ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Veratrum Ester Alkaloids, Isolation of, by Chromatography. S. M. Kupchan and C. V. Deliwala. (*J. Amer. chem. Soc.*, 1953, **75**, 4671.) The total alkaloids obtained from *Veratrum album* were separated into ether-soluble and ether-insoluble fractions. The inactive alkamines were removed from the former fraction to leave an amorphous alkaloid fraction which was dissolved in chloroform and chromatographed on sulphuric acid-washed alumina. Neogermitrine and germitetrine B were obtained in the eluates. By the same procedure, protoveratrine A and protoveratrine B were isolated from protoveratrine.

A. H. B.

***Veratrum fimbriatum* Gray, Alkaloids of.** M. W. Klohs, M. D. Draper, F. Keller, S. Koster, W. Malesh and F. J. Petracek. (*J. Amer. chem. Soc.*, 1953, **75**, 4925.) Two new hypotensively active germine esters, germanitrine and germinitrine as well as the known veratrum alkaloids, neogermitrine, jervine and pseudojervine, were isolated from *Veratrum fimbriatum* Gray. An ester alkaloid, veratroylzygadenine, previously isolated from *Zygadenus venenosus* Wats was also obtained. The alkaloids were isolated from the total "amorphous bases" by countercurrent distribution. Alkaline hydrolysis of germanitrine yielded germine, acetic acid, *l*-α-methylbutyric acid and tiglic acid. On the basis of the analytical data and the hydrolysis products the empirical formula $C_{39}H_{59}O_{11}N$ was established. On methanolysis, germanitrine was converted to a diester, germinidine ($C_{37}H_{57}O_{10}N$), by the loss of the labile acetyl group. Alkaline hydrolysis of the triester germinitrine ($C_{39}H_{57}O_{11}N$) yielded germine, acetic acid, tiglic acid and angelic acid. The hypotensive activities expressed as μg./kg. of anæsthetised dog per minute required for a 10-minute intravenous infusion to lower the mean arterial blood pressure 30 per cent. were found to be germanitrine (0·12 μg.), germanidine (0·77 μg.), germinitrine (0·41 μg.) and veratroylzygadenine (1·1 μg.).

A. H. B.

ANALYTICAL

Alkaloids, Assay of, with Antimony Triiodide (Caille and Viel's Reagent). S. Besson and J. J. Brignon. (*Ann. pharm. franç.*, 1953, **11**, 535.) The reagent may be prepared by the following method. Dissolve 40 g. of potassium iodide in 67 ml. of water and dissolve 5 g. of antimony trichloride in 20 ml. of hydrochloric acid. Add the antimony trichloride solution drop by drop to the potassium iodide solution, stirring constantly, and add sufficient water to produce 100 ml. It is essential that no precipitate of basic antimony salt should appear at any time during the mixing of the solutions, since this spoils the reagent by setting off a progressive precipitation. The solution should be stored over powdered antimony, otherwise iodine is evolved and the reagent loses its sensitivity. Alkaloidal solutions may be assayed by precipitation with a slight excess of the reagent, filtering, washing, drying and (1) weighing the precipitate, (2) treating the precipitate with alkali, or dissolving it in tartaric

acid and adding alkali, and extracting the alkaloid with chloroform or (3) extracting the precipitate with a warm 10 per cent. solution of sodium hydroxide, neutralising and determining the quantity of iodine by titration with alkaline potassium permanganate solution. Alternatively, the quantity of antimony remaining in the reagent after removal of the alkaloidal precipitate may be determined and the amount of alkaloid calculated from the quantity of antimony in the precipitate. In experiments with this reagent, the whole of the caffeine was obtained in a pure crystalline state from an extract of cola, but in the extraction of strychnine from extract of nux vomica, it was necessary to re-precipitate the alkaloid from a solution of the first precipitate in order to obtain a pure product. G. B.

Ammonia, Determination of, with Nessler's Reagent. J. Büchi, R. Alther and M. Soliva. (*Pharm. Acta Helvet.*, 1953, **28**, 237.) A comparative study of a number of formulæ proposed for Nessler's reagent led to the following conclusions. About 15 minutes is required for the development of maximum colour, and this remains constant for 1 hour. The colour strength increases with the amount of reagent. An excessive amount of alkali increases the tendency to become turbid, and this is also the case when sodium hydroxide is replaced by potassium hydroxide. The optimum concentration of sodium hydroxide is 8·5 per cent. The concentration of potassio-mercuric iodide is not important, but excess of potassium iodide decreases the sensitivity and increases the tendency to turbidity. The formula recommended is as follows. 4·25 g. of potassium iodide is dissolved in 5 ml. of boiled water and treated with 6 g. of mercuric iodide, which dissolves after shaking. A cooled solution of 15 g. of sodium hydroxide in 75 ml. of boiled water is prepared, the two solutions are mixed and the volume is made up to 100 ml. After about 4 days the solution is filtered through a fine porcelain filter, and kept in a clean, hard glass bottle protected from light. G. M.

Aneurine in Pharmaceutical Products, Determination of. F. J. Bandelin and J. V. Tuschhoff. (*Analyt. Chem.*, 1953, **25**, 1198.) Investigation showed that aneurine could be quantitatively precipitated as the reineckate from aqueous solutions although nicotinic acid, nicotinamide and pyridoxine also produced insoluble reineckates under certain conditions. Under the conditions of the method developed, aneurine could be selectively precipitated from mixtures of other vitamins of the B complex group in a buffered solution having a *p*H of 4·5. In injectable solutions and elixirs for oral administration buffering was usually unnecessary and precipitation could be carried out after dilution with water; in multivitamin formulæ containing nicotinic acid, nicotinamide, or pyridoxine, extraction and/or dilution with acetate buffer was necessary. Certain heterocyclic amines and quaternary ammonium salts, which produce insoluble reineckates, interfered with the method. R. E. S.

Atropine, Determination of, with Reinecke Salt. H. Bräuniger and H. W. Raudonat. (*Pharm. Zentralh.*, 1953, **92**, 277.) Small quantities (1 to 10 mg.) of atropine may be determined in presence of lactose by precipitation with Reinecke salt, provided that the dilution is not too great. The alkaloid, in about 10 ml. of water, is treated with 1 ml. of dilute sulphuric acid and 2 ml. of a 2 per cent. solution of Reinecke salt. After 30 minutes standing at 0° C., the precipitate is filtered off and washed with 20 ml. of ice water. It is then dissolved in 5 ml. of acetone and the solution is used for photometric determination.

Alternatively the precipitate is dissolved in 2·5 ml. of acetone, diluted with 40 ml. of water and heated for 10 minutes with 2 ml. of alkaline sodium potassium tartrate solution. After cooling, the solution is titrated with 0·1N silver nitrate, 1 ml. being equivalent to 8·45 mg. of anhydrous atropine sulphate. G. M.

Ergot, Colorimetric Assay of. E. Thielmann, W. Land and H. Kaiser. (*Arch. Pharm. Berl.*, 1953, **268**, 379.) The proposed method is as follows: 0·5 g. of powdered (and not defatted) ergot is heated for short period with a 10 per cent. solution of tartaric acid in 50 per cent. methanol, and filtered through a plug of cotton wool, the filter being washed twice with 5 ml. of the solution. The turbid filtrate is treated with a 10 per cent. aqueous solution of zinc acetate, and the mixture is made up to 25 ml. 1 volume of this clear solution is treated with 2 volumes of reagent (0·2 g. of *p*-dimethylaminobenzaldehyde, 0·1 g. of crystalline ferric chloride, 120 g. of sulphuric acid (sp.gr. 1·836), and 35 g. of water) and after 15 minutes the colour is determined with a filter S61 (maximum absorption at 619 mμ). The alkaloidal concentration is then determined from a standard curve. It is shown that a loss of alkaloid results from defatting the drug. The ergotamine and ergobasine groups of alkaloids may be separated by paper chromatography, using a mixture of ethyl orthoformate (or ethyl formate), dilute ethanol and *iso*butanol (5:9:2). The position of the spots is determined by observation in ultra-violet light, and they are then cut out and eluted with a methanol solution or tartaric acid. G. M.

Opium Alkaloids, Separation by Surface Chromatography. M. L. Borke and E. R. Kirch. (*J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 627.) An adsorbent paste, prepared by mixing silicic acid (10 g.), heavy magnesium oxide (10 g.), plaster of paris (4 g.), phosphorescent zinc silicate (0·25 g.) and phosphorescent zinc cadmium sulphide (0·25 g.) and then triturating with 38 ml. of phosphate buffer (*p*H 6·6) is spread on glass strips (2·5 × 43 cm.) to about 1 mm. thickness and dried at 80° to 90° C. Stock solutions of the pure alkaloids are then spotted on the strips which are developed, by the ascending technique, with 1:4-dioxane in a chamber saturated with the solvent. Following development, the strips are exposed to ultra-violet light and the separate alkaloids identified by marking the areas of highest alkaloidal concentration and calculating the R_F values. R_F values found for the pure alkaloids were: morphine 0·39, codeine 0·62, papaverine hydrochloride 0·88, narcotine 0·92 and narceine and meconic acid 0·00. R_F values obtained from chromatographing mixtures of the pure alkaloids, opium extracts and a galenical corresponded well with those given above. The method detects as little as 5 to 20 μg. of papaverine hydrochloride, narcotine or meconic acid and 50 μg. of morphine, codeine or narceine. The spots of papaverine hydrochloride and narcotine are differentiated by their colours, being violet and blue respectively. J. R. F.

Phenazone, Identification Reaction of. K. C. Güven. (*Pharm. Acta Helvet.*, 1953, **28**, 252.) A solution of phenazone in dilute acid gives, with potassium ferrocyanide, a white precipitate which is coloured a greyish black on the addition of a small quantity of iodine-potassium iodide solution. This reaction is not given by amidopyrin, quinine, caffeine, theobromine, papaverine, strychnine or by derivatives of phenazone. The reaction is sensitive to 0·02 g. It appears that the cause of the coloration is similar to that of iodide of starch. G. M.

Quaternary Ammonium Bases, Colorimetric Determination of. K. R. Gottlieb. (*Dansk. Tidsskr. farm.*, 1953, **27**, 199.) A number of quaternary ammonium bases can be determined colorimetrically with bromothymol blue. Details of the method, as applied to a cetrimide ointment, are as follows. A quantity of the ointment, corresponding to about 0·2 mg. of cetrimide, is treated with 15 ml. of warm water, and the mixture is swirled round to dissolve the compound. After cooling, 5 ml. of 2N sodium carbonate solution and 5 ml. of bromothymol blue indicator solution are added. The mixture is shaken with 20 ml. of chloroform for 2 minutes, and the extraction is repeated with 2 quantities, each of 10 ml., of chloroform. The mixed chloroform solutions are made up to 50 ml., and the solution is filtered through paper, the first 25 ml. being rejected. The absorption of the remainder of the solution is then determined at 420 mμ. A blank test is done similarly with the same amount of ointment, but with 5 ml. of water in place of bromothymol blue solution. The extinction is proportional to the amount of cetrimide, being equal to 0·165 for 0·200 mg. The method may also be used for cetylpyridium chloride, and benzalkonium chloride. At a lower *p*H value the reaction is less specific and at *p*H about 8 a positive reaction is given by deca- and hexa-methonium chlorides, diphenhydramine, and other compounds. G. M.

Vegetable Oil Stability, Determination of. M. J. Golden. (*J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 545.) A simple colorimetric peroxide test is proposed for the determination of the peroxide content of oils, using a ferrous thiocyanate reagent. The oil (0·1 ml. equivalent to approximately 0·092 g.) is taken, 5 ml. of ether-mineral oil mixture (25 per cent. ether in mineral oil) is added to dissolve the oil, followed by 7 ml. of ferrous thiocyanate reagent; the air in the test tube is replaced by carbon dioxide and the mixture is shaken for one minute. The lower layer varies in colour from pink to dark red according to the amount of peroxide present, the colour being measured photoelectrically using a Klett filter No. 56. Satisfactory correlation was found between the values obtained by the peroxide test method and those of the modified Wheeler peroxide and Swift stability methods at 25° C. and 97·7° C.; the method could also be used to determine quantitatively the peroxide content of oils at 125° C.
R. E. S.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Digitoxin. P. Demoen and P. Janssen. (*J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 635.) Using a method of chromatography on silica gel, gitoxin and other glycosides may be separated from impure digitoxin. The glycosides are eluted with ethyl acetate, saturated with water and containing 0·5 per cent. of methanol. The accuracy of the method due to Stoll is increased by the use of a colorimetric method of determining the glycosidal content of each fraction of the eluate. In examining a number of samples, significant differences were observed in the value of $E_{1\text{ cm.}}^{1\text{ per cent.}}$ at the ultra-violet absorption maximum (219 to 220 mμ), measurements being made on solutions in 50 per cent. methanol. It is suggested that the variation is due to partial decomposition of samples on drying for analysis and the influence of probable impurities and of resonating structures of the chromophore. Many data in the literature of the colour reactions for digitoxoside are invalid because the effects of time, temperature and impurities were not taken into account or because an unsuitable wavelength was used for colour measurements. Published figures for the 3 : 5-dinitrobenzoic

acid and picric acid reactions are not directly comparable and there is disagreement among the figures quoted for the *m*-dinitrobenzene reaction. When commercial samples (undried) were analysed by ultra-violet absorption, and by the Baljet alkaline picrate and Keller–Kiliani methods, the standard deviation of the mean was least in the Keller–Kiliani test, although the probable errors in this method were the greatest. This suggests that the samples differed more in the nature and content of the butenolide ring structure than in digitoxose content. Absorption maxima in the Keller–Kiliani test occurred at 590, 500, 470 and 415 ± 5 mμ. In the Baljet test the maximum colour was developed after 4 to 7 minutes at 20° C., at the absorption peak of 490 mμ. It is concluded that the physical and chemical properties of pure digitoxin are still largely undetermined. G. B.

TOXICOLOGY

Strychnine, Toxicological Determination of. W. Roth, L. Arrigoni and L. Fischer. (*J. Amer. pharm. Ass. Sci. Ed.*, 1953, **42**, 308.) Strychnine may be removed from toxicological samples by digestion with pancreatin at *p*H 8, followed by dilution with water and acidification with acetic acid, which brings the alkaloid into solution and aids filtration. The filtrate is evaporated and extracted with benzene in the presence of sodium carbonate solution; the residue, after evaporation of the benzene, is reduced with zinc and hydrochloric acid and treated with sodium nitrite. The red colour is measured in a colorimeter at 530 mμ and the quantity of strychnine read from a standard curve prepared by submitting known quantities of strychnine to the process. The method was tried with artificial toxicological samples containing known amounts of strychnine ranging from 0·1 to 1·0 mg. in 10 g. of ground liver, and gave an average recovery of 98·9 per cent., compared with 84·6 per cent. for the Haines modification of the Dragendorff method. G. B.

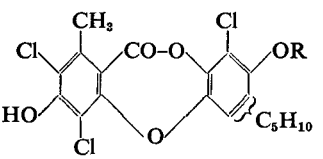
BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Aneurine, Thermal Destruction of. K. T. H. Farrer. (*Aust. J. exp. Biol. med. Sci.*, 1953, **31**, 247.) The thiochrome reaction has been used to determine aneurine in experiments to assess the comparative thermal stability of aneurine mononitrate and aneurine hydrochloride in two buffer solutions and in a yeast extract at 100° C., comparative results were also obtained for the mononitrate and natural aneurine in processed cheese and in yeast extract at normal storage temperatures. In phosphate and phosphate-citric acid buffer solutions at 100° C., the mononitrate behaved in the same general way as the hydrochloride in its sensitivity to *p*H, buffer anions and concentration of buffer salts; the mononitrate was, however, generally less stable than the hydrochloride. In yeast extract solutions at 100° C., both nitrate and hydrochloride behaved similarly and were destroyed at the same rate. In processed cheese the mononitrate was slightly more stable than naturally occurring aneurine after storage at normal temperatures, but in yeast extract no difference in stability could be detected. R. E. S.

Ketosteroids, Paper Partition Chromatography of. K. Savard. (*J. biol. Chem.*, 1953, **202**, 457.) The paper partition chromatography of C_{19} and C_{21} ketosteroids was studied using propylene glycol as the stationary phase and ligroin (and similar hydrocarbons) as the mobile phase. The range of compounds and their respective mobilities indicate that the ligroin–propylene glycol system possesses many of the features essential for good chromatography in this category of steroids. This system was also shown to be applicable to the separation of the C_{17} ketosteroids of urinary origin. By relating the mobility of the ketosteroids indirectly to the distance travelled per unit time, a numerical expression of chromatographic mobility (R_T) is proposed which may have wide use as a helpful tool in the expression of chromatographic sequence on paper of steroids for which the conventional R_F term cannot serve. The relationship of the relative mobility (R_T) to the chemical structure is in inverse proportion to the polarity of the oxygen functions of the steroid molecule. These structure/mobility generalisations include C_{21}-ketosteroids (oxygen function at C-20) move faster than the corresponding C_{19} steroids (with similar oxygen function at C-17); derivatives of the androstane (allo) series move faster than corresponding derivatives of the etiocholane (normal) series. The mobility sequence of steroids with oxygen function at a fixed position (particularly the C-3 position) is saturated ketone > α:β-unsaturated ketone > hydroxyl in the polar conformation > hydroxyl in the equatorial conformation. A. H. B.

Nidulin and "Ustin": Two Chlorine-containing Metabolic Products of *Aspergillus nidulans*. F. M. Dean, A. Robertson, J. C. Roberts and K. B. Raper. (*Nature, Lond.*, 1953, **172**, 344.) Kurung's original strain of *Aspergillus ustus* (Bain.) (*Science*, 1945, **102**, 11) was concluded to be a non-ascosporic strain of *Aspergillus nidulans*. When this mould was grown at 30° C. on a medium containing Czapek-Dox salts, 4 per cent. of glucose and 0·1 per cent. of marmite, some "ustin" was produced in the substrate and much fatty material found in the mycelium. When it was grown on the same substrate at a much lower temperature, the amount of fat was considerably reduced and, from the dried mycelium, a crystalline compound for which the name "nidulin" is proposed was isolated as the main product. Pure nidulin, $C_{20}H_{17}O_5Cl_3$, crystallised from ethanol in colourless rhombs and from light petroleum in slender, shining rods, m.pt. 180° C. It was shown to be the monomethyl ether of "ustin." The name "nornidulin" is suggested for "ustin." The following partial structures were suggested for nidulin and nornidulin:



Nidulin (R = CH_3). Nornidulin or "ustin" (R = H).

Nidulin completely inhibits the growth of *Myco. tuberculosis* for 4 weeks at a dilution of between 1 in 5,000 and 1 in 10,000; the test was carried out in presence of serum, by modified Long's medium and the floating-pellicle method. A 0·1 per cent. solution also inhibits the growth of the human parasitic fungi *Trichophyton tonsurans* and *Microsporum audouini*, but shows little or no activity towards quite a wide range of other micro-organisms. It is inactive against bacteriophage. A. H. B.

Oxytetracycline (Terramycin) and Chlortetracycline (Aureomycin), Avidity of, for Metallic Cations. A. Albert. (*Nature, Lond.*, 1953, **172**, 201.) The ionisation constants of the two antibiotics were determined experimentally; the pK_a values for oxytetracycline (0·001M) being 3·10(±0·02), 7·26 (±0·02) and 9·11 (±0·03); and for chlortetracycline (0·005M) 3·30 (±0·02), 7·44 (±0·01) and 9·27 (±0·03). Experiments showed the formation, first of a 1:1-complex, and later, as the *p*H rose during titration, of a 2:1-complex, that is, one containing two molecules of the antibiotic to one atom of metal. A table is given of the stability constants of the metallic complexes of the antibiotics; the constants for oxytetracycline and chlortetracycline were almost identical within experimental error. The constants, and the order of preference for the various metals, were much the same as in the common amino-acids with two important exceptions: (i) the position of Fe^{2+} was elevated above its usual position between Mn^{2+} and Zn^{2+}, and (ii) Fe^{3+} was the most strongly bound ion. R. E. S.

BIOCHEMICAL ANALYSIS

Adrenaline and Noradrenaline, Determination of. H. Persky and S. Roston. (*Science* 1953, **118**, 381.) Adrenaline and noradrenaline formed separate condensation products with ethylenediamine, the adrenaline derivative fluorescing with 5 times the intensity of that from noradrenaline. The fluorescence spectrum of each condensation product was determined for two exciting frequencies, namely the 365 mμ and 436 mμ lines of the mercury arc; the intensity of fluorescence of adrenaline derivative at its peak emission was 2·2 and 3·5 times greater respectively than that of the noradrenaline derivative at 365 and 436 mμ. The fluorescence emission of the adrenaline derivative on excitation with the 436 mμ line was almost twice that obtained with the 365 mμ line, whereas the noradrenaline derivative did not show such an increase; these differences were used as a basis for a quantitative differentiation of adrenaline and noradrenaline. An outline of a method using a photoelectic fluorimeter and filter combinations is given. R. E. S.

Amino-acids in Cerebrospinal Fluid, Paper Chromatography of. A. P. Prior and T. P. Whitehead. (*Nature, Lond.*, 1953, **172**, 358.) It was found that protein passes through a sulphonated polystyrene resin of the bead type unchanged. Thus, when a biological fluid is passed through a suitable cation exchange resin, proteins and anions are removed and the amino-acids retained. These amino-acids may be displaced with ammonia. The method used consisted of activating the resin (Zeo-Karb 225) with twice its volume of 10 per cent. w/v hydrochloric acid, washing free of acid and then placing in a column approximately 15 cm. by 0·5 cm. The cerebrospinal fluid (1 ml.) was then passed through the column and 15 ml. of water used for washing it through. The amino-acids were displaced with 15 ml. of N ammonia solution, the first 3 ml. of eluate being ignored. The next 12 ml. were evaporated under reduced pressure at 37° C. and the dry residue dissolved in water and used for paper chromatography. Arginine required a much greater concentration of ammonia for its removal from the column. Combining this technique with the circular filter-paper method of paper chromatography enabled results to be obtained within 4 hours of the fluid being received, and required only approximately 30 minutes of bench time. Preliminary results showed that the method was suitable for serum and urine analysis. A. H. B.

Anthrone, Determination of Blood Sugar with. S. J. Prokhovnik and J. F. Nelson. (*Aust. J. exp. Biol. Med. Sci.*, 1953, **31**, 279.) A rapid method for the determination of blood glucose is described. A sample of blood containing 20 to 300 μg. of glucose is pipetted into 5·0 ml. of distilled water and 0·2 ml. of sodium hydroxide (0·6 N) and 0·2 ml. of zinc sulphate (10 per cent.) are added and the mixture, after standing for 10 minutes, is centrifuged; 2·0 ml. of the supernatant liquid is cooled, 4 ml. of anthrone reagent (0·2 per cent. sulphuric acid) is added and the mixture is heated in a boiling water bath for 7 minutes. After rapid cooling the colour developed is measured spectrophotometrically at 620 mμ; the glucose concentration of the sample is obtained from a calibration curve prepared by using known amounts of glucose. The method is suitable for the estimation of 6 to 100 μg. of glucose; it gives a more accurate measure of blood-glucose concentration than most existing methods and is not affected by the anticoagulants normally used for blood preservation. R. E. S.

Bilirubin, Estimation of. W. G. Dangerfield and R. Finlayson. (*J. clin. Path.*, 1953, **6**, 173.) A method for the estimation of serum bilirubin is proposed in which the serum is treated with diazo reagent and a mixture of caffeine sodium benzoate and phosphate buffer; azobilirubin is formed rapidly and is measured photoelectrically in comparison with an azobilirubin standard previously prepared from bilirubin dissolved in 0·1N sodium carbonate solution. Details of the proposed method are given. A graph of the optical density of azobilirubin solutions prepared from dilutions of a 20 mg./100 ml. bilirubin standard against the concentration of bilirubin was practically linear between 2 mg. and 20 mg. bilirubin per 100 ml.; departure from linearity at 40 mg./100 ml. was slight. The addition of alkaline solutions containing 2 to 8 mg. of bilirubin per 100 ml. to both normal and jaundiced sera gave "recoveries" of 95 per cent. to 103 per cent. Estimations of serum bilirubin made by the proposed caffeine method showed no discrepancies when compared with results obtained by the technique of King and Coxon (*J. clin. Path.*, 1950, **3**, 248). R. E. S.

Calcium in Serum, A Photoelectric Micro Method, with Ethylenediamine Tetra-acetate. J. Lehmann. (*Scand. J. clin. Lab. Invest.*, 1953, **5**, 203.) The The direct titration of calcium in serum with ethylenediamine, does not give a sharp visual end-point. Two procedures are described for the accurate spectrophotometric registration of the end-point. The first is based on the difference in the maximum light absorption at different wavelengths, 505 to 510 mμ for a calcium purpurate complex and 550 mμ for the purpurate in the absence of calcium. The light from the titration vessel falls on two photocells with a colour filter selecting the range 505 mμ and another 550 mμ. A deflection of the galvanometer is seen as long as a colour change at 505 mμ occurs. When the end-point is reached the needle is steady. The standard error of an estimation was $\pm$ 0·04 to 0·05 mg. per cent. calcium, and the values were 0·8 mg. per cent. lower than the Kramer and Tisdall method. A second method of registration of the end-point was to titrate the solutions in a photoelectric absorptiometer at a wavelength of 505 to 510 mμ and to record the extinction values on millimetre paper. The end-point was interpolated between the two slopes of the curve obtained just before and after the end-point. G. F. S.

Calcium in Serum, Estimation of. L. R. Davis and M. J. H. Smith. (*J. clin. Path.*, 1953, **6**, 198.) The direct volumetric assay of calcium in serum using ethylenediamine tetra-acetic acid and ammonium purpurate as indicator was investigated. Serum (1 or 2 ml.) was diluted with 50 ml. of calcium-free

water, 0·8 ml. of 9N sodium hydroxide solution and 5 drops of a saturated solution of ammonium purpurate were added, and the mixture was titrated with standard ethylenediamine tetra-acetic acid (disodium salt) solution; the end-point was more difficult to judge than in aqueous solutions. Serum samples were therefore titrated to the same purple end-point as control mixtures containing the same amounts of sera, which had been deliberately over-titrated to a definite purple. Advantages claimed for the method are speed and simplicity of manipulation, although the difficult end-point obtained with serum was a serious limitation. Tables of results show a proportion of inaccurate results and it is considered that the method is unsuitable. R. E. S.

Cobalt in Biological Materials, Detection and Determination of. M. Mokranjac and B. Medakovič. (*Acta pharm. Jugoslav.*, 1953, **3**, 5.) It was observed that the colour produced in the determination of cobalt with nitroso-R salt is destroyed by direct sunlight in 4 to 5 hours and in ordinary room light the intensity fades by about 30 per cent. in 10 to 11 hours. Cobalt was extracted from very dilute solutions by the so-called zincate method. Iron was separated by precipitation with ammonia, and milk of lime added to precipitate cobalt, zinc, copper and nickel. The precipitate was dissolved in dilute hydrochloric acid, and the copper removed with hydrogen sulphide. The residue after evaporating the solution to dryness was treated with 2 ml. of nitric acid, evaporated to dryness and dissolved, with the aid of heat, in a mixture of 9 ml. of water, 0·5 ml. of hydrochloric acid (50 per cent.) and 0·5 ml. of nitric acid (50 per cent.), filtering if necessary. 2 ml. of nitroso-R salt solution (0·1 per cent.) and 2 g. of sodium acetate were added and the solution boiled for exactly 45 seconds. 1·5 ml. of hydrochloric acid was added, the solution again boiled for 45 seconds, cooled, and the colour intensity measured. This process extracted the cobalt quantitatively, in contrast to the dithizone method, which is only applicable to small quantities (thousandths of a mg.) of cobalt and which is difficult to carry out in the presence of zinc, nickel and copper. G. B.

Gastric Analysis, Tubeless. J. Harkness and J. A. Durant. (*J. clin. Path.*, 1953, **6**, 178.) The technique depends upon the displacement of the quininium cation from a quininium-resin indicator compound by the hydrogen cation of the free hydrochloric acid of the gastric juice at *p*H 3 or lower; the quinine hydrochloride formed is rapidly absorbed from the small intestine and quinine is excreted in the urine where it appears within 15 minutes of the ingestion of the resin. The quinine excreted is estimated quantitatively in the urine by a method based on its fluorescence in ultra-violet light. Details are given of the preparation of the quininium resin indicator and of the fluorimetric estimation of the quinine in the urine. Various sources of errors which may arise are considered together with the precautions necessary to exclude them. The test should be of value for achlorhydria in patients with anæmia and suspected gastric carcinoma. R. E. S.

17-Ketosteroid Mixtures, Quantitative Analysis of. B. L. Rubin, R. I. Dorfman and G. Pincus. (*J. biol. Chem.*, 1953, **203**, 629.) A quantitative method is described for the analysis of 17-ketosteroid mixtures on a semimicro scale. It is based on paper chromatographic methods using two different solvent systems, heptane-phenyl Cellosolve and heptane-propylene glycol and it has been designed chiefly for application to the analysis of the principal 17-ketosteroids in urine. Strips of Whatman No. 1 filter paper, of a defined size,

previously extracted with 1 : 1 methanol-benzene, are used. The papers are first impregnated with a 1 : 1 mixture of either propylene glycol and methanol or phenyl cellosolve and methanol. Micro quantities of the test determinations are applied to a defined position and the solution dried on the paper. Descending chromatograms are run, with, as mobile phase, heptane previously equilibrated with phenyl cellosolve or propylene glycol. The chromatograms are run in a tank at 23° ± 2° for 12 hours using the cellosolve solvents, or 24 or 72 hours using the heptane-propylene glycol. The chromatographic behaviour and resolving of a number of 17-ketosteroids is described. A determination of the $\Delta^{9(11)}$-unsaturated 17-ketosteroids can be accomplished by oxidizing the androsterone and etiocholane-3α-ol-17-one zones with perbenzoic acid.

G. F. S.

Lactic Acid, Determination of. R. P. Hullin and R. L. Noble. (*Biochem. J.*, 1953, **55**, 289.) Results are given of investigations into the procedure of Barker and Summerson (*J. biol. Chem.*, 1941, **138**, 535). The conditions under which the oxidation of lactic acid to acetaldehyde were carried out in the first stage of the method were of critical importance; when the oxidation was performed in open tubes, the result varied greatly with the time of heating employed, contrary to the findings of Barker and Summerson. If interference due to pyruvic acid was encountered, the recommended method involved a dilution making it applicable to 2 to 10 μg. of lactic acid. With the changes given, 1 to 8 μg. of lactic acid could be estimated with an accuracy of ±2 per cent.

R. E. S.

Nitrates in Biological Fluids, Determination of. S. J. Mellette, W. A. Brodsky and L. Palmer. (*J. Lab. clin. Med.*, 1953, **41**, 963.) A method is given for the rapid spectrophotometric determination of nitrates in urine and plasma. 5 ml. of a brucine solution in sulphuric acid (0·1 per cent.) is added to 2 ml. of sample, the mixed solutions are allowed to stand for 5 minutes and 5 ml. of water is added. After cooling for 10 minutes the colour intensity is read at 440 mμ. Details are also given of the determination in the presence of nitrites which are removed with sulphamic acid. Results indicate that nitrates can be detected in amounts of 5 to 30 μg., and in amounts of 10 to 60 μg. in the presence of nitrites. Close correlation between optical density and concentration of nitrate was obtained, the analytical error being of the order of 2 to 4 per cent. between duplicate or triplicate determinations. The nature of the colour complex formed by brucine in the presence of nitrates is discussed. R. E. S.

Pentobarbitone, Fate of, in Man and Dog, Estimation in Biological Material. B. B. Brodie, J. J. Burns, L. C. Mark, P. A. Lief, E. Bernstein and E. M. Papper. (*J. Pharmacol.*, 1953, **109**, 26.) A method is described for the estimation of pentobarbitone and other oxy-barbiturates in biological fluids and tissues. The pentobarbitone is extracted from the acidified biological material including plasma, urine and tissues, with petroleum ether containing a small amount of *iso*amyl alcohol, the aqueous phase being saturated with sodium chloride. The pentobarbitone is then transferred to a *p*H 11 buffer and the optical density at 240 mμ determined in an ultra-violet spectrophotometer. Comparisons are made with a standard solution diluted with *p*H 11 buffer. Studies of the metabolic fate of pentobarbitone in two human subjects showed it to be almost entirely metabolised in man. Less than 1 per cent. was excreted unchanged in the urine, but a metabolite, identified as *d*-5-ethyl-5-(3-hydroxy-methylbutyl)

barbituric acid was present in an amount equivalent to 15 per cent. of the administered pentobarbitone. After intravenous administration the plasma level declined rapidly for the first two to three hours, the drug leaving the plasma and being distributed throughout the body. This was followed by a slower rate of fall, 4 per cent. per hour, representing biotransformation of the drug. Unlike thiopentone, pentobarbitone was not extensively localised in body fat and this largely explains why pentobarbitone is not as short acting as thiopentone. In man pentobarbitone was metabolised more slowly than thiopentone, while the reverse was true for the dog. G. F. S.

Picric Acid and Mepacrine in Urine, Chromatographic Test for. U. Gallo. (*Boll. chim.-farm.*, 1953, **92**, 287.) Picric acid and mepacrine give a yellow colour to the skin which simulates jaundice and these drugs have been taken by malingerers. Chemical separation from the urine is difficult and time-consuming, but they can readily be detected by paper chromatography. The author used Whatman No. 1 paper and as eluant butanol saturated with water, and 2 parts per thousand of mepacrine and 5 parts per thousand of picric acid in both normal and jaundiced urine. After 12 hours the front of the solvent had traversed 27 to 30 cm. The strips of paper were dried in a current of hot air and examined in ultra-violet light. Mepacrine gave a bright yellow fluorescence, R_F at 25° C. = 0·66 in water, 0·70 in normal urine and 0·73 in jaundiced urine. Picric acid gave a less bright but characteristic brick-red fluorescence R_F at 25° C. in water 0·49, in normal urine 0·57 and in jaundiced urine 0·60. Biliary pigments in jaundiced urine remained fixed, R_F at 25° C. = 0, and gave only a weak white fluorescence. H. D.

Sodium and Potassium in Biological Fluids, Determination of. E. R. Holiday and J. R. K. Preedy. (*Biochem. J.*, 1953, **55**, 214.) A direct-reading flame photometer is described for the estimation of sodium and potassium in urine and serum. The efficiency of various filter combinations for the sodium emission at 589 mμ and the potassium at 766 mμ was assessed. The dilution and estimation of samples containing 2 to 5 μg. of sodium or potassium per ml. had a mean standard deviation of 0·03 μg./ml. The estimation of sodium and potassium in urine and serum with standard solutions of sodium or potassium chlorides by direct comparison was assessed by addition experiments and by comparison with chemical methods and serial dilutions. Urinary sodium and potassium estimations were found to be free from interferences at sample dilutions between 1:300 and 1:1000; at higher concentrations values for both sodium and potassium were depressed. Serum sodium estimations were free from interference, but serum potassium estimations (dilution 1:100) were subject to a systematic error of +13 per cent. of the chemical estimation. Estimations accurate to ±1·5 per cent. could be made by applying an arithmetical correction; details of the results are given. R. E. S.

CHEMOTHERAPY

Alkyl Benziminazole, Inhibition of Influenza Virus by. I. Tamm, K. Folkers, C. H. Shunk, D. Heyl and F. L. Horsfall. (*J. exp. Med.*, 1953, **98**, 245.) The known inhibitory effect of 2:5-dimethylbenziminazole on the multiplication of influenza viruses suggested a systematic investigation of other alkyl dimethylbenziminazoles. The amount of virus of Lee strain present after 36 hours incubation in infected allantoic fluid was determined by hæmagglutination titration on geometric means of 6 cultures. The nature and position of the

substituent groups affected the activity. No increase was shown by compounds with a single methyl group at position 1 or 2 of the iminazole ring but substitution of 4 methyl groups in the benzene ring gave a compound with much more activity than unsubstituted benziminazole. Substitution by ethyl instead of methyl at positions 5 and 6 of the benzene ring also gave enhanced activity, and so did lengthening of the alkyl side chain from methyl to ethyl at position 2 of the iminazole ring. Further lengthening of the alkyl side chain had no effect. Attempts to block the inhibitory effect of benziminazole and 2:5-dimethylbenziminazole by means of purines or cyanocobalamin were unsuccessful. The activity of benziminazole and its mono- and di-methyl derivatives against Lee virus appeared usually to be correlated with their activity against *Lactobacillus lactis* Dorner, but the 2:5-dimethyl compound was inactive against the latter, and the activity of 2-alkyl substituted 5:6-dimethyl derivatives against *L. lactis* varied with the lenth of the alkyl side chain. The five most active derivatives were 2-propyl-5-methyl- 2:4:5:6:7-pentamethyl-, 2-ethyl-5-methyl-, 2-butyl-5-methyl-, and 2-*iso*propyl-5-methyl-. These gave 75 per cent. inhibition of multiplication of the virus at concentrations of about 0·0002M. H. T. B.

p-Aminosalicylic Acid, Antitubercular Compounds Related to. W. O. Godtfredsen, E. J. Nielsen, R. Reiter, E. Schønfeldt and I. Steensgaard. (*Acta chem. scand.*, 1953, **7**, 781.) A number of derivatives of the phenyl esters of *p*-aminosalicylic acid and *p*-aminothiolsalicylic acid were prepared, together with *p*-aminosalicylic acid esters of various alcohols. Tables showing the chemical properties of a number of compounds of 4-aminosalicylic acid and 4-aminothiolsalicylic acid with various phenols are given. The esters were prepared by reduction of the corresponding nitro compounds; the nitrocompounds were prepared by fusing together 4-nitrosalicylic acid and the phenol or thiophenol concerned in the presence of phosphorus oxychloride or by allowing 4-nitrosalicyl chloride to react with the phenol or thiophenol in boiling toluene. The nitro compounds were reduced to the corresponding amino compounds either catalytically by means of Adam's catalyst or by means of stannous chloride. R. E. S.

Ethylenephosphoramide, Chemotherapy of Tumours in Rats with. M. L. Crossley, J. B. Allison, R. P. Parker, E. Kuh and D. R. Seeger. (*Proc. Soc. exp. Biol. N.Y.*, 1953, **83**, 438.) A number of ethylenimines, *NN'N''*-triethylenephosphoramide, *NN*-diethyl-*N'N''*-diethylenephosphoramide, *N*-pentamethylene-*N'N''*-diethylenephosphoramide, *N*-(3-oxapentamethylene)-*N'N''*-diethylenephosphoramide, *N*-tetramethylene-*N'N''*-diethylenephosphoramide, *NN'N''*-tris-(tetramethylene)phosphoramide, *NN'N''*-triethylenethiophosphoramide and *N*-(3-oxapentamethylene)-*NN''*-diethylenethiophosphoramide have been investigated for therapeutic effectiveness in the treatment of transplantable tumours in rats. Solutions were given daily by the intraperitoneal route, some subcutaneously, over periods of 2 to 9 weeks to young male rats. Rats in which tumours had regressed were kept to see if the cure was permanent and if they were resistant to further tumour implants. All the compounds except *NN'N''*-tris(tetramethylene)phosphoramide, were effective against implanted Sarcoma 231 and Flexner-Jobling carcinoma. The animals were cured and were normal except that their testes were reduced in size. Large doses caused testicular damage. Sarcomas 3 and 7 were not affected. The results and chemical relationships are discussed. There is no convincing evidence for oncolytic drug specificity. G. F. S.

Hydrazides, Non-pyridinoid Heterocyclic, as Antitubercular Substances. R. I. Meltzer, A. D. Lewis, F. H. McMillan, J. D. Genzer, F. Leonard and J. A. King. (*J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 594.) The hydrazides of the following heterocyclic carboxylic acids were prepared:—2-phenylcinchoninic, 2-pyrrolidyl carboxylic, indole-2-carboxylic, iminazole-2-carboxylic, 1:2:3-triazole-4-carboxylic, 3-methylisoxazole-5-carboxylic, 2-thienoic, 2-thienylacetic and its 5-chloro derivative and 2-furoic and its benzo, tetrahydro, 5-nitro, 5-chloro, 5-hydroxy and 4-*iso*propyl derivatives. A number of *N'*-substituted derivatives of furoic hydrazide were also prepared. Methods of preparation and melting points of the hydrazides are given. All these compounds were tested *in vitro* against *Mycobacterium tuberculosis* H37Rv and D4M3 and in mice infected with virulent bovine D4M3 strain. Many of the compounds were inactive *in vivo*, but some showed activity of the same order as isoniazid. G. B.

PHARMACY

NOTES AND FORMULÆ

Aurothioglycanide (Lauron). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1953, **152**, 1338.) Aurothioglycanide is α-auromercaptoacetanilide, $C_6H_5{\cdot}NH{\cdot}CO{\cdot}CH_2{\cdot}SAu$, and occurs as a greyish-yellow powder, m.pt. 238° to 241° C., insoluble in water, acids, bases, benzene, ether and chloroform. It yields a deep red colour when treated with a 4 per cent. solution of selenium oxychloride in sulphuric acid and loses not more than 0·2 per cent. of its weight when dried at 105° C. for 4 hours. On ignition, it yields 53·3 to 55·0 per cent. of gold, equivalent to 98·0 to 101·0 per cent. of aurothioglycanide. It is used as a suspension in oil in the treatment of rheumatoid arthritis by injection into the gluteal muscle. G. R. K.

Chloroprocaine Penicillin O (Depo-Cer-O-Cillin Chloroprocaine). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1953, **152**, 1339.) Chloroprocaine penicillin O is a crystalline, water-insoluble salt of 2-chloroprocaine and penicillin O(allylmercaptomethylpenicillin). When injected intramuscularly as an aqueous suspension, it produces penicillin blood levels approximately equal to those produced by similar injections of procaine benzylpenicillin, but which persist for about 24 hours in contrast to the 12- to 18-hour levels obtained with procaine benzylpenicillin. The majority of patients sensitive to salts of benzylpenicillin will tolerate chloroprocaine penicillin O without allergic reactions, although some patients may be sensitive to both. Aqueous suspensions of chloroprocaine penicillin O may be kept at room temperature for 3 weeks without significant loss of potency and without caking. If stored in a refrigerator, they should be carefully warmed and well-shaken before injection. G. R. K.

PHARMACOLOGY AND THERAPEUTICS

***l*-Adrenaline and *l*-Noradrenaline, Similarity of Acute Hæmodynamic Response to.** M. E. Zanetti and D. F. Opdyke. (*J. Pharmacol.*, 1953, **109**, 107.) Some divergence of results recording the cardiovascular actions of *l*-adrenaline and *l*-noradrenaline exists in the literature. Some investigators show little difference between the two, others have found striking differences, especially on the heart rate and output. The authors consider that the methods of observation and recording may have been responsible for this variance of opinion. In some cases the data have been obtained intermittently during continuous infusion of the drugs, in others the recording methods have not been suitable for the elucidation of the integrated action of one of the drugs.

To overcome these shortcomings the acute effects of equivalent doses of the two drugs were observed continuously over a period of 60 seconds after the end of injection. Aortic and atrial pressure pulses were recorded with optical manometers with frequencies of *ca.* 100 and 50 cycles per second respectively and carotid and femoral arterial blood flow with optically recording rotameters. The experiments were conducted on healthy mongrels anæsthetised with morphine and intravenous barbitone sodium. Intravenous injection of 25 μg. of the amine irrespective of the weight of the dog was made through the femoral vein into the inferior vena cava. No significant differences were observed, with equivalent doses of the two amines, between the acute changes in the length of the cardiac cycle, aortic systolic and disastolic pressures and the stroke index (calculated by the Hamilton-Remington method when diastolic pressure ceased to fluctuate from cycle to cycle). Similarly, carotid and femoral arterial blood flows showed no detectable differences between *l*-adrenaline and *l*-noradrenaline. The extent of the changes in these last two factors was observed to depend largely on the initial vasomotor tone of the vascular bed prior to the administration of the drug. With both amines the change in femoral blood flow indicated an initial reflex vasodilatation in the femoral bed, which later gave way to a flow decrease below that of the control before injection.

G. P.

Arfonad: Controlled Hypotension during Anæsthesia. I. W. Magill, C. F. Scurr and J. B. Wyman. (*Lancet*, 1953, **264**, 219.) Arfonad (*d*-3:4-(1′:3′-dibenzyl-2′-ketoimidazolido)-1:2-trimethylene thiophanium *d*-camphor sulphonate) was used to produce controlled hypotension during anæsthesia in 5 people undergoing: stellectomy, radical mastectomy, laminectomy, thyroidectomy, and mastoidectomy. The drug was given by intravenous drip containing 1 mg. of arfonad per ml., given at the rate of approximately 3 mg./minute. The blood pressure level was readily controllable by varying the rate of drip. After the drip was stopped recovery was rapid and the blood pressure remained stable during several hours post-operative observation. Very satisfactory results were obtained and no undesirable side effects or complications were observed.

S. L. W.

Arfonad: Controlled Hypotension in Neurosurgery. S. Anderson and W. McKissock. (*Lancet*, 1953, **265**, 754.) Arfonad is a vasodepressor with ganglion-blocking and direct vasodilator activity. The results are reported of the trial of arfonad in 52 major craniotomies. For operations on the cerebral blood vessels hypotension is essential. It is superior to the methonium compounds; being short-acting it gives the anæsthetist greater control of the blood pressure, but still reduces the hæmorrhage and intracranial tension to the satisfaction of the surgeon. The dangers of hypotension still exist, however, and arfonad should be used only in selected cases as a calculated risk. It is given by intravenous drip, at a concentration of 1 mg./ml. The amount employed and the rate of flow is adjusted in accordance with the blood pressure estimations. All to whom the drug was administered responded by a fall in blood pressure, and the hypotensive state was easily maintained. Blood pressure was restored usually after a lapse of from 1 to 20 minutes after stopping the infusion.

S. L. W.

Bacterial Pyrogens, Tolerance to, in the Rabbit. D. M. Tennent and W. H. Ott. (*J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 614.) 3 preparations of a pyrogen from *Salmonella newport* were injected into 30 groups of rabbits. Tolerance to the pyrogen developed in all rabbits which had received

sufficient pyrogen to cause a rise of body temperature of 0·22° C. or more. After a single dose of pyrogen, rabbits became progressively more tolerant during the following week, and additional doses had little effect on the rate of development of tolerance. Animals recovered in an average of 3 weeks from the date of the last dose of pyrogen, but individual recovery times showed very considerable variation. Rabbits showing tolerance are unsuitable for use in tests, since failure to detect pyrogenic samples may result. To avoid the necessity of resting all rabbits 3 to 4 weeks between tests, it is suggested that each rabbit should have the usual 3 to 4 day rest period and one extra day for each 0·1° C. temperature rise in the previous test. G. B.

Carbomycin, Clinical Trial of. M. Finland, E. M. Purcell, S. S. Wright and B. D. Love, Jr. (*New Engl. J. Med.*, 1953, **249**, 310.) Carbomycin is an antibiotic derived from *Streptomyces halstedii*. Active against gram-positive cocci, it also affects the gonococcus, some saprophytic mycobacteria, the rickettsiæ and large viruses; it is inactive against fungi. Preliminary studies had shown that blood levels after oral doses of carbomycin approaching the maximum that could be tolerated were irregular, and that it was less active than erythromycin. The antibiotic was not therefore tried on any patients who were critically ill; 43 of them had pneumonia, 2 had staphylococcal infections, 2 had streptococcal infections and 1 had *Escherichia coli* septicæmia and pyelonephritis. In 2 the drug was given by intramuscular injection, 1 receiving a single dose of 400 mg., the other receiving an initial dose of 400 mg. followed by 5 doses each of 200 mg. every 3 hours. The remainder had the drug by mouth, an initial dose of 500 mg. being followed by doses of 250 mg. or 500 mg. every 3 or 4 hours. Defervescence occurred within 48 hours in 21 patients but fever persisted for 4 days or longer in 13. Pulmonary consolidation or infiltration cleared within a week in 15 patients but persisted after 2 weeks in 18 and after 3 weeks in 4. White cell counts returned to normal within a week in 20 but were last found elevated after 4 or more days' treatment in 20 and after 8 or more days' treatment in 8. Pneumococci isolated from 20 patients were inhibited by concentrations of 0·1 to 0·8 μg./ml. In 1 patient after 4 days' treatment the minimum inhibiting concentration was 4 times as great as that before treatment was started. The highest serum concentration found, namely 0·5 μg./ml., occurred in one-third of the patients; levels of 0·25 to 0·12 μg./ml. were found in 22 per cent. In 44 per cent. the drug could not be detected (below 0·12 μg./ml.). The only untoward effects noted were gastro-intestinal symptoms in 11/43 patients receiving the drug by mouth. The compound produced no favourable response in the patients with staphylococcal infections. In one person treated intramuscularly the temperature rose to 104° F.; the second had a convulsion after the 6th dose. Carbomycin is not highly effective in the fevers treated; it is distinctly inferior to other antimicrobial agents now available and the authors consider that it is specifically contra-indicated in the treatment of pneumococcal infections. H. T. B.

Cholinesterase, Role at Myoneural Junction. J. M. Barnes and J. I. Duff. (*Brit. J. Pharmacol.*, 1953, **8**, 334.) After the addition of diethyl *p*-nitrophenyl phosphate (paraoxon) to the isolated phrenic nerve diaphragm preparation of the rat there are fasciculations and an initial increase in the size of the contractions to indirect stimuli, which then rapidly diminish to their former size. With tetanic stimuli the normal diaphragm responds by a sustained contraction, but this is lost when the substance is added, while its sensitivity to acetylcholine, which reduces the contractions, is increased. The addition of a high concentration of acetylcholine protects the preparation from the free action of the

compound. When the chemical is removed by washing, the response to tetanus gradually returns after 30 to 40 minutes and the enhanced sensitivity to acetylcholine is lost. Further addition of more chemical to such a preparation did not produce an increase in contractions or fasciculations, but again response to tetanus was lost and the sensitivity to acetylcholine reappeared. The changes in response of the diaphragm to this chemical are attributed to progressive inhibition of cholinesterase, the speed of which depends on the concentration of the inhibitor. Homogenates of diaphragms which had been exposed to the chemical substance showed inhibitions of cholinesterase and pseudocholinesterase of the same order as in rat red cells. The reaction to tetraethyl pyrophosphate (TEPP) was similar. With di*iso*propyl phosphorofluoridate and di-*iso*propyl *p*-nitrophenyl phosphate the initial response was the same after removal of the inhibitor but reversal did not appear in 4 hours. With the dimethyl derivative reversal occurred rapidly and subsequent doses caused a period of enhanced contraction. Diaphragms removed from rats poisoned by diethyl *p*-nitrophenyl phosphate, while responding to single stimuli, were unable to hold a tetanus. The significance of these findings is discussed. G. F. S.

Coronary Vasodilator Drugs, Clinical Comparison of. H. I. Russek, K. F. Urbach, A. A. Doerner and B. L. Zohman. (*J. Amer. med. Ass.*, 1953, **153**, 207.) The effectiveness of coronary vasodilator drugs was compared by recording their effect on the electrocardiographic response to a standard exercise given by selected patients with coronary artery disease who had been found over a period to give a relatively constant response to a given amount of exercise. 50 were men aged from 32 to 77 and 2 were women aged 49 and 52. The drugs tested were glyceryl trinitrate, papaverine hydrochloride, ethanol (as whisky), aminophylline, β-pyridylcarbinol tartrate (ronicol), tolazoline hydrochloride, tetraethyl-ammonium chloride, octyl nitrite, dioxyline phosphate, khellin, heparin, dicoumarol, pentaerythritol tetranitrate and morphine. Only 3 of the drugs were found worthy of continued use as vasodilators in angina pectoris and other states of coronary insufficiency, namely, glyceryl trinitrate, papaverine and pentaerythritol tetranitrate. Glyceryl trinitrate is the drug of choice for treatment of the acute attack and for prophylaxis just before contemplated exertion. For prolonged protection 10 to 20 mg. of pentaerythritol tetranitrate 3 times a day is best; it is effective in a higher proportion of patients than papaverine, its action is more prolonged and it is less expensive. Ethanol had no electrocardiographic effect but it prevented anginal pain or reduced its severity. Morphine acted similarly.

H. T. B.

Dextran: Antigenic Properties in Man. P. H. Maurer. (*Proc. Soc. exp. Biol., N.Y.*, 1953, **83**, 879.) Trials in 55 healthy volunteers have confirmed that dextrans are antigenic in man. 5 samples of native dextran were used (3 from *Streptococcus dextranicum* and 2 from strains of *Leuconostoc mesenteroides*) and 4 samples of clinical dextran. Within 3 weeks after injection of 1 mg. of dextran, precipitins and cutaneous wheal and erythema sensitivity developed. The possibility that individuals showing precipitins and skin sensitivity to dextran may have produced antibodies to certain types of pneumococcal polysaccharides which cross react with dextrans in other species (rabbit and horse) was ruled out, and it would appear that the allergic reactions previously reported are due to the antibodies to dextran. Data are presented showing that significant amounts of antibodies to the various dextrans may persist for as long as one year after immunisation. S. L. W.

Nalorphine as an Antagonist to the Intestinal Spasm Produced by the Addicting Analgesics. C. M. Gruber, Jr. and C. M. Gruber. (*J. Pharmacol.*, 1953, **109**, 157.) Hart and McCawley (*J. Pharmacol.*, 1944, **82**, 339) found that nalorphine (*N*-allylnormorphine) antagonised the intestinal spasm caused by morphine on the Thiry-Vella loop of the dog. This antagonism has been confirmed for morphine and extended to racemorphan (dromoran), hydromorphone (dilaudid), metopon, pethidine, methadone and alphaprodine (nisentil) on modified Mann loops of trained unanæsthetised dogs. When nalorphine was given before the analgesic the usual increase in intestinal tone did not develop and if given after the analgesic a prompt antagonism of the increase in tone caused by the analgesic occurred. In some of the experiments an increase in the rhythmical contractions of the gut was seen after administration of the nalorphine. G. P.

Neuromuscular Blocking Agents, Actions of, on "Red" and "White" Muscle in the Dog. M. I. Gluckman. (*Arch. int. Pharmacodyn.*, 1953, **94**, 320.) A comparison has been made of the actions of quinine hydrochloride, magnesium chloride, tubocurarine chloride and decamethonium bromide on the anterior tibial and gastrocnemius muscles of the dog, stimulated indirectly through the sciatic nerve or by direct stimulation of the previously denervated muscle. The drugs were injected intra-arterially. Quinine increased the maximal twitch of both muscles stimulated indirectly and the twitch of the denervated anterior tibialis. Magnesium chloride depressed the twitch and the effect was less in the denervated muscle. With tubocurarine and decamethonium the sensitivity of the animals varied greatly. Both antagonism to and potentiation of partial curarisation by decamethonium was demonstrated in both muscles, but antagonism to curare depression by decamethonium was easier in the anterior tibialis, the dose of decamethonium being an important factor. It was never possible to demonstrate an antagonism by curare to partial decamethonium paralysis; curare after decamethonium caused a deepening of the block in both muscles. In a few experiments tubocurarine caused a contracture of denervated muscle and this also occurred with decamethonium, which could be prevented by a previous dose of curare. Decamethonium did not reverse the curare-like action of magnesium at the neuromuscular junction, nor was the magnesium depression reduced by a previous dose of decamethonium. Magnesium also did not block decamethonium contracture in the denervated muscle. Quinine which normally potentiated the twitch caused a further depression if administered during partial decamethonium paralysis. In the denervated muscle, quinine prevented the contracture produced by decamethonium. Throughout, the anterior tibialis showed the greater susceptibility to change. Individual fibre response may explain why compounds which act by depolarisation and by competition can produce the same type of inhibition and it is considered that while quinine and curare have similar actions the activity of magnesium involves other actions. G. F. S.

Penethamate, Fatal Anaphylactic Shock Due to. F. J. Pick and J. F. Patterson. (*Brit. med. J.*, 1953, **2**, 605.) A woman aged 31 had suffered from bronchial asthma for 2½ years. Frequently there was an association between respiratory infections and asthmatic attacks. About 11 months after treatment with penethamate and adrenaline hydrochloride she developed another respiratory infection accompanied by asthmatic attacks. The asthma was immediately relieved by the injection of 0·5 ml. of adrenaline solution (1 in 1000) and this was followed by 500,000 I.U. of penethamate intramuscularly. A

slight headache and feeling of faintness passed off in a few minutes. The next day 0·85 ml. of hyperduric adrenaline solution and 500,000 I.U. of penethamate were given. After 5 minutes there was transient precordial pain, passing off in a few seconds. 10 minutes after the injection of penethamate she suddenly collapsed and became unconscious. Respirations became gasping and infrequent, and she died within a few minutes. The findings on post mortem examination were consistent with anaphylactic shock. It is suggested that the use of a slow-acting adrenaline solution allowed the fatal reaction to take place whereas the administration of the ordinary adrenaline solution with the penethamate the previous day prevented a severe reaction. H. T. B.

Phenyltoloxamine (Bristamin), Antihistaminic Properties of. J. B. Hoekstra, D. E. Tisch, N. Rakieten and H. L. Dickison. (*J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 587.) Phenyltoloxamine, *NN*-dimethyl-2-(α-phenyl-*o*-toloxy)ethylamine was tested *in vitro* and found to be 100 times more powerful than papaverine in preventing histamine-induced spasm of the rabbit ileum or barium chloride-induced spasm of the guinea-pig ileum. In similar experiments, diphenhydramine and tripelennamine were 20 and 10 times more powerful than papaverine. For the protection of guinea-pigs against an aerosol of histamine phosphate, 0·19 mg./kg. of phenyltoloxamine was required, compared with 0·06 mg./kg. for tripelennamine and 0·22 mg./kg. for diphenhydramine. Phenyltoloxamine was at least as effective as tripelennamine in blocking the vasopressor response to histamine in dogs, and some blocking action against acetylcholine and arecoline was observed especially at high dosage levels. Instillation of a 0·1 per cent. solution induced local anæsthesia of the rabbit cornea, phenyltoloxamine being approximately as potent a local anæsthetic as procaine. The effects of the drug on respiration, uterus *in situ*, salivary glands and heart were investigated in dogs and effects on the eye and irritation of the conjunctival sac, in rabbits. No toxic effects were observed at therapeutic dosage levels, and acute toxicity tests showed that phenyltoloxamine is considerably less toxic than tripelennamine or diphenhydramine. In chronic toxicity tests, dogs tolerated 40 mg./kg./day without suffering untoward effects.

G. B.

Streptomycin Resistance of Tubercle Bacilli in Experimental Animals. E. Wolinsky and W. Steenken, Jr. (*J. Bact.*, 1953, **66**, 229.) The frequency of the development of resistance to streptomycin in tubercle bacilli *in vivo* was studied in guinea-pigs, mice and rabbits, the animals being treated for prolonged periods with streptomycin alone or with streptomycin and aminosalicyclic acid after being infected with a variety of virulent human and bovine strains of *Mycobacterium tuberculosis*. In guinea-pigs, after daily injections for 90 days, 41 out of 43 strains had retained their original sensitivity while the other 2 showed only slight resistance. In animals with chronic lesions, which would be expected to contain multiplying bacteria for a long period, all of 5 strains were fully sensitive after 300 days. The effect of simultaneously administering aminosalicylic acid was investigated in mice. Previous reports that drug resistance occurs in mice treated with streptomycin were not confirmed; a few of the strains examined showed a significant increase in resistance and the authors suggest that the difference may be connected with the size of the infecting inocula. Administration of aminosalicylic acid with the streptomycin had very little, if any, effect. Similar results were obtained in rabbits with chronic caseous and cavitary pulmonary lesions more resembling those in man.

H. T. B.