutes. After removal of the acid layer, the tetrachloride solution is dried by shaking strongly with 50 ml. of water. Washing is repeated 3 times, each time with 50 ml. of water. The carbon tetrachloride solution is washed with sodium sulphate and the extinction is determined at 370 mμ. Correction is made for a blank test carried out in a similar manner. The method cannot be used for quantities of acetophenone less than 0·015 mg., as the reaction with dinitrophenylhydrazine is then too slow. For the determination of lobeline an aqueous solution, containing 1 to 2·5 mg. of lobeline hydrochloride, is treated with 20 ml. of buffer solution (*p*H,8·0) and made up to 70 ml. with water. The flask containing the mixture is then connected to a condenser with a receiver containing 10 ml. of water. After heating on a water-bath for 15 minutes, the solution is distilled over, at a rate of not more than 3 ml. per minute, until 30 ml. of distillate has been collected. The condenser is rinsed with a little water, the solution is made up to 100 ml., and 5 ml. of this solution is treated as above. For determining unchanged lobeline in partially decomposed solutions it is necessary first to remove any free acetophenone by shaking the slightly acidified solution twice with carbon tetrachloride. Direct determination of acetophenone in such solutions is unsatisfactory. The presence of alcohol interferes with the determination, giving results which are too high. G. M.

***p*-Aminosalicylic Acid, Identification of.** F. v. Bruchhausen, H. Karbe and W. Kunz. (*Arch. Pharm., Berl.*, 1950, **283**, 110.) The *p*-aminosalicylic acid used therapeutically is 4-amino-2-hydroxybenzoic acid. Some samples on the market have been found to be 5-amino-2-hydroxybenzoic acid. The following reactions may be used to identify the latter acid. Viebock reaction: by boiling with acid, the 5-aminosalicylic acid is converted into *p*-aminophenol; the solution is treated with hypochlorite and then with phenol; a blue colour is produced. Vlezenbeek reaction: to 0·01 g. of the compound is added 0·1 g. of resorcin and 1 ml. of concentrated sulphuric acid, and the mixture is heated to 180°C. After cooling, the mixture is poured into 5 ml. of water, made alkaline, cooled and a few drops of iodine solution added; a blue-violet colour appears, owing to formation of resorufin and resazurin. Reduction test: 5-aminosalicylic acid reduces ammoniacal silver solution; the true *para* compound does not. G. M.

Antrycide, Determination of. A. Spinks. (*Biochem. J.*, 1950, **47**, 299.) Four methods for the determination of antrycide are described. Two are colorimetric methods which may only be used for aqueous solutions of the drug. The antrycide solution (up to 10 μg. of antrycide/ml.) is well shaken with a solution of congo red WS, phosphate buffer (*p*H7) and butyl alcohol. After separation the upper layer is transferred to a colorimeter and the extinction coefficient is measured against pure butyl alcohol using a green filter. In the second method the antrycide solution is well mixed with a solution of suramin and phosphate buffer and set aside for 5 minutes. A solution of 2-*p*-dimethylaminosteyryl-6-acetamidoquinoline methochloride, SQ 24 (12·5 mg./100 ml.) is then added and the extinction coefficient is measured against distilled water at 505 mμ. The third method utilised the fluorescence of solutions of antrycide. The plasma to be analysed is compared with ordinary plasma and plasma containing a known amount of antrycide. The solutions are treated with trichloracetic acid, set aside for 10 minutes, centrifuged and the upper layer examined in a fluorimeter. For amounts varying from 1 to 7 μg. of antrycide the recovery was from 89 to 100 to 119 per cent. In the fourth method the plasma is diluted with water, treated with trichloracetic acid and centrifuged, and the upper layer is mixed with buffered eosin reagent and solution of *n*-butyl alcohol in chloroform. After vigorous shaking the lower layer is pipetted off, filtered and examined in a Coleman fluorimeter. Concentrations of 40 μg. of antrycide/1. may be determined with satisfactory accuracy. The method is fairly specific, most other tertiary and quaternary bases not reacting. It can also be applied to tissues and urine.

A. D. O.

Carbon Monoxide, Spectrophotometric Determination of. N. C. Klendshoj, M. Feldstein and A. L. Sprague. (*J. biol. Chem.*, 1950, **183**, 297.) This method depends on the different value of the ratio between the optical densities of reduced hæmoglobin (3·15) at 555 and 480 mμ and that for carboxyhæmoglobin (1·94). Mixtures of the two pigments give intermediate values. 1 ml. of oxalated blood was diluted to 100 ml. with 0·4 per cent. ammonia and 3 ml. was placed in a cuvette with 10 mg. of sodium hydrosulphite. The solution was gently mixed and the densities at the two wavelengths were measured using 0·4 per cent. ammonia as a blank on a Beckman DU quartz spectrophotometer in 1 cm quartz cells. The ratio D_{555}/D_{480} was calculated from a calibration curve prepared from samples of blood containing known amounts of carbon monoxide. All determinations were checked by the Slyke's gasometric method and good agreement was obtained, especially when the content of carbon monoxide was less than 20 per cent. Methæmoglobin did not interfere with the results, but the method was not practicable when samples had become hæmolysed.

A. D. O.

Cocaine, Colour Test for. E. Rathenasinkam. (*Analyst*, 1950, **75**, 169.) The colour reaction with alkalis of the nitro-compound produced by nitration of cocaine with a mixture of nitric and sulphuric acids is used as a basis of a colour test. To about 0·5 mg. of the substance about 100 mg. of potassium nitrate and 10 drops of sulphuric acid were added, the mixture being heated in a boiling water bath for 10 minutes, cooled, and diluted with water to about 30 ml. The mixture was extracted once with chloroform, made alkaline with ammonia and extracted again with chloroform. The residue left on evaporation of the chloroform was dissolved in about 2 ml.

of acetone and 1 to 2 drops of a 10 per cent. solution of sodium hydroxide were added; cocaine gave an intense purple colour. Amylocaine hydrochloride treated similarly gave no colour; procaine hydrochloride, a light reddish-violet colour, changing quickly through brown to greenish-yellow; benzocaine, no colour; homatropine hydrobromide, a light reddish colour; atropine sulphate, a strong violet colour. R. E. S.

Cortisone and Related 17:21-Dihydroxy-20-Ketosteroids, a Quantitative Colour Reaction for. C. C. Porter and R. H. Silber. (*J. biol. Chem.*, 1950, **185**, 201.) 17:21-Dihydroxy-20-ketosteroids react with phenylhydrazine and sulphuric acid to give a yellow colour. The steroid is dissolved in 1 ml. of methyl alcohol and is heated at 60°C. with 8 ml. of phenylhydrazine solution (65 mg./100 ml. sulphuric acid, 1·63 : 1) for 20 monutes. When cool the optical density is measured at 410 mμ against a reagent blank. To correct for interfering steroid compounds a second determination is made omitting the phenylhydrazine. The difference in the two readings is proportional to the quantity of 17:21-dihydroxy-20-ketosteroid in the sample. With plasma it is frequently necessary to apply an additional correction for the opalescence of the solutions. With 1 to 25 μg. of pure cortisone acetate the optical density was directly proportional to the concentration of steroid, and the method was sensitive to about 1 μg. It was possible to estimate precisely the amount of cortisone acetate in a solution containing 20 μg. each of this steroid, methyltestosterone, œstradiol and pregnenolone, in spite of the interference which these substances caused. Other interfering substances such as fructose and dehydroascorbic acid can be eliminated in the preliminary preparation of the sample. With extracts of adrenal cortex it was found difficult to dissolve out the keto-steroids. When they were added to blood and urine they could readily be extracted with chloroform, the results showing about 80 to 95 per cent. recovery. Only after prolonged administration of the compounds to rats was it possible to detect them in the urine. A. D. O.

Digitalis, Chemical Assay of. F. Neuwald. (*Arch., Pharm., Berl.*, 1950, **283**, 93.) The method of Knudson-Dresbach, based on the reduction of alkaline picrate, gives high results in the determination of digitalis glucosides. This is not due to any reducing effect of the sugar fraction, but to some other compound present in the leaves. The author has modified this method by first separating the genins, as follows: 1 g. of coarsely powdered drug is infused with 100 ml. of water at 90°C. for 15 minutes, cooled, filtered and made up to 100 ml.: 40 ml. of this solution is treated with 2 ml. of 10 per cent. solution of lead acetate, and made up to 50 ml. After a short time the mixture is filtered, and to 25 ml. of the filtrate is added 1 ml. of 10 per cent. solution of disodium phosphate. After filtration, the residue is washed well with water. To the filtrate is added 1 ml. of 0·1N hydrochloric acid, and water to about 50 ml., and the mixture is boiled gently for 30 minutes, cooled, and neutralised to litmus with ammonia. The liquid is evaporated to dryness on the water-bath, and the residue is digested successively with 10 ml., then 5 ml., and 5 ml. of chloroform, the chloroformic solutions being washed with water. The chloroform is evaporated off and the residue dissolved in 5 ml. of methyl alcohol: 5 ml. of freshly prepared alkaline picrate reagent is added and, after 30 minutes, the extinction coefficient is determined at 20°C., using filter S50. The method is standardised against pure digitoxin solution. In applying this method it was found that the chloroform-insoluble fraction gave a strong reaction, although it did

not give the Legal reaction. This fraction was very toxic, but with frogs the stoppage of the heart occurred in diastole, whereas with the heart-active glucosides and genins it stops in systole. Thus, there is in the leaf a toxic substance which is not a typical heart poison. G. M.

Emodins in Drugs, Determination of. R. Fischer and E. Buchegger. (*Pharm. Zentralh.*, 1950, **89**, 261.) The determination of total emodins in rhubarb, frangula and cascara is carried out as follows: 0·3 to 0·5 g. of the finely powered drug is heated on the water-bath, under a reflux condenser, for 10 minutes with 15 ml. of methyl alcohol, 0·05 ml. of dilute sulphuric acid and 5 to 10 drops of hydrogen peroxide (30 per cent.). The mixture is then evaporated to 2 ml. and heated under a reflux condenser with 25 ml. of chloroform. After filtering through cotton wool, the filtrate is concentrated to 10 ml. and treated with a little sodium bicarbonate and anhydrous sodium sulphate. This chloroformic solution is passed through a column containing in succession 4 g. of sodium bisulphite, 2 g. of kieselguhr and (as bottom layer) 4 g. of ammonium carbonate. The liquid passing through is passed over a second column containing 8 g. of calcium carbonate. Washing with chloroform is continued until the liquid coming out of the first column is colourless. The second column is then washed with 5 + 5 + 3 ml. of alcohol (96 per cent.). When the red colour has almost reached the bottom of the column, a few drops of potassium hydroxide solution are added to the receiver in order to show when emodin commences to come through the column, when the washing is stopped immediately. The calcium carbonate is removed from the tube, and the red band treated with hydrochloric acid and extracted with chloroform. The chloroform solution is passed through a column containing 8 g. of finely powdered calcium chloride to remove impurities, the chloroform solution is evaporated to dryness and the residue is weighed. The ammonium carbonate used for the column should be purified by extraction with boiling alcohol, and drying at 60° to 70°C. Free emodins may be determined in a similar manner by direct extraction of the drug with chloroform, while treatment with sulphuric acid alone, followed by chloroform extraction, gives free and combined emodins. Anthranols are then calculated from the difference between total emodins and free + combined emodins. For senna leaves the first stage of extraction is as described above, but if the chloroform solution has a greenish colour instead of orange brown, it must, after drying, be treated with a little sodium peroxide. Immediately after the colour has changed to brown the peroxide should be filtered off. In this case the final residue is dissolved in 5 per cent. sodium hydroxide solution and 2 per cent. ammonia solution and made up to 200 ml. The emodin is then determined colorimetrically, using chrysophan as standard, with a filter absorbing at 475 or 560 mμ. G. M.

Emulsions, Use of Chlorophyll for Breaking. I. C. Edmundson and B. J. Wilkins. (*Analyst*, 1950, **75**, 169.) During the extraction of alkaloids in alkaloidal assays of solanaceous drugs it was noticed that the emulsions became worse as the chlorophyll was extracted. Quantities ranging from a few drops to a few ml. of a 5 per cent. solution of chlorophyll in chloroform were therefore added to the emulsions. Breaking began immediately on shaking and was complete within a short time; the treatment was used with success on tinctures and extracts which contained chlorophyll. Commercial spirit-soluble chlorophyll was used, a blank determination showing it to have

no effect on the final titre. Any tendency to emulsification in the subsequent acid extractions can be prevented if the acid layers are bulked together with any emulsion and shaken, although one extra chloroform wash may be needed to remove all the chlorophyll. R. E. S.

Strychnine and Brucine, Chromatographic Separation of. R. Fischer and E. Buchegger. (*Pharm. Zentralh.*, 1950, **89**, 146.) For the assay of nux vomica or semen ignatii, 2 g. of the drug is shaken for 3 hours with 60 g. of chloroform and 2 ml. of 2N sodium hydroxide. The mixture is filtered through cotton wool in a covered filter, and 30 g. of the chloroform solution is taken. This is concentrated and shaken out with 20, 15 and 15 ml. of 1 per cent. hydrochloric acid, the combined aqueous extracts being washed with 15 ml. of chloroform. The solution is made alkaline with ammonia, and the bases are shaken out into three 15 ml. quantities of chloroform. The chloroformic solution, after drying with sodium sulphate, is evaporated to dryness and the residue is taken up in 10 ml. of warm neutral trichlorethylene. The solution is passed through a column of 10 g. of alumina in a tube of 9 mm. diameter, the column being sucked dry and washed with two 5 ml. quantities of solvent. This trichlorethylene liquor is rejected. Strychnine is then eluted from the column by 75 ml. of a mixture of carbon tetrachloride containing 9 per cent. of acetone, the first 15 ml. of eluate being rejected. After removal of the solvent, the strychnine is dissolved in 10 ml. of 0·01N hydrochloric acid and determined by titration. Brucine is then eluted from the column by 25 ml. of ethyl alcohol and determined in a similar manner. G. M.

ORGANIC CHEMISTRY

***p*-Aminosalicylic acid, Manufacture of.** C. van der Stelt and W. T. Nauta. (*Pharm. Weekbl.*, 1950, **85**, 474.) In the manufacture of *p*-aminosalicyclic acid by carboxylation of *m*-aminophenol with carbon dioxide at high pressure, a certain amount of 4-amino-6 hydroxy*iso*phthalic acid is formed. This compound is only slightly soluble in water and organic solvents, and may best be identified by the melting-points of its methyl and ethyl esters, 146° to 147°C. and 140° to 141°C. respectively. The MLD50 for mice is between 2 and 2·4 g./kg. G. M.

Citric Acid, Production of, by *Aspergillus niger*. F. W. Kunstmann. (*Pharm. Zentralh.*, 1950, **89**, 259.) The yield of citric acid from a glucose culture may be doubled by suitable preliminary treatment of the organism. Strains of *Aspergillus niger* should be selected by culture in acid medium (*p*H 1·6 to 1·4) at 28°C.; the spores formed are inoculated on to molasses-agar at *p*H 6·0 and 35°C.; the spores produced after 3 to 4 days are then used as inoculum. The culture solution should contain 0·01 per cent. of zinc sulphate and 0·28 per cent. of pure ferric chloride. G. M.

Phenacetin, Acet-4-chloranilide as Impurity in. J. Hald. (*Dansk Tidsskr. Farm.*, 1950, **24**, 183, 195.) A sample of phenacetin which had been found to cause methæmoglobinæmia in patients, was found to contain 18 per cent. of acet-4-chloranilide. In a number of samples examined, quantities ranging from 0·02 to 0·6 per cent. were found. Up to 1·5 per cent. does not depress the melting-point of phenacetin below the official limit. The presence of this material is due to the reaction of *p*-chloronitrobenzene (the usual

starting point for phenacetin) with sodium ethoxide being incomplete, so that the *p*-nitrophenetole contains unchanged chloronitrophenol. On reduction and acetylation this gives acet-4-chloranilide. The latter compound was actually isolated from impure specimens of phenacetin by a long process of purification, but its presence may be detected by a determination of combined chlorine in the phenacetin. For this the author prefers the method of Zacherl and Krainick, involving distillation with chromic acid and titration of the chloride with mercuric nitrate, using diphenylcarbazone as indicator. G. M.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Ayfivin and Bacitracin, Resolution of Crude Products in Similar Series of Peptides. G. G. F. Newton and E. P. Abraham. (*Biochem. J.*, 1950, **47**, 257.) Ayfivin is an antibiotic produced by a strain of *Bacillus licheniformis*. The crude material was resolved by counter-current distribution and the behaviour of its components was compared to those of bacitracin. In a solvent system composed of amyl alcohol-*n*-butyl alcohol and 0.05M phosphate buffer at *p*H 6.8 to 7, crude ayfivin resolved into at least seven components, but with a system comprised of *sec.*-butyl alcohol and dilute acetic acid the resolution of the components was very poor. Three of the components, designated A, B and C, were found to be polypeptides possessing antibacterial activity. Component A, the major constituent, was equally as active as C and four times as active as B. The distribution curve of commercial bacitracin in the first system was similar to that of partially purified ayfivin. A mixture of ayfivin A and the corresponding component of bacitracin could not be resolved. Ayfivin B and C. also behaved in a similar way to two other components of bacitracin. The active components of bacitracin and ayfivin are probably identical and it is suggested that the active components of the latter should be called bacitracin A, B and C. A. D. O.

Insulin, Infra-red Evidence of Chain Configuration in Natured and Denatured. A. Elliott, E. J. Ambrose and C. Robinson. (*Nature, Lond.*, 1950, **166**, 194.) A sample of crystalline insulin was cast from formic acid solution at high temperature and the frequency of the infra-red absorption band characteristic of the C=O peptide link measured. The curve obtained by plotting wave number against optical density was similar to that obtained with insulin denatured by boiling in dilute hydrochloric acid solution. The same material, redissolved in *m*-cresol and recast as a film, gave a curve similar to that obtained with insulin natured by precipitation from aqueous phenol. Since synthetic polypeptides cast from solution in *m*-cresol are predominantly in the folded (α) form, but when cast from solution in formic acid are in the extended (β) chain configuration, the results support the view that natured insulin consists of polypeptide chains in the same α-fold as α-synthetic polypeptide and that denaturation is accompanied by extension into the β-form. The type of α-fold enables the α-β transformation to occur without any marked change in the side-chain packing but merely by rotation of the -CO-NH- groups about bonds attached to the asymmetric carbon atom; no rotation of side-chains about the chain axis is required and the transformation could occur without breaking the disulphide linkages, which must be preserved if denaturation is to be reversible. G. R. K.

Phosphate, Inorganic, Mechanism of Absorption from Blood by Tissue Cells. G. Popják. (*Nature, Lond.*, 1950, **166,** 184.) Rabbit liver was perfused *in situ* with homologous plasma to which a small amount of carrier free inorganic $^{32}PO_4^{3-}$ had been added (2 to 5 microcuries per litre) with or without 0·01M sodium azide. The amounts of phosphorus-32 which disappeared from the perfusion fluid were measured at 2-minute intervals and the amount of inorganic phosphorus absorbed by the liver calculated from the known inorganic phosphorus (^{31}P + ^{32}P) content of the perfusion fluid. The average absorption was 14 μg./minute in the control perfusion and 3·6 μg./minute in the perfusion with azide, a difference amounting to a 74 per cent. inhibition. Since azide inhibits phosphorylation, the simplest interpretation of the results is that the transfer of inorganic phosphate across cell membranes requires phosphorylating reactions. The concentration of inorganic phosphorus in tissue cells is higher (10 to 12 mg. per cent. in the liver) than in the plasma (3 to 4 mg. per cent.) and a transfer of phosphate against the concentration gradient requires expenditure of energy. The phosphorylating reactions on the cell membrane rather than those within the cytoplasm are concerned in the transfer of phosphate from the extracellular to the intracellular phase.

G. R. K.

Pyrogens, Decrease of, on Storage. J. Dorche and M. Castaing. (*Ann. pharm. franc.*, 1950, **8,** 365.) The pyrogenic character of a solution generally decreases considerably on storage over a period of months. Those which were found not to do so all contained glucose, and it appears that in this case the pyrogenic substance was derived from the glucose and not from the water. G. M.

Streptomycin, A New. R. G. Benedict, H. H. Stodold, O. R. Shotwell, A. M. Borud and R. A. Lindefelser. (*Science*, 1950, **122,** 77.) The streptomycin described in this paper is produced by an organism which differs from previously recorded sources. The aerial mycelia of the mould slowly change from greyish white to a flesh colour and the name of *Streptomyces griseo-carneus* n.sp. is therefore suggested for it. In certain characteristics the compound resembled the known streptomycins, but by Winston and Eigens' method, it was completely separable from mannosidostreptomycin and streptomycin in artificially prepared mixtures. Investigation of the structure suggested the name of hydroxystreptomycin. Its specific rotation (trihydrochloride) differed from both streptomycin and mannosidostreptomycin, and, on catalytic reduction, it absorbed the amount of hydrogen required to form a dihydro compound. When the trihydrochloride was assayed against *B. subtilis* it had the equivalent activity of streptomycin base 748 μg./mg. (streptomycin trihydrochloride 842 μg./mg.). A probable structure has been assigned to the compound but larger amounts of material are necessary to confirm it. A. D. O.

Tetanus Toxoid, Concentration of. M. B. Jacobs and M. A. Behan. (*J. Amer. pharm. Ass., Sci. Ed.*, 1950, **39,** 466.) Tetanus toxoid was precipitated at *p*H 3·5 to 4·0 with trichloroacetic acid. About 800 ml. of toxoid is mixed with 80 ml. of N trichloroacetic acid and set side for 30 minutes. The solution plus precipitate was then carefully distributed in four 250-ml. centrifuge cups and centrifuged sufficiently to pack the precipitate. The supernatant liquid was drained off and the contents of all the cups was dissolved in 10 ml. of phosphate buffer (*p*H 8). The concentration was determined by Ramon's limit of flocculation test and adjusted with 0·85 per cent.

saline solution. Thiomersalate (1 in 10,000) was added as a preservative, and the toxoid was sterilised by passing it through a Berkefeld filter. Toxoid prepared in this way passed the tests for potency and limits and toxicity of the U.S. National Institutes of Health. Not more than 17 per cent of the toxoid was lost in processing, and the flocculation time was only slightly increased. Nearly all the nonspecific nitrogen was removed. A. D. O.

Tetanus Toxoid, Purification of. M. B. Jacobs. (*J. Amer. pharm. Ass., Sci. Ed.*, 1950, **39**, 469.) The purity of a concentrated toxoid may be considered from the points of view of either the ratio of the flocculation units/mg. of nitrogen (Lf/mg.N) in the purified toxoid to the Lf./mg.N of the total protein (total undialysable nitrogen) in the crude preparation, or to the Lf./mg. of total nitrogen. Using the latter ratio the author has investigated products prepared by the method previously described (*J. Amer. pharm. Ass., Sci. Ed.*, 1950, **39**, 466). It was found that, by this method and some slight modifications of it, it was possible to remove about 99 per cent. of nitrogen. The modified processes, which entailed extra washings of the precipitate or similar treatment, enabled a purer product to be obtained but resulted in greater working losses. A. D. O.

BIOCHEMICAL ANALYSIS

Acetaldehyde in Blood, Spectrophotometric Determination of. T. N. Burbridge, C. H. Hine and A. F. Schick. (*J. Lab. clin. Med.*, 1950, **35**, 983.) A method for the rapid determination of blood acetaldehyde levels is described, which has permitted as many as 48 determinations to be completed in a 5-hour period. The separation of acetaldehyde from blood is accomplished by diffusion in a Conway cell. A semicarbazide solution is used as the reactant and the resulting acetaldehyde semicarbazone is determined spectrophotometrically with a Beckman DU spectrophotometer. When a 2 ml. blood specimen is analysed, acetaldehyde in concentrations as low as 0·2 μg./ml. can be determined with an accuracy of ± 3·5 per cent. Blood samples of 0·5 to 2 ml. are adequate for the amount of acetaldehyde encountered in normal blood and the blood of patients undergoing antabuse therapy. Normal acetaldehyde blood levels as determined by this method range from 40 to 110 μg per cent. Diffusion eliminates interference from all non-volatile agents, and there is no indication of interference from the possible presence of acetone. S. L. W.

Adrenal Cortical Hormones ; Analysis by Paper Partition Chromatography and Occurrence in the Urine of Normal Persons. A. Zaffaroni, R. B. Burton and E. H. Keutmann. (*Science*, 1950, **111**, 6.) The corticoids were sufficiently soluble in polar solvents and their α-ketol group was sufficiently detectable to obviate the use of Girard T reagent. The best solvent systems were benzene-formamide and toluene-propylene glycol. The paper strips were dipped in the polar solvent, the excess removed, the samples applied to the strips and the chromatograms developed by the descending method with benzene or toluene saturated with its respective polar solvent. The papers were dried and the positions of the corticoids revealed by treatment with alkaline silver nitrate (10 ml. of 0·1N silver nitrate, 10 drops of concentrated ammonium hydroxide and 5 ml. of 10 per cent. sodium hydroxide) followed after maximum colour production with a 5 per cent. sodium thiosulphate solution. As little as 10 to 15 μg. of a corticoid could be detected. Cortisone,

but no other corticoid tested, gave an intense blue colour in amounts over 15 μg. when the chromatogram was sprayed with a 0·3 per cent. solution of iodine in a 5 per cent. potassium iodide solution. The benzene-formamide system was most suitable for preliminary fractionation of complex corticoid mixtures, for the resolution of individual $C_{21}O_3$ compounds and to analyse the acetates and propionates of cortisone and 17-hydroxycorticosterone. The toluene-propylene glycol system gave wider and more rapid separation of $C_{21}O_5$ compounds. The method was applied to urine collected from 5 patients. The urines were extracted with ether at *p*H 1, and the extracts washed with dilute alkali and acid and separately analysed. The presence of cortisone and 17-hydroxycorticosterone was strongly indicated by the agreement in chromatographic behaviour of the urinary compounds and the known steroids, the similar movements of their esters, the characteristic colour reactions and the typical ultraviolet absorption curves. The normal 24-hour excretion of each was estimated to be between 20 and 40 μg. No other cortical hormone was found in appreciable amount. G. R. K.

Adrenaline in Biological Media, Micro-determination of. E. Sinodinos and R. Vuillaume. (*Bull. Soc. Chim. biol.*, 1950, **32**, 409.) A new colour reaction of adrenaline is obtained by adding to an acid solution of adrenaline, *p*-nitraniline and sodium nitrite, and making alkaline. The colour is greenish-blue, although it becomes brownish if the proportion of adrenaline is too high. The sensitivity is 1 μg. Details are as follows: to 1 ml. of adrenaline solution, containing not more than 20 μg., 0·1 ml. of saturated solution of *p*-nitraniline in N sulphuric acid, 1 drop of 1 per cent. solution of sodium nitrite and 2 drops of strong sodium hydroxide are added. The colour reaches its maximum intensity immediately, and is stable for several hours. Determination in adrenal gland: the gland is rubbed down with sand and 5 per cent. solution of trichloracetic acid and, after filtering, the determination is carried out on the filtrate. Determination in blood; the blood is deproteinised with trichloracetic acid, and the filtrate is passed through alumina at *p*H 8. After elution at *p*H 2 to 3, the reaction is applied directly. It is necessary to apply a factor to allow for the loss which occurs during these operations (the value of this factor is not stated). A similar colour is given by certain adrenaline derivatives; in the case of nor-adrenaline only 1/10 the strength of that with adrenaline. Although the presence of nor-adrenaline has been reported in adrenal gland, the amount appears to be too small to have any appreciable affect on the results of this method. G. M.

***p*-Aminosalicylic Acid, Determination of.** R. Fleury. (*Bull. Trav. Soc. Pharm. Bordeaux*, 1950, **88**, 68.) For the determination of *p*-aminosalicylic acid in pus, a volume of the latter is diluted with an equal volume of physiological salt solution. After centrifuging, the sediment is treated with saline solution until no more of the acid can be detected in the liquid. The solution is diluted from 20 to 500 times, and 5 ml. of it is treated with one drop of 10 per cent. solution of ferric chloride. The colour is then compared with a series of freshly-prepared standards containing from 0·1 to 0·033 per cent. of *p*-aminosalicylic acid. The results obtained by this method are equally as accurate as those obtained by diazotisation, and it has the advantage that it is applicable directly to acetaminosalicylic acid, the form in which *p*-aminosalicylic acid is eliminated. For determination in urine, it is only necessary to dilute the sample from 50 to 100 times, and then adjust to *p*H 3 by the addition of hydrochloric acid. When *p*-aminosalicylic acid (9 g.) is injected

into an abscess, it is eliminated completely in 36 hours. Diffusion in the abscess is very slow at first and is only complete after 8 hours. During this period urinary elimination is very low. G. M.

Benzylpenicillin, a New Method for the Spectrophotometric Determination of. H. Pénau, G. Hagemann, Y.-G. Leclére and R. Viennet. (*Ann. pharm. franc.*, 1950, **8**, 450.) Commercial penicillin salts may be assayed for benzylpenicillin by the following method. Prepare a solution of the dried material in a mixture of 95 parts of alcohol (95 per cent.) and 5 parts of water, and determine the optical density at 263·0 mμ, 280·0 mμ and 322·0 mμ. Unless the optical density at 322·0 mμ is greater than that at 280·0 mμ, in which case the substance has undergone decomposition and the result will not be valid, determine the ratio of the optical densities at 263·0 mμ and 280·0 mμ and calculate the percentage of benzylpenicillin by reference to a standard curve prepared with a chromatographically pure sample of benzylpenicillin. The determination is accurate to ±2 per cent., and avoids the use of aqueous solutions in which there is rapid deterioration and shift of absorption maxima. G. B.

Globulin and Total Protein in Cerebrospinal Fluid, Microphotelometric Determination of. H. B. Salt. (*J. Lab. clin. Med.*, 1950, **35**, 976.) A method is described for the precipitation of globulin by methyl alcohol (45 per cent.) at *p*H 6·6 in the presence of ghatti gum in 1 hour at 37°C. Albumin is not precipitated under these conditions provided its concentration is below the critical level of 125 mg./100 ml. A similar procedure is described for the precipitation of total protein by salicylsulphonic acid in the presence of ghatti gum in 5 minutes at room temperatures. Details are given (together with a sectional plan and elevation) of a photoelectric apparatus whereby the precipitated proteins, held in colloidal suspension by the gum, are determined directly by light dispersion measurements. Dispersimetric values are correlated with globulin and with total protein concentrations by means of empirically established curves. Spectral red light is normally used, but for greater sensitivity the wave-band may be changed to spectral violet. The analytical procedures described are applicable to diluted blood serum as well as to cerebrospinal fluid and give reliable results for quantities of protein from 0·1 mg. to 1·4 mg. per aliquot analysed. S. L. W.

Insulin Preparations, Commercial, A Comparison of Three Methods for the Assay of. D. M. Young, D. B. W. Reid and R. G. Romans. (*Canad. J. Res. Sect. E*, 1950, **28**, 19.) Commercial preparations including crystalline and non-crystalline insulin, protamine zinc insulin and globin insulin with zinc were assayed by three methods, (i) determining blood-sugar levels after subcutaneous injection into 32 rabbits using the "twin cross-over" design; (ii) determining the blood-sugar levels after intravenous injection into 16 rabbits using the 4 × 4 Latin square design; and (iii) determining the number convulsing within 75 minutes after subcutaneous injection into 288 mice, using a two-level quantal response design. There was satisfactory agreement between the results by the three methods. The average standard error of the result was: method (i), 13 per cent., method (ii), 10 per cent., and method (iii), 9 per cent. G. B.

Penicillin in Urine, Determination of. H. H. Pénau, E. Saïas, N. de Chezelles and D. Benoist. (*Ann. pharm. franc.*, 1950, **8**, 444.) A quantity of urine containing not less than 5 units of penicillin is adjusted

to *p*H 2 and extracted twice with chloroform in the presence of sufficient ammonium sulphate to depress considerably the solubility of penicillin in the aqueous phase. The chloroform solution, after decolorisation, if necessary, with charcoal, is extracted with aqueous sodium hydroxide, and the penicillin titrated iodimetrically. Concentrated urine requires a preliminary washing with chloroform at *p*H 4, and this does not remove an appreciable quantity of penicillin. The urinary concentration of penicillin corresponds fairly closely to the blood level, determined microbiologically, and curves relating urinary penicillin concentration with time may be used in estimating blood levels maintained with various salts of penicillin. G. B.

Vitamin D, Spectrophotometric Estimation of. H. E. Cox. (*Analyst*, 1950, **75**, 521.) The following method is recommended for the determination of vitamin D in foodstuffs. Dissolve the unsaponifiable matter in 10 ml. of *cyclo*hexane, and to 0·2 ml. of the solution add 1·8 ml. of a freshly prepared, alcohol-free reagent containing 20 per cent. w/v of antimony trichloride and 4 per cent. of acetyl chloride. If vitamin A is present, a blue colour is produced, and the size of sample is adjusted so that the colour fades in about 4 or 5 minutes. Determine the absorption at 500 mμ after 6 minutes, and calculate the vitamin D content from the datum $E^{1\ per\ cent.}_{1\ cm.} = 1880$ for pure vitamin D_3. If the composition of the material is known, as when the test is used for control of manufacture, a correction can be applied for the colour produced by other sterols. The test is four times more sensitive than the assay by determination of absorption at 265 mμ, and is not so susceptible to interference by other sterols and vitamin A. When there is five or more times as much vitamin A as vitamin D present, the vitamin A should be removed chromatographically, before assaying for vitamin D. G. B.

PHARMACY

NOTES AND FORMULÆ

Acetomeroctol (Merbak). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **143**, 814.) Acetomeroctol, 2-(acetoxymercuri)-4-(1:1:3:3-tetramethylbutyl)phenol, $C_{16}H_{24}O_3Hg$, is a white solid, m.pt. 155° to 157°C., almost insoluble in water, soluble in alcohol, ether and chloroform, and sparingly soluble in benzene. When 3 ml. of chloroform containing 0·1 g. of iodine is gradually added to a solution of 0·2 g. of acetomeroctol in 3 to 5 ml. of chloroform, the iodine colour is immediately discharged, a momentary green appears and yellow mercuric salts are precipitated. When acetomeroctol is extracted with water and filtered the filtrate gives no colour or precipitate with sodium sulphide (absence of mercuric ion); the loss in weight on drying *in vacuo* over anhydrous calcium sulphate for 24 hours is not more than 0·75 per cent. Acetomeroctol contains 43·0 to 43·2 per cent. of mercury calculated with reference to the dried substance. It is assayed by heating under a reflux condenser with monoethanolamine, separating the globule of mercury thus obtained by centrifuging, dissolving in nitric acid and estimating by titration with potassium thiocyanate. Acetomeroctol is applied locally in 0·1 per cent. solution containing 50 per cent. of alcohol and 10 per cent. of acetone as an antiseptic for the control of superficial infection. G. R. K.

Bismuth Glycolylarsanilate (Milibis). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **143**, 895.) Bismuth glycolylarsanilate, the product

of reaction between sodium *p*-*N*-glycolylarsanilate and bismuth nitrate, is an odourless, yellowish-white to flesh-coloured amorphous powder which decomposes on heating. It is very slightly soluble in water and alcohol and insoluble in benzene, chloroform and ether; a saturated aqueous solution has *p*H 2·8 to 3·5. When a solution in acidified water is boiled, cooled and treated with bromine solution, a white precipitate is obtained, which on extraction with ether yields tan-coloured crystals of 2:4:6-tribromoaniline, melting at 118° to 121°C. (presence of arsanilic acid). It contains not more than 0·5 per cent. of free arsanilate, calculated as arsanilic acid and determined by titration with sodium nitrite in ice-cold acid solution, and not more than 3 per cent. of moisture when dried at 105°C. for 24 hours. The content of arsenic is 14 to 16 per cent., of bismuth 36 to 42 per cent., and of arsanilic acid 42·2 to 44·8 per cent. Arsenic is assayed by digesting with acid, adding sodium potassium tartrate, neutralising with sodium hydroxide and titrating with iodine in the presence of sodium bicarbonate; bismuth is assayed by digesting with nitric acid, diluting, treating with diammonium phosphate and weighing the precipitate so obtained; and arsanilic acid is assayed by refluxing with hydrochloric acid, cooling in ice and titrating with sodium nitrite. Bismuth glycolylarsanilate is an amœbicide given as tablets in an average adult dose of 0·5 g. thrice daily.

G. R. K.

Choline Bicarbonate. (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **143,** 814.) Choline bicarbonate, (2-hydroxyethyl)trimethylammonium bicarbonate, $CH_2OH.CH_2.N(CH_3)_3.HCO_3$ is prepared by passing carbon dioxide into a solution of choline until the *p*H falls below 9, concentrating, again passing in carbon dioxide until the *p*H falls to 8·5 to 8·9, and evaporating *in vacuo*. It is a very hygroscopic, white solid with an amine-like odour, very soluble in water and alcohol, and slightly soluble in benzene, chloroform and ether; a 10 per cent. aqueous solution has *p*H 8·5. It gives a white curdy precipitate with phosphotungstic acid and an emerald green colour with cobaltous chloride and potassium ferrocyanide. When treated with hydrochloric acid, choline bicarbonate evolves carbon dioxide; the resulting solution after gentle heating and diluting gives no colour or precipitate with hydrogen sulphide; ash not more than 0·05 per cent. It contains 98 to 105 per cent. of choline bicarbonate and is assayed by treating an aqueous solution with ammonium reineckate, filtering, washing the precipitate with water and alcohol, drying by suction, dissolving in acetone and measuring the absorption at 5260Å with a spectrophotometer; the content of choline is obtained from a standard curve obtained by similar determinations on a series of solutions of choline chloride reference standard U.S.P. Choline bicarbonate is recognised for clinical trial as an adjunct in the treatment of fatty infiltration of the liver; the total daily dose is 8 g. or more.

G. R. K.

Isopropylarterenol Hydrochloride (Isuprel Hydrochloride). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **144,** 238.) Isopropylarterenol hydrochloride is α-(*iso*propylaminomethyl)protocatechuyl alcohol hydrochloride or 1-(3′ : 4′-dihydroxyphenyl)-2-*iso*propylaminoethanol hydrochloride, $C_6H_3(OH)_2.CHOH.CH_2.NH.CH(CH_3)_2,HCl$. It is a white, odourless, slightly bitter, nonhygroscopic, crystalline solid, m.pt. 166° to 172°C., soluble in water and alcohol and very slightly soluble in benzene and ether; aqueous solutions become pink on standing. A 1 per cent. aqueous solution

is clear and colourless and has *p*H 4·5 to 5·5. It may be distinguished from amphetamine, ephedrine, methamphetamine, naphazoline, phenylpropanolamine, phenylpropylmethylamine and tuaminoheptane by the dark brown to black colour which develops when it is added to a 10 per cent. solution of ammonium molybdate in sulphuric acid; the addition of ferric chloride to an aqueous solution gives an intense green colour which becomes olive-green on standing (distinction from hydroxyamphetamine and phenylephrine, which give a purple colour). Isopropylarterenol hydrochloride loses not more than 1·0 per cent. of its weight when dried to constant weight over phosphorus pentoxide; ash, not more than 0·2 per cent. It contains 5·40 to 5·80 per cent. of nitrogen (determined by semi-micro Kjeldahl) and 14·1 to 14·8 per cent. of hydrogen chloride (determined by adding silver nitrate and weighing the silver chloride precipitated). A 0·0004 per cent. solution in water exhibits an ultra-violet absorption maximum at 2800 Å, and has $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ of 113 ± 3, equivalent to 97 to 103 per cent. of isopropylarterenol hydrochloride. Tablets and solution are assayed by measuring the transmission at 2800 Å and calculating the content of isopropylarterenol hydrochloride form a standard curve. Isopropylarterenol hydrochloride is a sympathomimetic amine closely related in its action to adrenaline and noradrenaline. It is effective in the treatment of mild and moderately severe asthma. When inhaled it exerts a mild expectorant action. It is administered sublingually in a dose of 10 to 15 mg. or by inhalation in a dose of not more than 0·5 ml. of a 0·5 per cent. solution. G. R. K.

Isopropylarterenol Sulphate (Isonorin Sulphate). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **144,** 239.) Isopropylarterenol sulphate is α-(*iso*propylaminomethyl)protocatechuyl alcohol sulphate or 1-(3′:4′-dihydroxyphenyl-2-*iso*propylaminoethanol sulphate, $(C_6H_3(OH)_2.CHOH.CH_2.NH.CH(CH_3)_2)_2.H_2SO_4$. It is a white, odourless, slightly bitter, hygroscopic, crystalline solid, m.pt. 118° to 122°C., freely soluble in water, slightly soluble in alcohol and very slightly soluble in benzene and ether; aqueous solutions become pink on standing. A 1 per cent. aqueous solution is clear and colourless, and has *p*H 3·5 to 4·5. Isopropylarterenol sulphate responds to the colour tests for isopropylarterenol hydrochloride. When dried to constant weight; over phosphorus pentoxide it loses not more than 7·0 per cent. of its weight; ash, not more than 0·3 per cent. It contains 5·20 to 5·60 per cent. of nitrogen, determined by semi-micro Kjeldahl. A 0·0004 per cent. aqueous solution exhibits an ultraviolet absorption maximum at 2800Å and has $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ 106 ± 3, equivalent to 97 to 103 per cent. of isopropylarterenol sulphate.
G. R. K.

Mercaptomerin Sodium (Thiomerin Sodium). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **143,** 895.) Mercaptomerin sodium is the disodium salt of N(γ-carboxymethylmercaptomercuri-β-methoxy)propylcamphoramic acid and occurs as a white, hygroscopic solid which decomposes when heated to 150° to 155°C. It is freely soluble in water, soluble in alcohol and almost insoluble in ether, benzene and chloroform. When a solution is treated with sodium acetate and cobalt nitrate followed by potassium iodide, a deep orange colour is produced. The presence of allylcamphoramic acid is shown by treating an aqueous solution with sodium sulphide and an excess of hydrochloric acid, boiling and filtering; the white crystals obtained on concentrating the filtrate melt between 171° and 173°C. Mercaptomerin sodium contains 3·60 to 3·98 per cent. of

sodium, 31·4 to 34·8 per cent. of mercury and 14·4 to 15·6 per cent. of mercaptoacetic acid: the molecular ratio of mercaptoacetic acid to mercury is not less than one. When dried over phosphorus pentoxide at 100°C. for 3 hours it loses not more than 2 per cent. of its weight. The content of mercury is determined by electrolysing an aqueous solution containing sodium sulphide for twenty-four hours at a current density of 0·5 amp. using a rotating platinum cathode, and measuring the increase in weight of the cathode. Mercaptoacetic acid is assayed by adding potassium iodide and glacial acetic acid and titrating with potassium iodate. Mercaptomerin sodium is a mercurial diuretic and is administered subcutaneously as a 14 per cent. aqueous solution in a dose of 0·5 to 2 ml. G. R. K.

Methoxyphenamine Hydrochloride (Orthoxine Hydrochloride). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **143,** 897.) Methoxyphenamine hydrochloride is β-(*o*-methoxyphenyl)*iso*propylmethylamine hydrochloride, $CH_3O.C_6H_4.CH_2.CH(CH_3).NH(CH_3),HCl$. It occurs as an odourless, bitter, white, crystalline powder, melting at 124° to 128°C.; it is freely soluble in water, alcohol and chloroform and slightly soluble in benzene and ether, the *p*H of a 5 per cent. solution in water being 5·3 to 5·7. When distilled with hydriodic acid, methyl iodide is obtained in the distillate; when the residual solution in the flask is cooled to 0°C., treated with diazotised *p*-nitroaniline and made alkaline, a bright orange-red precipitate forms (presence of hydroxyphenyl group). Methoxyphenamine hydrochloride loses not more than 0·5 per cent. of its weight when dried *in vacuo* over phosphorus pentoxide for 24 hours; ash, not more than 0·5 per cent. It contains 98 to 102 per cent. of methoxyphenamine hydrochloride (determined by the Kjeldahl method) and 16·25 to 16·65 per cent. of chlorine. Methoxyphenamine hydrochloride is a sympathomimetic agent and is administered as tablets in doses of 50 to 100 mg. every 3 or 4 hours. G. R. K.

Tripelennamine Citrate (Pyribenzamine Citrate). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **142,** 569.) Tripelennamine citrate, *N*:*N*-dimethyl-*N'*-benzyl-*N'*(α-pyridyl)ethylenediamine citrate, is a bitter, white, crystalline powder, m.pt. 106° to 110°C. It is freely soluble in water, and alcohol, very slightly soluble in ether and almost insoluble in benzene and chloroform; a 1 per cent. solution has *p*H 4·25. It gives a flocculent pink precipitate with ammonium reineckate, and a dipicrate melting between 184° and 186°C. When treated with sulphuric acid it turns brown but does not dissolve (distinction from the hydrochloride). When dried *in vacuo* over phosphorus pentoxide for 24 hours, it loses not more than 0·5 per cent. of its weight; ash not more than 0·3 per cent. It contains 98 to 102 per cent. of tripelennamine citrate and is assayed by weighing the dipicrate obtained by treatment with trinitrophenol. Tripelennamine citrate is a histamine antagonist and has the same action as the hydrochloride; it is, however, more palatable. The average dose is 75 mg. 4 times a day. G. R. K.

PHARMACOGNOSY

Eucalypt Kinos, Chromatographic Analysis of. W. E. Hillis. (*Nature, Lond.*, 1950, **166,** 195.) Butyl alcohol-acetic acid-water (40-10-50 per cent.) gave poor resolution of the components of the eucalypt kinos, and phenol-water, although more suitable did not give sharp resolution. The most suitable

solvents were (a) a mixture of phenol and 2N acetic acid containing 0·3 per cent. of sodium chloride; (b) a mixture of equal volumes of phenol and an aqueous solution 2N with respect to both acetic and hydrochloric acids; and (c) ethyl alcohol-benzene-water (40-20-40 per cent.). Solvents (a) and (b) gave comparable R_f values but (b) was more suitable for old samples of kino. Solvent (c) resolved only those compounds which had a high R_f value in the phenol solvents. The kino of *E. calophylla* R. Br. (marri) was resolved into at least 12 components (6 fluorescent and 6 detected by Tollen's reagent), that of *E. corymbosa* Sm. into at least 13 (5 fluorescent, 7 by Tollen's reagent, 1 by both), and that of *E. goniocalyx* F. v. M. into at least 11 (4 fluorescent, 5 by Tollen's reagent, 1 by both and 1 carbohydrate). Aromadendrin had an R_f value of 0·79 and showed a green fluorescence in ultraviolet light. G. R. K.

Frangula Extract, Chromatographic Examination of. P. F. Jørgensen. (*Dansk Tidsskr. Farm.*, 1950, **24**, 111.) By a series of fractionations on various columns, a number of fractions were obtained from liquid extract of frangula. These are summarised in the table below, with their probable modes of origin.

	Compound	Formed from	Reaction
1.	Anthrone-anthranol glucoside complex		——
2.	Anthraquinone glucoside complex A	1	Hydrolysis and oxidation
3.	Anthraquinone glucoside complex B	1	Hydrolysis and oxidation
4.	Chrysophanic acid anthrone-frangula emodinanthrone monomethyl ether	1	Hydrolysis
5.	Frangula emodinanthrone	1	Hydrolysis
6.	Difrangulin	3	Hydrolysis
7.	Frangulin	6	Hydrolysis
8.	Condensation product of partly oxidised frangula emodinanthrone	5	Oxidation
9.	Chrysophanic acid frangula emodin monomethyl ether	4 2 3	Oxidation Hydrolysis Hydrolysis
10.	Frangula emodin	2 8 5 7	Hydrolysis Oxidation Oxidation Hydrolysis

G. M.

PHARMACOLOGY AND THERAPEUTICS

Adrenocorticotrophic Hormone in Poliomyelitis. L. L. Coriell, A. C. Siegel, C. D. Cook, L. Murphy and J. S. Stokes. (*J. Amer. med. Ass.*, 1950, **142**, 1279.) This is a report of a clinical investigation undertaken to obtain answers to the following questions: (1) Is the "alarm reaction" evoked by poliomyelitis? (2) What is the physiological action of the drug in poliomyelitis? (3) Does the drug modify the course of the disease when administered in the early stages? A total of 70 patients with poliomyelitis was studied 35 receiving the drug and 35 a placebo (isotonic sodium chloride solution). The drug was given intramuscularly at 6-hourly

intervals for 4 or 5 days. The results showed (1) that the "alarm reaction" is mobilised in poliomyelitis as shown by the eosinophil response; (2) that in these patients the drug produced a physiological effect as evidenced by a further depression of the eosinophil count and by decreased excretion of 17-ketosteroids; (3) that there was no demonstrable effect on the treated as compared with the untreated group when evaluated on the basis of temperature response, paralysis, progression of paralysis, or early residual effects. Statistical analysis showed that the drug has no beneficial or obvious deleterious effect on the course of poliomyelitis when treatment is begun after the onset of symptoms. S. L. W.

Adrenocorticotrophic Hormone Therapy in Inflammatory Diseases of the Eye. J. A. Olson, E. H. Steffenson, R. R. Margulis, R. W. Smith and E. L. Whitney. (*J. Amer. med. Ass.*, 1950, **142,** 1276.) Of 7 patients with inflammatory eye diseases 4 had acute plastic iritis and 2 had keratitis and anterior uveal tract involvement, 1 of the 2 with secondary glaucoma, 1 patient had a recent chorioretinitis with absolute scotoma. The drug was given intramuscularly at intervals of either 4 or 6 hours in dosages of 10 to 20 mg. Total dosages ranged from 185 to 432 mg. and treatment was continued over periods of from 3 to 13 days. In all patients attempts were made to avoid early relapses by withdrawing the drug slowly after the last 24 to 36 hours of treatment. All the patients responded abruptly and favourably to the treatment. Symptomatic relief and distinct objective changes were achieved as early as the second hour in 1 patient and by the 4th hour in the other patients. 2 of the patients with acute plastic iritis were "cured" and showed no evidence of relapse in 5 and 7 weeks. Glycosuria in 2 patients and auricular fibrillation in 1 patient were observed during the period of administration. S. L. W.

Antimycin A, an Antibiotic with Insecticidal and Miticidal Properties. G. S. Kido and E. Spyhalski. (*Science*, 1950, **112,** 172.) Antimycin is derived from an unidentified species of *Streptomyces* and is a potent fungicide. It is an optically active nitrogenous phenol of the molecular formula $C_{28}H_{40}O_9N_2$. For antimycin to exert its poisonous effect against insects it must be ingested. Certain insects such as the German cockroach and the larva of the webbing clothes moth (*Tineola biselliella,* (Hum.)) are immune; larvaæ of *Attagenus perceus,* (Oliv.) will not feed on fabrics treated with it. In one-hundredth of the concentration, antimycin A will afford the same protection for fabrics as the normal amount of sodium aluminium silicofluoride against larvæ of the black carpet beetle. It is more effective than methoxychlor in controlling Mexican bean larvæ and di(*p*-chlorophenyl)-methyl carbinol against red spider mite. A. D. O.

Aureomycin, Treatment of Pneumococcal Pneumonia with. H. F. Dowling, M. H. Lepper, H. H. Hussey, E. R. Caldwell and H. W. Spies. (*J. lab. clin. Med.*, 1950, **35,** 215.) 174 cases of pneumococcal pneumonia were treated with aureomycin. 250 mg. was administered orally every 3 hours, or 500 mg. every 6 hours until temperature fell and remained normal for 48 to 72 hours. Temperature fell more rapidly than in penicillin-treated patients. The fatality rate in cases of typed pneumococcal pneumonia was 1·4 per cent., compared with 5·2 per cent. in penicillin-treated cases, but this difference is not statistically significant because of the relatively small number of patients treated witth aureomycin. G. B.

Bacitracin, Treatment of Amœbiasis with. H. Most, J. W. Miller, E. B. Grossman and N. Conan. (*J. Amer. med. Ass.*, 1950, **143,** 792.) 51 patients with amœbiasis varying in severity from an asymptomatic condition to fulminating dysentery were treated with bacitracin given as tablets. The total daily dose varied from 40,000 to 120,000 units and treatment extended for from 5 to 20 days. In 34 patients there was apparent parasitic cure after the first course of treatment, but the remainder relapsed; of these, 9 received a second course and of these, 6 again relapsed. Extension of therapy beyond 10 days or increase in dose above 80,000 units did not significantly enhance the probability of cure. In 8 patients who were moderately or severely ill, the clinical response was notable in that the dysentery was brought under control within a few days, the amœbæ disappeared from the stools and surface lesions, and complete healing occurred in from four to fourteen days. In 3 patients, *E. histolytica* reappeared in the stools 5, 8 and 350 days after the last dose of bacitracin, but were unaccompanied by clinical activity. The toxicity of bacitracin is negligible and the drug is apparently little absorbed. G. R. K.

Chloramphenicol, Hæmopoietic Changes during Administration of. I. F. Volini, I. Greenspan, L. Ehrlich, J. H. Gonner, O. Felsenfeld and S. O. Schwartz. (*J. Amer. med. Ass.*, 1950, **142,** 1333.) In 3 patients, 2 with typhoid and 1 with brucellosis, profound blood and marrow changes were observed during chloramphenicol therapy. These changes consisted of a precipitous fall in the total leucocyte count, which occurred by the 7th day in 1 case and continued as long as the drug was administered. The leucopenia resulted primarily from a decrease in the number of granulocytes in the marrow due to a maturation arrest without significant alteration in the monocytes or lymphocytes. The total dosages employed were 53 g. in 18 days in one case, 53 g. in 19 days in the second case, and 26 g. in 9 days in the third case. The treatment was remarkably effective in controlling the infection in all the patients; an immediate precipitous rise in granulocytes and leucocytes followed discontinuance of the chloramphenicol therapy. The toxic manifestations of chloramphenicol in man will have to be studied more extensively before it can be considered an absolutely safe therapeutic agent, particularly when large total dosages are employed over relatively short time periods. S. L. W.

Chloramphenicol, Placental Transfer of. W. C. Scott and R. F. Warner. (*J. Amer. med. Ass.*, 1950, **142,** 1331.) In order to determine whether or not there was placental transfer of chloramphenicol 12 normal pregnant patients were given the drug during term labour in doses of 2 g. orally at 2-hour intervals until delivery occurred. At the time of delivery 10 ml. of maternal venous blood was drawn and 10 ml. of fœtal blood was collected from the umbilical cord. Colorimetric determinations revealed that 10 of the 12 had a therapeutically effective serum concentration in fœtal cord blood within 71 minutes after initial dosage. This level was maintained for at least 135 minutes and by 2-hourly repetition of the dose could be maintained as long as 625 minutes. No evidence of toxicity was exhibited by any of the patients or infants. S. L. W.

Dicoumarol Labelled with Carbon-14, Tracer Experiments in Mammals with. J. W. T. Spinks and L. B. Jaques. (*Nature, Lond.*, 1950, **166,** 184.) Fourteen mice were each given 0·25 mg. of labelled dicoumarol intravenously and sacrificed at various times after injection. No significant radioacivity

was detected in the lungs, kidney or tissues in general, but great activity was found in the liver, gall bladder, fæces and urine; 80 per cent. of the activity disappeared from the blood in the first hour following injection. A series of rabbits similarly treated with 10 mg. of labelled dicoumarol gave a similar distribution pattern, with a rapid disappearance of dicoumarol from the blood and a rapid increase of activity in the liver; it was shown that the activity in the liver was essentially all due to unchanged dicoumarol, whereas although a large percentage of activity was recovered in the urine at the end of the experiment, none of this was due to dicoumarol. In the mice, the dicoumarol remained in the liver for one day and the prothrombin time remained high for 3 days, whereas in the rabbits the corresponding periods were 7 days and 8 days respectively. When mice were injected with the labelled dicoumarol together with vitamin K, activity appeared in the liver immediately, but disappeared much more quickly than from the livers of animals not receiving the vitamin. The results show that the liver is the target organ for the action of dicoumarol and suggest that the period of time during which it remains in the liver is related to its effectiveness in interfering with the formation of prothrombin. G. R. K.

Mephenesin in Infantile Cerebral Palsy. C. H. Frantz. (*J. Amer. med. Ass.*, 1950, **143,** 424.) Mephenesin (3-ortho-toloxy-1 : 2-propanediol) given by mouth over a 7-month period was well tolerated by 23 out of a test group of 27 children with infantile cerebral palsy. 4 doses of mephenesin a day were given, 20 minutes before meals and 30 minutes before retiring. Children under 10 received 1 g. a day initially as 4 tablets of 250 mg., and children over 10 were started at 1·5 g. daily. From 5 to 7 days the dose was increased by adding 1 (or more) tablet per meal. Thereafter, monthly, the dosage was increased to tolerance. The average doses over a prolonged period were between 0·75 and 3 g. Vertigo, nausea, vomiting, irritability and listlessness were undesirable side effects noted in children receiving doses up to 2 g. Vertigo was in most cases easily controlled by a change in dosage. No undesirable results were shown in blood and urine studies. Of 16 athetoid children 14 showed beneficial effects. Children with tension athetosis seem to give a more consistently favourable response than other groups. Children with spasticity and rigidity did not respond satisfactorily.

S. L. W.

Morphine, Pharmacological Properties of New Derivatives of. P. Chabrier, R. Giuducelli and K. Kristensson. (*C. R. Acad. Sci., Paris,* 1950, **231,** 289.) The two new compounds described are the dibromomethylate of morpholylethylmorphine, and dihydromorpholylethylmorphine. The dibromomethylate shows a toxicity 30 times as great as morpholylethylmorphine itself. Thus toxicity of the bases, which decreases in the order codeine, morphine, morpholylethylmorphine, is reversed in the methyl bromide compounds. This fact is the more remarkable as codeine is 7·5 times more toxic than morpholylethylmorphine. With all three, bromomethylation has the effect of reducing considerably, in intensity and duration, the inhibitory respiratory action; decreasing their convulsive power; and causing curarising properties. The latter is, for rabbits, about 10 times as great with the morpholylethylmorphine compound as for morphine and codeine. G. M.

Phenosulfazole (Darvisul) in Acute Poliomyelitis. M. J. Fox and E. Z. Hornberger. (*J. Amer. med. Ass.*, 1950, **143,** 535.) 29 patients who

had bulbar and spinobulbar forms of poliomyelitis were treated with phenosulfazole, *N*-(2-thiazolyl)-phenol sulphonamide, in a dose of 400 mg./kg. of bodyweight daily; the drug was given both intravenously and orally. Among this group the mortality rate was 34·5 per cent. During the same period 19 patients did not receive the drug and showed a mortality rate of 42·1 per cent. There appeared to be no alteration in the length of temperature elevation or the length of hospitalisation in the group receiving the drug, and instances of drug toxicity were not noted. The authors conclude that phenosulfazole has little to offer in the treatment of poliomyelitis, since the mortality rate in both the treated and untreated groups fell within the expected range for a severe epidemic. S. L. W.

Pituitary Adrenocorticotrophic Hormone Therapy in Ophthalmological Conditions. D. M. Gordon and J. M. McLean. (*J. Amer. med. Ass.*, 1950, **142**, 1271.) Pituitary adrenocorticotrophic hormone was given to 6 patients with the following diseases:—severe corneal œdema (corneal dystrophy), secondary glaucoma, chronic iridocyclitis, retinitis pigmentosa and acute choroiditis. The usual dosage was 25 mg. intramuscularly 3 or 4 times daily. The duration of treatment was controlled by the short supply of the drug and the response of the patient; in no instance was treatment continued for longer than 9 days. The circulating eosinophil count offers a good index of the dosage. The response of the patients with iridocyclitis and choroiditis was dramatic, that of the patient with retinitis pigmentosa apparently temporarily beneficial. The other 2 patients failed to show any favourable response to short courses of treatment. No adverse systemic effects were noted other than temporary hypertension, glycosuria and mild abdominal distension. Every patient showed a definite fall in circulating eosinophils. S. L. W.

Pituitary Posterior Lobe Extracts, Assay of. G. A. Stewart. (*Analyst*, 1950, **75**, 542.) For oxytoxic activity, various modifications of the *in vitro* guinea-pig uterus method are in use, with different assay patterns, and different concentrations of magnesium and calcium in the Ringer's solution. The most precise isolated uterus method appears to be that using the non-pregnant rat uterus in a modified Locke's solution having one half the usual calcium and one quarter the usual glucose concentration, with a 4-point design. The depression of blood pressure in fowls anæsthetised with phenobarbitone sodium is an effect of the oxytocic hormone. This is the basis of a quick and reliable *in vivo* assay, and the vasopressor hormone does not affect the results appreciably unless the ratio vasopressin/oxytocin is greater than 10. Vasopressor activity may be estimated by recording the blood pressure in spinal cats or anæsthetised dogs, and antidiuretic activity may be assayed by delay in excretion of water in rats, or inhibition of diuresis in dogs. It should not be assumed that commercial pituitary extracts all contain the hormones in the same relative proportions. Separate tests for oxytocic, vasopressor and antidiuretic activity should be made. G. B.

Pyrogens, Action of, in Rabbits. J. Dorche and M. Castaing. (*Ann. pharm. franc.*, 1950, **8**, 353.) Methods, based on leucocytosis in rabbits, have been proposed as a test for pyrogens. The authors find that pyrogenic solutions, when injected intravenously, produce a leucopenia of short duration followed by hyperleucocytosis affecting the polynuclear leucocytes. On account of the instability of the blood of the rabbit, it does

not appear that any test of this kind would be more sensitive than that of hyperthermy. Untreated rabbits rarely show a percentage of polynuclear leucocytes greater than 50, and any higher value, if confirmed 3 or 4 hours after the injection, is generally parallel with the hyperthermy. G. M.

Pyrogens, French Official Test for. J. Dorche, G. Bouthier, M. T. Ardiet and M. Castaing. (*Ann. pharm. franc.*, 1950, **8**, 358.) In using the French official test, it is essential to take certain precautions. Some animals show an abnormal reaction, small ones tending to be hyposensitive and large ones hypersensitive. The sensitivity of the animals should be tested by a suspension of typhoid bacilli (200 million /ml.), heated at 115°C. for one hour. This suspension retains its activity for a long period. A dose of 0·5ml./kg. of body weight should produce a rise of temperature of 0·9 to 1·5°C. Alternatively a freshly prepared and sterilised dilution (1:20) of T.A.B. vaccine may be used. Any type of thermometer may be used, but it should be inserted to a depth of 75 mm. Strongly hypotonic solutions should be made isotonic. In 30 negative tests, the range was within ±0·3°C. in 84 per cent. of the cases. The official limit of 0·6°C. may therefore be considered reasonable. G. M.

Terramycin in the Treatment of Venereal Disease. F. D. Hendricks, A. B. Greaves, S. Olansky, S. R. Taggart, C. N. Lewis, G. S. Landman, G. R. MacDonald, and H. Welch. (*J. Amer. med. Ass.*, 1950, **143**, 4.) Terramycin at the proper dosage effects a satisfactory cure rate in the treatment of gonorrhœa, though the dose required for cure is somewhat higher than has been necessary with chloramphenicol. From 1 to 2 g. of terramycin hydrochloride given by mouth in divided doses gives a cure rate of 80 to 100 per cent. (based on treatment of 73 cases), while single doses of 750 mg. of chloramphenicol give similar cure rates. Clinical healing of lesions of both syphilis and granuloma inguinale occurs promptly with terramycin hydrochloride given orally in doses of 60 mg./kg. of bodyweight daily. S. L. W.

Terramycin Hydrochloride, Clinical Observations on the Use of. E. Q. King, C. N. Lewis, H. Welch, E. A. Clark, J. B. Johnson, J. B. Lyons, R. B. Scott and P. B. Cornely. (*J. Amer. med. Ass.*, 1950, **143**, 1.) Terramycin hydrochloride was administered to 30 patients with various types of infection; these included penumococcal pneumonias, urinary tract infections due to *Escherichia coli* and *Aerobacter aerogenes*, whooping cough bacteræmia due to *Salmonella*, pneumonitis and lung abcess with mixed bacterial infections. The drug was given by mouth according to the following dosage schedules. In patients of 14 years and older without urinary tract infections 750 mg. every 6 hours; with urinary tract infections 500 mg. every 6 hours; in children of 9 years and younger 500 mg. every 4 hours. Assayable amounts of terramycin were found in the blood and urine within 1 hour and for 5 hours after administration of 750 mg. of the drug by mouth. There was a good response to the treatment in the majority of cases and the results obtained in urinary tract infections were promising. There was a low incidence of side reactions most of which were mild and subsided with continued treatment; they included nausea, abdominal pain, slight headache, mild transient erythema, vomiting and diarrhœa. In an occasional instance severe gastro-intestinal distress, including diarrhœa, may necessitate withdrawal of the drug. S. L. W.