ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Ambergris, Identification of. P. J. Hardwick and E. Q. Laws. (*Analyst*, 1951, **76**, 662.) The note records a simple chemical means for the identification of genuine ambergris which consists of ambreine, *epi*coprostanol, arachidic acid, ambroporphyrines, a ketonic fraction and a liquid paraffin; the method used entails the chromatographic separation of ambreine and *epi*coprostanol. The dried sample is extracted with ether, the fatty acids removed by shaking with aqueous alkali, the extract being evaporated to dryness and the residue dissolved in light petroleum. For the adsorption column alumina (Brockmann Grade III) is used and the light petroleum solution is adsorbed on it with the aid of a further quantity of light petroleum. A narrow yellow band, fluorescing green in ultra-violet light, moves rapidly down the column, the elution being continued until this band is washed into the receiver. The column is developed with a 1 + 1 mixture of ethyl ether and light petroleum when immediate separation of broad yellow bands at the top of the column begins and a series of bands that are fluorescent in ultra-violet light appears, a green and a yellow one travelling rapidly down the column. The green and yellow fluorescent fractions are collected separately, the yellow fraction containing the ambreine; a red fraction remaining in the column contains *epi*coprostanol which is eluted with benzene. The ambreine could be prepared in crystalline form by slow evaporation of the solvent; the crystals were colourless needles, m.pt. 83° C., showing a positive optical rotation and giving a rose colour with the Liebermann reagent. The ash of good quality ambergris ranged from 0·5 to 5·0 per cent., although a very inferior material from the outside of a large mass contained 57 per cent. of ash, consisting essentially of the phosphates of calcium and magnesium with a small proportion of sodium chloride and traces of copper, silicon, manganese and chromium. R. E. S.

Colchicine, Colorimetric Estimation of. J. S. King. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 424.) Pure colchicine alkaloid (2·5 mg.) is dissolved in 25 ml. of N hydrochloric acid in a 125-ml. Erlenmeyer flask. A funnel is inserted into the neck of the flask, which is then warmed on the steam-bath for 1 hour, removed, cooled to room temperature and the volume made up to 25 ml. with N hydrochloric acid. Graduated amounts containing up to 0·5 mg. of this solution are made to 5 ml. with N hydrochloric acid and 0·1 ml. of a 5 per cent. solution of ferric chloride is mixed with each. These solutions are then read in a colorimeter for standard transmittance at 470 mμ. This establishes the standard curve, which follows Beer's law up to 0·5 mg. Samples of unknown colchicine content are run in the same way, after separation of interfering materials. The jade green colour is formed immediately, and its intensity makes it suitable for identification of as little as 4 μg. of colchicine on a spot-plate. The procedure has been successfully applied to pharmaceutical mixtures, and a detailed example of such an application is given. S. L. W.

Dithizone as an Indicator in the Volumetric Determination of Zinc. J. P. Mehlig and A. P. Guill. (*Anal. Chem.*, 1951, **23**, 1876.) This work was

performed to establish the conditions for the use of dithizone as an indicator in the titrimetric determination of zinc with potassium ferrocyanide. In the titration, the zinc solution, buffered to *p*H 4·0 by potassium hydrogen phthalate, was run from a burette into the standard potassium ferrocyanide solution with constant shaking until 1 drop of dithizone in chloroform turned pink. It was not possible to titrate the zinc solution with the standard ferrocyanide solution since the ferrocyanide, unless in considerable excess, did not discharge the pink colour of the indicator in a reasonable time: the dithizone zinc complex was stable within a *p*H range of 3·5 to 5·0. All cations which form insoluble ferrocyanides in the presence of hydrochloric acid interfere with the titration and may be removed by the Waring procedure (*J. Amer. chem. Soc.*, 1904, **26**, 4) consisting of precipitation of copper with aluminium strips followed by precipitation of zinc sulphide in formic acid solution, the separated zinc sulphide being dissolved in hydrochloric acid. R. E. S.

Ethanol in Ether, Determination of, by Infra-red Absorption. A. A. Colon and H. A. Frediani. (*J. Amer. pharm. Ass. Sci. Ed.*, 1951, **40**, 607.) A rapid and convenient method for the determination of small amounts of ethanol in ether depends upon the absorption band in the region of 2·83 μ. As this band is due to the –OH group allowance must be made for the water present. For the preparation of calibration curves a pure sample of ether may be prepared as follows. Ethanol and water are removed from absolute ether by treatment with anhydrous calcium chloride followed by sodium ribbon added until evolution of gas ceases, filtration and distillation. Known proportions of ethanol and water are added, the absorptions are determined and calibration curves prepared. When a sample of ether is to be examined, its water content is determined by the Karl Fischer method and its absorption is determined at 2·83 μ. Using the calibration curves the ethanol content is calculated. Samples whose absorption is too high for convenient measurement may be diluted with ethanol-free ether. The accuracy of the method is about ± 2 per cent. G. B.

Hydrastine in Hydrastis, Fluorimetric Determination of. E. Brochmann-Hanssen and J. A. Evers. (*J. Amer. pharm. Ass. Sci. Ed.*, 1951, **40**, 620.) Hydrastine and canadine, but not berberine, are extracted from finely powdered hydrastis with ether in the presence of ammonia. The alkaloids are taken into aqueous solution and 5 ml. is oxidised by treatment with 10 ml. of nitric acid at 50° C. for 30 minutes, the solution is diluted and the blue-green fluorescence measured in a suitable fluorimeter. The fluorescence, which is due to hydrastinine and opianic acid, oxidation products of hydrastine, is dependent on temperature and fades on exposure to ultra-violet radiation. The hydrastine content is calculated from a standard curve prepared with pure hydrastine. The method is rapid and reasonable accuracy can be achieved. Impurities extracted from the hydrastis do not interfere. G. B.

Opium, Colorimetric Determination of Morphine in. A. Mariani, S. Guarino and O. Mariani Marelli (*Annali Chim.*, 1951, **51**, 661.) The official methods for the determination of morphine in opium are all long, troublesome and give doubtful results. The colour reactions devised by Guarino (*Arch. sci. biol.*, 1946, **31**, 115; *Quart. J. Pharm. Pharmacol.*, 1948, **21**, 67) form the basis of a colorimetric method which is rapid and accurate. The reaction adopted is that of oxidation by excess of iodic acid in an acid medium and subsequent reaction with a trace of ferric chloride in a solution made alkaline with sodium bicarbonate or ammonium carbonate and then determining the absorption in

a spectrophotometer. The greatest absorption is at the wavelength of 510 to 520 μ and this should be chosen for the test, and as the colour is so deep that a high dilution is necessary for reading, the yellow colour of the solution of opium can be neglected except for deeply coloured samples, low in morphine. In the latter case a reading of the solution after adding the sodium bicarbonate should be taken and deducted from the final reading after adding the ferric chloride. The most delicate part of the reaction is the time allowed for oxidation with iodic acid before making alkaline. With pure morphine this should be 30 seconds, but with an opium solution made with lime it is slower and 2 to 4 minutes should be allowed. In a solution in the neighbourhood of 0·8 mg. in 50 ml. the curve follows Beer's law perfectly and $E_{1\text{ cm.}}^{1\text{ per cent.}} = 57{\cdot}14$. The authors compared the method of the Italian Pharmacopœia with their method using the lime solution obtained in the Italian Pharmacopœia method after the addition of hydrochloric acid, and also using the hydrochloric acid solution obtained by Eder and Wäckerlin's method (*Quart. J. Pharm. Pharmacol.*, 1937, **10**, 680). The last results were from 25 to 30 per cent. higher than those of the official method and 15 to 19 per cent. higher than the second method. This they attribute to the incomplete extraction of the morphine in the official concentration, and the loss of morphine in the precipitation. They recommend that 1 g. of opium be extracted with lime water sufficient to make a volume of 100 ml. and that 1 ml. of this solution be diluted with 9 ml. of 0·1 N hydrochloric acid. Good concordance was obtained on repeated determinations as well as with the addition of known quantities of morphine to extracts of opium. H. D.

Particle Size of Fine Powders, Determination of. C. Rossi and R. Baldacci. (*J. appl. Chem.*, 1951, **1**, 446.) The sedimentation curves for suspensions of kaolins from Central Europe, Italian bentonites and clays have been obtained by using a hydrostatic balance provided with a normal plunger. The concentration of the suspension should not exceed 2 per cent. by volume, so that for a powder of medium density (e.g., 2·5 g./ml.) an upper limit of 5 per cent. by weight is permissible; boiling or evacuation are necessary to remove all gas bubbles, and deflocculants are also added depending on the material under examination, although further detailed treatment may also be required to produce a final suspension which is satisfactory for measurements of specific gravity against time. Investigations undertaken to establish the accuracy of the method, and the theoretical calculation of the sedimentation curves are briefly described and the possibility of applying Stokes' law to the mass settling of kaolins is also discussed. The possibility (*a*) of determining the average diameter of powder particles by means of the tangent to the curve at zero time, and (*b*) of deducing from experimental sedimentation curves both the curves relating to size distribution (the number of particles as a function of equivalent diameter) are emphasised. Regular curves from sedimentation measurements, particularly with kaolins, were obtained only when conditions were such as to avoid flocculation, achieved by adding suitable electrolytes in quantities which determined the smallest sedimentation volume. The average equivalent diameters of the powders examined were deduced from sedimentation curves and were compared with statistical measurements of dimensions made by direct microscopic observations on two kaolins; Bohemian kaolin had an equivalent average diameter of 2·8 mμ and Zettlitz kaolin an equivalent average diameter of 2·11 mμ, these figures corresponding to results of 2·7 and 2·18 mμ respectively, calculated from equations using the sedimentation curves.

R. E. S.

Quercetin in Rutin, Chromatographic Estimation of. J. Naghski, C. S. Fenske Jr. and J. F. Couch. (*J. Amer. pharm. Ass. Sci. Ed.*, 1951, **40**, 613.) Quercetin may be separated from commercial samples of rutin chromatographically and estimated spectrophotometrically. A solution of commercial rutin is dissolved in methanol or *iso*propanol, placed on the filter paper and developed with ethyl acetate saturated with water, using a descending chromatographic technique. Quercetin ($R_F = 0{\cdot}90$) is eluted while rutin ($R_F = 0{\cdot}05$) is retained on the paper. The quercetin solution is evaporated and the residue dissolved in absolute ethanol, aluminium chloride is added and the absorption determined at 440 mμ. The result is calculated from a standard curve. Recovery of quercetin is more complete from mixtures with rutin than when starting with quercetin alone. Certain flavonols which may be present in commercial rutin interfere, but this can be suppressed by using ethyl acetate, 35, benzene, 15, water, 50 as the developing solvent. G. B.

ORGANIC CHEMISTRY

***p*-Aminosalicylic Acid, Derivatives of.** W. Hückel and K. Janecka. (*Arch. Pharm., Berl.*, 1951, **284**, 341.) A description is given of the methods of preparation, and properties, of the following derivatives of *p*-aminosalicylic acid:—2-methoxy-4-aminobenzoic acid; methyl *p*-*N*-dimethylaminosalicylate; *p*-acetylaminosalicylic acid; methyl *p*-acetylaminosalicylate; 4-acetylamino-2-acetoxybenzoic acid; ethyl 4-acetylamino-2-acetoxybenzoate; *p*-acetylaminosalicylyl chloride; methyl *p*-acetylaminosalicylate, with the corresponding ethyl and *iso*propyl esters; methyl and ethyl *p*-aminosalicylates; ethyl *p*-acetylaminothiolsalicylate; and ethyl *p*-aminothiolsalicylate. G. M.

Iodinated Phenyl- and Pyridyl-alkanoic Acids as Contrast Agents. S. Archer, J. O. Hoppe, T. R. Lewis and M. N. Haskell. (*J. Amer. pharm. Ass. Sci. Ed.*, 1951, **40**, 143.) Compounds of the following series were synthesised:—(1) α-(3:5-diiodo-4-aminobenzamido)alkanoic acids, (2) α-(4-amino-3:5-diiodobenzenesulphonamido) alkanoic acids, and (3) α-(3:5-diiodo-4-pyridone) alkanoic acids. Series (1) was prepared by condensing *p*-nitrobenzoyl chloride with the α-amino acid, reduction and iodination. Series (2) was prepared by condensing acetamidobenzenesulphonyl chloride with the amino-acid, removing the acetyl group and iodinating. Series (3) was prepared by condensation of 3:5-diiodo-4-pyridone with the α-bromo acid, and an analogous series was prepared from 3:5-diiodo-4-pyridinethiol. The compounds were tested as gall-bladder contrast agents in the cat and the following observations were made for series (1) and (2). The amino group (or other group such as hydroxyl) in the aromatic ring merely serves to facilitate formation of the iodinated compound and has little influence on cholecystographic effect. The alkyl group is of the greatest importance in obtaining a good contrast medium, although the bridge which links carboxyl to the iodinated nucleus has some effect. The most active compounds were derived from heptoic acid in series (1) and hexoic acid in series (2). Diiodopyridonealkanoic acids were not absorbed when administered orally, but the diiodopyridylthioalkanoic acids passed into the gall bladder and were effective agents. G. B.

Phenyl-alkyl-carbinols, Derivatives of Basically Substituted. A. C. Kjær and P. V. Petersen. (*Acta chem. scand.*, 1951, **5**, 1145.) Carbinols of general formula $C_6H_5{\cdot}C(R.)\,(OH){\cdot}CHR_2\ CH\ R_3R_4$ have been prepared in an investigation of substances with potential analgesic and spasmolytic action.

Those compounds in which R_1 was C_6H_5 or C_6H_{11}, R_2 either H or CH_3, R_3 was H and R_4 either $N(CH_3)_2$ or piperidino were prepared by the action of either phenylmagnesium bromide or *cyclo*hexylmagnesum bromide upon the corresponding aminoketones; the latter are readily accessible by typical Mannich reactions. Related alcohols of the type $C_6H_5C(R.)\ (OH)\cdot CHR_2\ CHR_3R_4$ in which R_3 was methyl are prepared by a Grignard reaction with the corresponding amino and ethyl esters. None of these alcohols exhibited any significant spasmolytic or analgesic effect. Ester hydrochlorides of these carbinols with acetic, propionic, butyric and benzoic acids were unstable, being hydrolysed in water within a few hours. The free bases corresponding to the ester hydrochlorides were immediately hydrolysed in aqueous solution. Attempts to prepare ethers from the carbinols by reaction with alkyl halides in the presence of sodium, were unsuccessful. Treatment with thionyl chloride in an attempt to replace OH by Cl resulted in dehydration and the formation of unsaturated compounds, which in some cases showed considerable spasmolytic activity. One chloro compound only was isolated $(C_6H_5\cdot)_2\ C\cdot Cl\cdot CH\cdot CH_3\cdot CH_2\cdot N(CH_3)_2\cdot HCl$ and this was readily hydrolysed to the carbinol. No ether could be prepared from the latter compound by the action of sodium propylate in absolute propanol. The formation of the ether $(C_6H_5)_2\cdot C(OCH_2COCH_3)\cdot CH(CH_3)\cdot CH_2\cdot N(CH_3)_2$, HBr by the reaction of bromoacetone on 1:1-diphenyl- 2-methyl-3-dimethylamino propanol is recorded; this compound was unstable in aqueous solution. J. B. S.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Neovitamin A Esters and Neoretinene.[1] P. D. Dalvi and R. A. Morton. (*Biochem. J.*, 1951, **50**, 43.) The fraction of vitamin A from fish liver oils reacting readily with maleic anhydride is the all-*trans* form, the remainder being neovitamin A, a *cis*-isomer in which only the double bond nearest to the $-CH_2OH$ or $-CH_2OCOR$ group possesses the *cis* configuration. The existence of neovitamin A makes it necessary to compare very closely the ultra-violet absorption shown by all-*trans* and neovitamin A, the precise form of the absorption curve being important in the analysis of liver oils when allowing for irrelevant absorption. Practical work on weakened alumina as adsorbent indicated that vitamin A ester concentrates could be enriched by chromatography until practically free from glycerides, sterol esters and other substances; repeated chromatographic adsorptions accumulated the neovitamin A esters in the strongly held fractions. The ultra-violet absorption spectra of the best neovitamin A ester fractions were measured in various solvents, the results being quoted on a basis of $E_{max.} = 1{\cdot}00$ and compared with the corresponding curves for all-*trans* vitamin A acetate. The natural neo-esters showed, relatively to the all-*trans* ester, higher absorption from 220 to 280 mμ and from 330 to 390 mμ; there was apparently a *cis*-peak near 250 mμ. In the region 280 to 330 mμ the differences between the neo-esters and the all-*trans* esters were small, but not negligible. Plotted on the basis of $E_{max.} = 1{\cdot}00$, the curves for neovitamin A alcohol showed higher intensity of absorption from 220 to 290 mμ and 330 to 390 mμ as compared with the all-*trans* free vitamin A. The differences, though small, were quite sufficient to influence corrections for irrelevant absorption if neovitamin A predominated over the all-*trans* form. Neovitamin A alcohol on oxidation over manganese dioxide (light petroleum solution) yielded neoretinene. The spectrum of neoretinene was slightly displaced in the direction of shorter wavelengths as compared with

retinene. It was concluded that the occurrence of neovitamin A in a fish liver oil could result in over-correction in spectroscopic assays if its presence were neglected. Many natural products contained about 25 per cent. of the total vitamin A in the neo form; if, however, this is confirmed it seems likely that for ester concentrates "over-correction" need not reach 5 per cent. The difficulty of saponification of neovitamin A esters was the only chemical difference observed in addition to the difference in speed of reaction with maleic anhydride. R. E. S.

Rutin and Quercetin, Relative Stability in Alkaline Solutions. E. B. Dechene. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 495.) Solutions of rutin and quercetin in aqueous ethanolic and ethanolic solutions of sodium hydroxide, ethylenediamine and hydrochloric acid in various concentrations were refluxed or allowed to stand at room temperature and the concentration of the flavonol in the solutions determined by the aluminium chloride colour reaction. Little or no decomposition of rutin occurred in aqueous-ethanolic solutions containing about 2 moles of sodium hydroxide or 30 moles of ethylenediamine per mole of rutin, and only slight decomposition occurred in solutions containing about 4 moles of sodium hydroxide per mole of rutin. Quercetin showed a rapid rate of decomposition in aqueous-ethanolic solutions containing about 2 moles of sodium hydroxide or 30 moles of ethylenediamine per mole of quercetin. Considerable decomposition of rutin occurred in solutions containing slightly less than 17 moles of sodium hydroxide or 60 moles of ethylenediamine per mole of rutin. Paper chromatograms of the alkaline solutions of rutin showed no detectable amounts of quercetin, indicating that the latter was decomposed as fast as it was produced by the hydrolysis of rutin. Considerable amounts of quercetin were detected on paper chromatograms of the acid solutions of rutin, indicating that rutin is readily hydrolysed in these conditions whereas quercetin is stable. G. R. K.

Starch and Ion Exchange Resin Chromatography for the Separation of ^{15}N-labelled Amino-acids. S. E. G. Aqvist. (*Acta. chem. scand.*, 1951, **5**, 1031.) Protein hydrolysates containing ^{15}N– labelled amino-acids from yeast and *E. coli*, obtained from 25 to 50 g. batches of protein, were separated on the cation exchange resin, Dowex 50, after being separated initially by electrodialysis into acidic, basic and neutral amino-acid fractions. Detailed information as to column size, packing and preliminary washing of the ion exchange resin are given. Each amino-acid mixture was applied to the column in 1·5 N hydrochloric acid and eluted in a large volume of the same solvent. Clear cut separation of all the common amino-acids was obtained by this method, with the exception that some overlays did occur with the three amino acids, methionine, *iso*leucine and leucine. Separation of protein hydrolysates corresponding to 250 to 300 mg. of protein and containing ^{15}N– labelled amino-acids was also effected on columns of potato starch following the general procedure described by Stein and Moore, though on a much larger scale. The starch column must be freed from organic impurities and metal ions before it is used in the separation of amino acids. A certain amount of fatty impurity, retained by the starch, may be eluted later with the amino-acid fractions, but this is thought to be preferable to the loss of resolving power which follows the more thorough preliminary extraction with solvents. Acid-propanol-butanol is recommended as the solvent system for these large-scale separations rather than benzyl alcohol as described by Stein and Moore (*J. biol. Chem.*, 1948, **176**, 337.) Identification of the different amino-acids separated was by paper chromatography. J. B. S.

Vitamin A, Spectroscopic Properties of All-*trans* Vitamin A and Vitamin A Acetate. H. R. Cama, F. D. Collins and R. A. Morton. (*Biochem. J.*, 1951, **50**, 48.) A detailed examination is made of the whole problem of the spectrophotometric estimation of vitamin A. The photoelectric spectrophotometer used was calibrated with potassium chromate and dichromate solutions; results are given which provide a basis for testing the performance of other instruments. Natural and synthetic vitamin A were found to be indistinguishable. The values of $E_{1\text{ cm.}}^{1\text{ per cent.}}$ and $\lambda_{max.}$ for vitamin A acetate and for vitamin A alcohol in *cyclo*hexane, light petroleum, ethanol and *iso*propanol are given. The values of $\epsilon_{max.}$ in *cyclo*hexane and light petroleum were the same for the free acetate and for the alcohol although values of $\epsilon_{max.}$ in ethanol and *iso*propanol for the alcohol were higher than for the ester. Detailed figures are given for the absorption intensities expressed as fractions of $E_{max.}$ at wavelength intervals of 1 mμ over the range 310 to 340 mμ together with the appropriate correction equations for eliminating irrelevant absorption in vitamin A acetate and alcohol solution in the solvents *cyclo*hexane, ethanol, *iso*propanol and light petroleum. All the absorption curves showed a second maximum at about 250 to 253 mμ, approximately 550. The value of the factor for converting $E_{1\text{ cm.}}^{1\text{ per cent.}}$ to I.U./g. was not strictly constant, but varied with the solvent and the state of combination of the vitamin A. A complete absorption curve for the reaction product of vitamin A and antimony trichloride has been obtained, the value of $\epsilon_{max.}$ being 144,900 $\pm$ 870 at 620 mμ. Detailed studies have been made of a sample of cod liver oil, two high potency oils and the International Standard Preparation of vitamin A acetate; it is concluded that the International Standard Preparation is perhaps not quite up to standard, results for $E_{1\text{ cm.}}^{1\text{ per cent.}}$ 328 mμ as low as 5·09 instead of 5·21 being recorded. It is emphasised that although for a wide range of products the $E_{1\text{ cm.}}^{1\text{ per cent.}}$ value at 325 to 328 mμ on the oil or the "unsponifiable fraction" is a good guide to potency, it is necessary in aiming at an accurate assay to take full account of the presence of anhydrovitamins A, vitamin A_2, kitol, epoxides and other substances. It is generally, except with low potency oils, a better procedure to use the whole oil since the correction procedure when applied to unsaponifiable materials tends to give low results. It is also necessary to determine the neo/all-*trans* ratio, so that in addition to the main test based on ultraviolet absorption, a number of subsidiary tests are essential.

Appendix. Correction Procedure. A correction procedure can be developed which makes use of all the wavelengths at 2 mμ intervals from 310 to 340 mμ. As only 3 wavelengths are necessary in order to correct for linear irrelevant absorption, 13 degrees of freedom are available for the determination of the error. The following formulæ are derived by the method of least squares. In the case of vitamin A acetate in *cyclo*hexane,

$$\bar{E} = \frac{21760\ \Sigma\ \alpha\ E\ -\ 19{\cdot}824\ \Sigma\ \lambda\ E\ -\ 13488\ \Sigma\ E}{1297{\cdot}422}$$

In the case of vitamin A alcohol in *cyclo*hexane,

$$\bar{E} = \frac{21760\ \Sigma\ \alpha\ E\ +\ 2{\cdot}848\ \Sigma\ \lambda\ E\ -\ 20735{\cdot}36\ \Sigma\ E}{1461{\cdot}4985}$$

where E is the measured extinction at wavelength λ, α is the ratio of the absorption of pure all-*trans*-vitamin A to the absorption at wavelength $\lambda_{max.}$, $\bar{E}$ = extinction actually due to vitamin A at $\lambda_{max.}$. R. E. S.

BIOCHEMICAL ANALYSIS

Amines, Paper Chromatography of. J. M. Bremner and R. H. Kenton. (*Biochem. J.*, 1951, **49**, 651.) The R_F values of filter paper chromatograms of a large number of amines in various solvents have been determined and tabulated. The values were obtained with one batch of filter paper and represent the means of several determinations. In agreement with Bates-Smith (*Biochem. Soc. Symp.*, 1949, No. 3, 62) the values were found to vary from batch to batch of filter paper, but to be reasonably constant with individual batches. Ninhydrin is an effective reagent for the detection of primary aliphatic amines on paper chromatograms. Some compounds containing a secondary aliphatic amino group also give the ninhydrin colour reaction, but tertiary amines do not appear to give the reaction. The results obtained suggest that amines likely to be present in biological materials could be separated and identified by paper chromatography. Relationships between the molecular structure of the amines studied and their R_F values are also discussed. J. R. F.

Formaldehydogenic Corticosteroids in Urine, Estimation of. J. Rabinovitch, J. Decombe and A. Freedman. (*Lancet*, 1951, **261**, 1201.) The significant features of the method described are as follows. The first morning specimen of urine is collected in a vessel which contains 300 mg. of sodium sulphite, 3 ml. of glacial acetic acid is immediately added and the container tightly corked, a 50-ml. sample of the specimen being used for the assay. This procedure inhibits bacterial growth and the α-ketol group is protected against oxidation. Acid hydrolysis of the urine liberates corticosteroids from conjugation, and maximum hydrolysis with little destruction is achieved by heating the urine for 15 minutes in a closed flask at 60° C. with 0·12 ml. of concentrated sulphuric acid and 60 mg. of sodium sulphite, cooling, and adjusting to a *p*H of 6 to 7. With other methods of assay the formaldehyde formed by treatment with periodic acid is removed from the reaction mixture for estimation, but by treating the solution directly with calculated quantities of silver sulphite and filtering off the silver iodide formed, separation of the formaldehyde is obviated and the solution is ready for colorimetric estimation. From a calibration curve obtained from estimation of urinary extracts with added known quantities of cortisone the corticosteroid content of the specimen may be calculated. A single estimation need not take longer than 6 or 7 hours, and four estimations can be done in 8 or 9 hours. The range of excretion in the night urine of normal men, established by this method, is from 55 to 190 μg. S. L. W.

Gentisic Acid in Serum, Determination of. J. Lowenthal. (*J. Lab. clin. Med.*, 1951, **38**, 916.) A method of determination is described based on the fact that gentisic acid in a buffered solution (*p*H 3 to 4) is oxidised by ferric ions, ferrous ions being produced. Potassium fluoride is added to render the excess of ferric iron colourless through complex formation; the concentration of the ferrous iron is then determined photoelectrically by the formation of the coloured ferrous iron-ortho-phenanthroline complex. The accuracy of the method was tested by adding various quantities of gentisic acid to serum; satisfactory recoveries of gentisic acid were obtained. Analyses on single samples of serum stored in the refrigerator for several days gave reproducible results. Normal serum not containing gentisic acid, gave values equivalent to 0·5 to 1·0 mg. per cent. of gentisic acid; this figure can be neglected in comparison with gentisic acid levels experienced in practice. R. E. S.

Vitamin B_{12}, Plate Assay with *Escherichia coli*. E. Harrison, K. A. Lees and F. Wood. (*Analyst*, 1951, **76**, 696.) A microbiological method for the cup-plate assay of vitamin B_{12} with a mutant of *Escherichia coli* as test organism is presented. The assay is claimed as an advance over methods previously published of this series because (*a*) a simple chemically defined medium is used, (*b*) changes in the E_H of the test medium have little effect on the response, (*c*) the zones of exhibition are reproducible and well defined in character and (*d*) specificity and general freedom from interfering effects and inexplicable variations are shown to a marked degree. The inoculum consisted of a small volume of culture grown overnight in peptone water; the assay plates could be incubated at any temperature between 27° and 37° C. The sensitivity of the method was such that zones of exhibition could be obtained with solutions containing 1 μg. of vitamin B_{12} per ml. The dose response line was rectilinear over the range 0·005 to 5·0 μg per ml. and hence a (2 + 2) assay design was normally used; results are given relating to the effects of times of incubation, and of standing before incubation. A streptomycin-resistant strain of the test organism was developed for the direct assay of the vitamin B_{12} content of *Streptomyces griseus* fermentation samples. Thymidine did not interfere with the assay, but methionine gave zones of exhibition, a solution of 1 mg. per ml. giving a zone of approximately 35 mm.; dilutions of 100 and 10 μg. per ml. also stimulated growth, but the zones were indistinct and not measurable. Standard errors of 0·14 to 0·23 mm. per zone were obtained, 0·18 mm. being the normal error encountered in assays on Petri dishes or large glass plates. R. E. S.

CHEMOTHERAPY

Cinnoline Compounds in *T. Congolense* Infections. E. M. Lourie, J. S. Morley, J. C. E. Simpson and J. M. Walker. (*Brit. J. Pharmacol.*, 1951, **6**, 643.) A series of quaternary quinoline, cinnoline and quinazoline compounds were tested for activity against *Trypanosoma congolense*, using mice as the experimental animals. Evidence was obtained suggesting activity in a substance having two cinnolinium residues joined by a simple linkage and of a number of such compounds tested the most active was N^1:N^3-bis(4′-aminocinnolyl-6′)-guanidine methiodide, designated "528." The therapeutic index represented by LD10/CD90 is 11·6 compared with 12·5 for antrycide, the average LD10 being 1·03 mg./20 g. and the average CD90 being 0·089 mg./20 g. as compared with 0·49 mg. and 0·039 mg. for antrycide. H. T. B.

Barbiturates, Anæsthetic Properties of some new *N*-benzylated and *NN′*-dibenzylated Compounds. F. Sandberg. (*Svensk. farm. Tidskr.*, 1952, **52**, 31.) The following compounds were prepared and tested for anæsthetic properties:— 5:5-dialkylbarbituric acid, 5-allyl-5-*iso*propylbarbituric acid, 5-allyl-5-phenylbarbituric acid, 5-ethyl-5-*iso*-amylbarbituric acid and their 1-benzylsubstituted and 1:3-dibenzylsubstituted derivatives. The introduction of an *N*-benzyl group into the compounds was attended with a reduction in hypnotic efficiency and in toxicity, and a reduction in duration of action. No general rules were apparent for the influence of a single radical in the 5-position upon the anæsthetic properties. The *NN′*-dibenzylated compounds did not exhibit anæsthetic activity. A. H. B.

PHARMACY

DISPENSING

Sodium *p*-Aminosalicylate, Intravenous. R. G. Douris and J. Bory. (*Thèrapie*, 1951, **6**, 371.) A 3 per cent. aqueous solution of sodium *p*-aminosalicylate, as recommended by Paraf for intravenous use, is almost isotonic. It may be sterilised by heating, since the proportion decarboxylated is 1·3 per cent. (100° C. for 1 hour), 1·6 per cent. (115° for ½ hour), or 2 per cent. (120° for 20 minutes), and this proportion of *p*-aminophenol does not appear unduly toxic. Oxidation products which are formed slowly in the cold and more rapidly on heating, are pyrogenic in rabbits. Oxidation may be prevented by the use of sodium bisulphite, sulphite or hyposulphite, but the quantities necessary are too large to permit prolonged therapeutic use. Alternatively, the solution may be stabilised with sodium formaldehyde sulphoxylate (patented).

G. B.

Sodium Bisulphite, Sterilisation of Solutions of. S. A. Schou and J. M. Rhodes. (*Dansk Tidsskr. Farm.*, 1951, **25**, 365.) The effect of sterilisation on solutions of sodium bisulphite is important, since this compound is sometimes added as a preservative to other solutions. On sterilisation, oxidation to sulphuric acid occurs with a consequent decrease in *p*H, since the buffer action is very small. In a solution containing 0·1 per cent. of sodium bisulphite and 0·8 per cent. of sodium chloride, the *p*H changed on autoclaving from 3·44 to 0·96. Bubbling nitrogen through the solution causes a rise in *p*H, owing to loss of sulphur dioxide: such solutions also become more acid on autoclaving, though to a smaller extent than those sealed in air. G. M.

GALENICAL PHARMACY

Adrenaline and Analogues, Rate of Loss of Potency in Solution. J. C. Munch, A. B. Sloane and A. R. Latven. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 526.) Solutions of adrenaline and vaponefrin containing 0·5 per cent. of chlorbutol and adjusted to *p*H 4 were packed in ½-oz. amber glass bottles and stored under a variety of conditions. In a refrigerator at 5° C., adrenaline showed no loss of potency in 5 months, whereas vaponefrin showed no significant change in colour or potency up to 40 months. In the dark at room temperatures which ranged from 20° to 35° C., adrenaline began to change colour in 2 to 4 weeks but showed no significant change in potency; vaponefrin showed no change in potency over 3 months, and after 40 months one bottle showed a slight colour and precipitate but a loss of only 7 per cent. in potency, or substantially the same as that shown by a companion bottle kept at 5° C. When stored on a laboratory shelf in direct sunlight, adrenaline showed no significant loss over a month but did develop marked colour change and some precipitation; vaponefrin began to develop a pinkish-brown colour and a sediment after a year and loss in potency varied from 16 per cent. after 8 months to 12 per cent. after 40 months. Adrenaline solutions stored at room temperature in direct sunlight in frequently opened bottles showed marked losses in potency and definite discoloration within a week. Vaponefrin solutions stored similarly showed little loss in potency or discoloration in the first 3 months; after 29 months the decrease in potency was about 16 per cent., and after 40 months, about 24 per cent. When stored at 37° C., adrenaline

maintained its potency for a week whereas vaponefrin lost only 25 per cent. of its potency after 104 months. At 55° C., adrenaline again maintained its potency for 1 week but showed marked changes in colour and a loss of over half its potency in 1 month. Vaponefrin showed no change in colour over 15 months and a loss of potency of only 22 per cent. in 40 months, similar to that shown by solutions stored in sunlight in frequently opened bottles. The assays were performed on dogs by the U.S.P. method. G. R. K.

PHARMACOGNOSY

Caffeine in Cacao Beans. W. G. C. Forsyth. (*Nature Lond.*, 1952, **169**, 33.) To determine whether caffeine exists in the cocoa bean free or as an L-epicatechin-caffeine complex, various extracts of fresh bean were subjected to paper chromatography using a variety of developers, and, in every case, caffeine was found only in the free form on the paper. Synthetic preparations of the complex were made (1 : 1 addition compound of caffeine and L-epicatechin), by mixing aqueous solutions of the two components. Chromatography on a paper strip caused the two components to separate and each had its usual R_F value even in neutral solvents. The caffeine could also be completely extracted from an aqueous suspension of the material by cold chloroform. The isolation of such an easily formed complex is thus not proof that it exists as such in the bean. A. H. B.

Ephedra (*Colonial Plant and Animal Prod.*, 1951, **2**, 119.) A review article dealing with the distribution and alkaloid content of the various species. Special attention is given to cultivation trials carried out in South Dakota, Kenya and Australia. In South Dakota it was found that 4 year old stems of *E. sinica* yielded the highest proportion of alkaloid and less expense was involved in handling the crop in the field. The stems were cut and dried in the sun like hay, then stacked or baled, this method giving a drug with a higher alkaloid content than oven-drying. In Kenya, one year old stems of *E. intermedia* and *E. gerardiana* dried at 50° C. had an alkaloid content varying between 1·54 and 1·69 per cent. The average ephedrine content of the total alkaloids was in the case of *E. intermedia* 30 to 40 per cent. and in *E. gerardiana* 70 to 80 per cent. Difficulties in the availability of the natural product from China stimulated interest in the synthetic production of ephedrine and as a result there has been a decline in interest in ephedra. Some manufacturers in this country favour the herb, but in order that the herb may hold its own against the synthetic alkaloid, it will be necessary to keep collection and transport costs as low as possible. G. R. A. S.

Morphine from Poppy Heads and Stalks. V. Ristic. (*Boll. chim.-farm.*, 1951, **90**, 472.) It has been suggested that morphine should be extracted from poppy heads and stalks, either to enable countries which do not produce opium to avoid the necessity of importation, or to increase the yield by extracting the heads after opium has been collected. A good deal of investigation is required before it can be said to be a commercial possibility. Various authors have reported finding between 0·02 to 0·9 per cent. of morphine in the heads, and similar amounts in the top 10 cm. of the stalks. Lower portions of the stalks are valueless. The heads lose morphine rapidly on storage. It is suggested that extraction plant should be set up in the neighbourhood of the poppy fields so that it would only be necessary to transport the extract to the factories, instead of the relatively weak and bulky raw material. H. D.

PHARMACOLOGY AND THERAPEUTICS

Adrenaline, Noradrenaline and Methedrine; Effects on Renal Circulation during Anæsthesia. H. C. Churchill-Davidson, W. D. Wylie, B. E. Miles and H. E. de Wardener. (*Lancet*, 1951, **2**, 803.) Inulin and *p*-aminohippurate clearances were estimated on 18 occasions in 14 patients before and after the administration of adrenaline, noradrenaline or methedrine given during varicose-vein ligation or herniorrhaphy. Inulin clearance was assumed to equal the glomerular filtration-rate and *p*-aminohippurate clearance the renal plasma-flow. Renal blood-flow was calculated from the renal plasma-flow and venous hæmatocrit. The patients were anæsthetised with ether or *cyclo*propane and 10 of the patients had been made hypotensive by pentamethonium bromide. Adrenaline and noradrenaline were given by continuous intravenous drip; adrenaline at the rate of 16 to 38 μg./minute and noradrenaline at the rate of 3·5 to 37 μg./minute. Methedrine was given intravenously in divided doses up to a total of 25 to 100 mg. The renal blood-flow was reduced in all cases by adrenaline and noradrenaline, but increased in 5 out of 6 cases by methedrine. These results show that the effects of adrenaline and noradrenaline are similar in anæsthetised and in unanæsthetised persons, the rise in blood pressure being accompanied by a consistent fall in renal blood-flow, whereas methedrine produced a rise in blood pressure and an over-all increase in renal blood-flow.

S. L. W.

Cardiotonic Drugs, Chick Embryo Assay. G. H. Bryan and C. H. Waldon. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 497.) 72-hour chick embryos were exposed by removing the shell and membrane from the air pocket, giving a frontal view of the clearly defined heart. The eggs were then submerged to three-quarters of their length in water at 37° C. when the hearts continued to beat regularly but more slowly than normal for 4 to 24 hours. The drugs, in solution in normal saline solution containing 10 per cent. of ethanol, were placed in the pocket formed by the shell, egg yolk and white and the embryo, and the time taken to stop the heart measured. 3 different dose levels were used for each drug and were chosen so as to give adequate responses in 450 seconds. The 19/20 confidence limits for the ED50 of digitoxin (± 14 per cent.) and olnerin (± 12 per cent.) paralleled the limits obtained for tincture of digitalis, but those for digoxin (± 40 per cent.) and tincture of strophanthus (± 70 per cent.) were extremely high. G. R. K.

Cortisone in Rheumatoid Arthritis, Synergistic Action of *p*-Aminobenzoic Acid on. L. L. Wiesel, A. S. Barritt and W. M. Stumpe. (*Amer. J. med. Sci.*, 1951, **222**, 243.) In view of the scarcity and cost of cortisone a search was made for a method of reducing the usual dosage in the treatment of rheumatoid arthritis. Its structural similarity to the œstrogens, whose inactivation by the liver is inhibited by the simultaneous administration of *p*-aminobenzoic acid, suggested an attempt to inhibit the destruction of cortisone in the body by a similar method. 15 patients suffering from rheumatoid arthritis were treated according to two different techniques, details of which are tabulated. The dosage of cortisone employed was, by itself, completely ineffective in controlling the manifestations of rheumatoid arthritis. On a daily intramuscular dose of 25 mg. of cortisone acetate, together with oral administration of 1·5 g. of sodium *p*-aminobenzoate every 2 hours until 12 g. had been taken, all the patients demonstrated great relief of pain and objective symptoms. None of

the undesirable side effects frequently present with the usual dosage of cortisone acetate was observed and only occasional mild heartburn resulted from administration of the sodium aminobenzoate. H. T. B.

Liquorice, Pharmacological Action of. D. Vincent. (*Thèrapie*, 1951, **6**, 448.) When a 1 in 10 decoction of dried liquorice root was injected intravenously into a chloralosed dog a rapid reduction of blood pressure was produced which lasted 20 to 30 minutes. In some dogs doses of 0·05 g. of the dry root per kg. of body weight produced a fall of 70 or 80 per cent. In other cases doses of 0·1 g. or even 0·2 g. per kg. produced less effect. Cardiac rhythm was only slightly affected and breathing was not changed. By various tests (use of physostigmine, cholesterinase, and the action on the dorsal muscle of the leech and the isolated intestine of the guinea-pig) the author showed that this action was not due to acetylcholine. Commercial ammoniated glycyrrhizin in 1 per cent. solution in corresponding doses did not produce similar falls in blood pressure. Previous injections of liquorice markedly diminish the action of acetylcholine on blood pressure and on the intestine of the guinea-pig. The drug inhibits the action of cholinesterases to an extent varying from 20 to 50 per cent. according to the source of the enzyme, so its effect in inhibiting the action of acetylcholine is not due to stimulating the enzyme. Liquorice also possesses antihistaminic properties which will be reported later. H. D.

Ouabain, Influence of Sex upon Response of Rat Heart to. A. Wollenberger and M. L. Karsh. (*J. Amer. pharm. Ass. Sci. Ed.*, 1951, **40**, 637.) Langendorff preparations of the isolated hearts of 4- to 5-month old rats were perfused with glucose-Ringer-Locke solution. When the contractions had become uniform ouabain was administered at the rate of 0·25 mg. every 3 minutes until the heart stopped in contracture. The minimal lethal dose, calculated with reference to the dry heart weight was 45 per cent. higher in females than in males in May-June, and 31 per cent. in November-December. This accords with the observed sex difference in LD50 for mature rats and shows that this may be attributed to the difference in sensitivity of the heart. G. B.

Primaquine in Experimental *Trypanosoma cruzi* Infection. T. Pizzi. (*Proc. Soc. exp. Biol., N.Y.*, 1951, **78**, 643.) Primaquine is 8-(4-amino-1-methyl butylamino)-6-methoxyquinoline and its effect in the treatment of *Trypanosoma cruzi* infection in mice has been investigated. The drug was given orally as a solution in water containing 1 mg./ml., the daily dose being 0·25 mg., and the number of trypanosomes in the blood was determined daily. During the early stages of the infection the primaquine has a strong suppressive action and most treated animals survived infection with a virulent strain. All showed a disappearance of, or a marked decrease in, the number of parasites in the blood. H. T. B.

Procaine Amide in Digitalis-induced Ventricular Tachycardia. L. I. Goldberg and M. de V. Cotten. (*Proc. Soc. exp. Biol., N.Y.*, 1951, 77, 741.) Auricular-ventricular block, with a slow ventricular rate, or ventricular tachycardia was induced in dogs by the administration of digitoxin to 12 and ouabain to 8 animals intravenously in divided doses. Rapid intravenous injection of a 10 per cent. procaine amide solution induced a reversion to normal sinus rhythm when administered to those in ventricular tachycardia. In most cases this was temporary and additional injections were required to maintain normal rhythm. Such injections produced no electrocardiographic signs

of cumulated toxicity. In several instances development of slow idioventricular rhythm and cardiac arrest followed the administration of the drug, demonstrating a danger of drug termination of digitalis-induced tachycardia. In agreement with other workers it was also found that the drug failed to increase the lethal dose of ouabain in an experiment with 7 cats. J. R. F.

***Veratrum viride*, Biological Estimation of.** R. Benigni. (*Boll. chim.-farm.*, 1951, **90**, 384. Craw has recommended the use of *Daphnia magna* for testing the activity of *Veratrum viride* and he calls a daphnia unit the quantity required to kill 1000 daphnias with the heart in systole in 2 hours and 22 minutes. The large number of animals, their minute size and the necessity of using the microscope to examine them, makes this a method unsuited for routine use. The author suggests the use of *Lebistes reticulatus* the "million" fish. This fish is a native of Venezuela and the West Indian islands, but has been spread over the tropics in the fight against malaria. The males are about 2·5 cm. long and the females 3·5 cm. They are obtainable commercially and are easily kept in an aquarium. They breed at the age of 3 months, are ovi-viviparous, and about once a month produce from 2 or 3 to 30 or 40 young. The method of testing is simple. In 5 or more 100-ml. beakers place 50 ml. of well-aerated water and in each place an adult lebistes, as far as possible all the same size, and add a series of doses of the preparation to be tested until a quantity is found which will kill a lebistes in 5 hours. The test is then repeated with doses more closely spaced until the minimum dose which will kill 3 fish out of 3 is found. This is called a lebistes unit (L.U.) and the author found that 5·7 of these units equals 10 daphnia units, and this is an average therapeutic dose by the mouth. Very concordant results are obtained by this method, and it is economical, as usually not more than 20 to 30 fish are used and those that survive can be used again after a sufficient interval. H. D.

BACTERIOLOGY AND CLINICAL TESTS

Boric Acid; Antibacterial Action in Tears. M. Novak and W. I. Taylor. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 430.) Bacteriostatic concentrations of boric acid in broth cultures of representative pyogenic bacterial species often found in the eye varies from 0·5 to 2 per cent. Higher concentrations, to a maximum of 4 per cent., showed a slow bactericidal action. Freshly collected as well as stored lachrymal secretions showed no inhibitory action on the antibacterial action of boric acid. The results suggest that tears actually increase the bactericidal action of boric acid to a slight degree. This action may be due to naturally occurring lysozyme. S. L. W.

Pyrogenic Properties, Attenuation of, During the Ageing of Sterile Microbial Preparations. J. Dorche and M. Castaing. (*Ann. pharm. franç.*, 1951, **9**, 583.) Sterile suspensions of *Salm. typhi*, *B. subtilis*, *E. coli*, and *Ps. æruginosa* were stored for periods of up to 2 years. They retained their pyrogenic properties with the exception of the *B. subtilis* suspension which rapidly decreased in pyrogenic power. A suspension of *Salm. typhi* appeared to be sufficiently stable for use as a standard pyrogen. G. B.

Pyrogenic Properties of Distilled Water and Microbial Cultures. J. Dorche, M. Carraz and M. Castaing. (*Ann. pharm. franç.*, 1951, **9**, 574.) Bacterial emulsions were prepared by suspending bacteria in normal saline, diluting to

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LETTER TO THE EDITOR

The lack of resolved fine-structure and of acid-alkali shift in curves (1) and (2), the absence of a band at *ca.* 250 mμ in curve (2) and the presence of resolved fine-structure and the 250 mμ band in (3) are fully consistent with our previous suggestion[6] that in vitamin B_{12} the benziminazole chromophore is co-ordinated to the cobalt atom.

G. H. BEAVEN.
E. R. HOLIDAY.

Medical Research Council,
Spectrographic Unit,
The London Hospital, E.1.
March 11, 1952.

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ABSTRACTS (*Continued from page* 340.)

about 200 million organisms per ml., as determined by opacity and autoclaving at 115° C. for half-an-hour. Organisms were also suspended in sterile water, incubated for 16 days at 37° C. and filtered or centrifuged. These preparations were injected into rabbits at the rate of 0·5 ml./kg. Emulsions, filtrates and supernatant liquids from the centrifuge were almost equally pyrogenic. The bacteria tested included 10 known pyrogenic species and some species of *Bacillus*, *Chromobacterium*, *Flavobacterium*, *Pseudomonas*, *Sarcina* and *Staphylococcus* which were isolated from pyrogenic samples of distilled water and classified. All proved to be pyrogenic in rabbits. For some species (for example *Ps. æruginosa*) there was a constant pyrogenic power, while for others (for example *B. subtilis*) it varied according to origin. No correlation was observed between pathogenic and pyrogenic power. G. B.

Pyrogens from Various Bacterial Species. L. G. Ginger, N. M. Nesset, B. Riegel and E. J. Fitzsimons. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 421.) Pyrogenic concentrates have been prepared from *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Serratia marcessens* and *Proteus vulgaris* by subjecting the cellular materials to tryptic digestion. Analysis of these concentrates has yielded information which indicates that there are essential differences in their chemical composition and biological activity; in particular, the concentrate prepared from *B. subtilis* differs distinctly from the other concentrates. At least three possible contaminants are associated with the pyrogenic polysaccharides, namely, nucleic acids, other nitrogenous material, and free lipid. The amount of contaminating nucleic acids in each concentrate is related to the initial nucleic acid content of the cellular material, while the amount of nitrogenous residues varies with the cellular species under consideration. The pyrogenic polysaccharides have been shown to consist of hexosamine, an unclassified reducing sugar, and a non-reducing fraction, but there seems to be no direct correlation between the amount of any of these constituents present in the concentrates and the pyrogenicity of the latter. It is possible that some yet undetermined component is responsible for the observed differences in biological activity. S. L. W.