ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Bromine and Chlorine in Organic Compounds, Microdetermination of. J. A. C. van Pinxteren. (*Pharm. Weekbl.*, 1953, **88**, 489.) The method is based on the Viebock process, using a current of nitrogen to carry over the gases produced by the action of sulphuric acid-potassium dichromate-silver sulphate on the organic compound. In order to determine bromine, the following absorption tubes are used in succession. The first two contain a mixture of equal volumes of 150 g. of chromic acid in 80 g. of water, and N potassium cyanide. The bromine is converted into cyanogen bromide, which is absorbed in two tubes (cooled in ice) the first containing N potassium iodide solution and the second containing glass beads moistened with the same solution. Chlorine does not interfere, as cyanogen chloride, though formed, does not react with potassium iodide. Between the first and the second pair of tubes is inserted a tube containing 1 per cent. solution of hydrazine sulphate to absorb any oxidising gases (ozone, chlorine dioxide). The iodine in the last two tubes is finally titrated with thiosulphate, when 2 atoms of iodine correspond to 1 of bromine in the original substance. The method can also be used to determine bromine + chlorine by inserting at the beginning of the absorption chain a tube containing N potassium bromide in N sulphuric acid, to convert the chlorine into an equivalent amount of bromine before passing through the remainder of the tubes. G. M.

Digitalis, Chemical Assay of. M. Langejan and J. A. C. van Pinxteren. (*Pharm. Weekbl.*, 1953, **88**, 529.) In the chemical assay of digitalis, purification of extracts by lead acetate alone is insufficient to remove substances which interfere with the colorimetric determination, and extraction with chloroform is incomplete. Three modified extraction procedures are described. 1. *By infusion*: 1·5 g. of powdered leaf is moistened with 1·5 ml. of water and, after 15 minutes, is treated with 148·5 ml. of water and heated for 15 minutes at 90° C. After cooling, 15 ml. of 15 per cent. lead acetate solution is added, and the supernatant liquid is decanted through filter paper. Of this 110 g. (= 1 g. of leaf) is extracted 4 times with 50 ml. quantities of chloroform-ethanol (1 : 1 by volume). The extracts are filtered through sodium sulphate, which is washed with 15 ml. of chloroform and, after evaporation of the chloroform, the residue is dissolved in 12·5 ml. of ethanol (96 per cent.) and made up to 50 ml. with water. This solution is passed through a column of alumina (6 to 7 cm. in height). 2. *By ethanol extraction*: 2 g. of the powdered leaf is shaken with 20 ml. of ethanol (48 per cent.) for 3 hours. After the addition of 5 ml. of 15 per cent. lead acetate solution, the mixture is filtered through sintered glass (3G3) and washed with water to about 40 ml.: 4 ml. of N ammonium phosphate solution is added, the mixture is centrifuged and the precipitate is washed with water. To the solution 40 ml. of ethanol is added, and the liquid is extracted with 3 quantities, each of 50 ml., of chloroform. The extracts are filtered through sodium sulphate, and washed through with 5 ml. of chloroform. After evaporation of the chloroform, the residue is dissolved in 25 ml. of

ethanol (96 per cent.), made up to 100 ml. with water, and passed through alumina as before. 3. *By cold maceration*: 1·5 g. of powdered leaf is rubbed down thoroughly with 5 ml. of water and, after 15 minutes, the mass is transferred to a bottle with 145 ml. of water. After shaking for 1 hour, 15 ml. of 15 per cent. lead acetate solution is added and the mixture is filtered. 110 g. of the filtrate (= 1 g. of leaf) is extracted with 4 quantities of 50 ml. of chloroform-ethanol (1 : 1 by volume), the extracts being filtered through sodium sulphate which is washed with 5 ml. of chloroform. The solution is evaporated to dryness and the residue is dissolved in 12·5 ml. of ethanol (96 per cent.) made up to 50 ml. with water, and passed through alumina. Two methods of colorimetric determination may then be employed: 1. *Determination of digitoxose with Bial's reagent*: 2 ml. of the reagent is added to 1 ml. of the glucoside solution, heated for 1 minute in a water bath, and cooled immediately after the addition of 10 ml. of ethanol (96 per cent.), and again cooling, the mixture is transferred to a 20 ml. measuring flask and washed in with 5 ml. of hydrochloric acid and 5 ml. of water, adjusted to 20° C. and made up to 20 ml. with water. The extinction is then measured in a 1 cm. cell at 620mμ. The process is standardised with pure digitoxin. 2. *Determination of aglycone with dinitrobenzoic acid*: To 5 ml. of the solution is added 5 ml. of 2 per cent. ethanolic solution of 3 : 5-dinitrobenzoic acid and 9 ml. of ethanol (96 per cent.). After cooling to 15° C., 2 ml. of N sodium hydroxide is added and the mixture is made up to 25 ml. with water. After keeping for 10 minutes in a water bath at 20° C. the extinction is measured at 550 mμ in a 2 cm. cell. Standardisation is against digitoxin. Results obtained by the three methods of extraction show fairly satisfactory agreement. G. M.

Formaldehyde, Determination of, in Presence of Phenols and Phenol Alcohols. J. I. de Jong. (*Rec. Trav. chim. Pays-Bas*, 1953, **72**, 356.) The method is based on a rapid and quantitative reaction of formaldehyde with cyanide ions according to the equations

$$CH_2O + CN^- \rightarrow CN{\cdot}CH_2O^-$$
$$CN{\cdot}CH_2O^- + H_2O \rightarrow CN{\cdot}CH_2OH + OH^-$$

Various modifications of the method have been described, but that of Pfeil and Schroth (*Z. anal. Chem.*, 1952, **34**, 333) using excess of cyanide and determining the excess by titration with mercuric acetate, using diphenylcarbazone as indicator, has been developed as the method of choice, being especially suitable for the determination of formaldehyde in the presence of phenols and phenol alcohols. The following procedure was adopted: 30 ml. of 0·1 M potassium cyanide was added to 1 to 2 moles of formaldehyde in water (20 ml.). After 5 minutes 10 per cent. aqueous pyridine (10 ml.) and 1 per cent. methanolic diphenylcarbazone (6 drops) were added, and the colour of the solution adjusted to orange yellow with N nitric acid. The mixture was titrated with 0·1 N mercuric nitrate until the colour of the solution changed sharply to rose violet. J. B. S.

Gamma Benzene Hexachloride, Determination of. J. Rosin and G. B. Radan. (*Analyt. Chem.*, 1953, **25**, 817.) Chromatography was preferred for the determination of the gamma isomer in commercial benzene hexachloride. The method of Aepli *et al.* (*Analyt. Chem.*, 1948, **20**, 610) was followed with the modification that the nitromethane was refluxed for 2 to 3 hours and redistilled to remove any residue on evaporation. Modified details for the method are given. A typical benzene hexachloride assay showed a total gamma content

corresponding to 34·4 per cent. of sample taken. The values obtained by this method checked with polarographic assays of the entire gamma content within ±5 per cent., provided that the sample did not have hepta- and octa-chloro-*cyclo*hexanes in such proportions as to make corrections in the polarographic interpretation too large to be accurate. R. E. S.

Ion Exchange in Paper Chromatography. J. B. Schute. (*Nature, Lond.*, 1953, **171**, 839.) When acids and alkaloids are chromatographed on paper with water as a solvent irreversible adsorption takes place due to ion exchange. The hydrogen ions of an acid in contact with paper exchanges with cations, such as sodium, potassium, calcium, magnesium, etc. (M_p^+), bound on carboxyl groups, silicate and other "insoluble material," according to the equilibrium $H^+ + M_p^+ \rightleftharpoons H_p^+ + M^+$. Blank paper chromatographed with water yields cations to the solvent, the concentration of which increases with the distance covered by the front, so that on washed paper part of the exchangeable cation has already been displaced by hydrogen ion; similarly the area of an acid spot increases on washing the paper with water. Chromatograms of salicylic acid on untreated paper are shown in which the front part of the acid spot contains salicylate. Unchanged salicylic acid is still present in this part, although the acid travels much more slowly than the solvent front. On paper which has been washed with acid and then with water, salicylic acid and sulphosalicylic acid chromatographed with water give normal chromatograms. Ion exchange is also possible with alkaloids, when they are chromatographed on paper with water, and separation of two alkaloids can be effected where one of the alkaloids is bound preferentially. J. B. S.

Phosphate Esters, Paper Chromatography of. E. Fletcher and F. H. Malpress. (*Nature, Lond.*, 1953, **171**, 838.) An enzymic method for the liberation of orthophosphate from phosphate esters already resolved on the chromatogram, is described. Ethanolic calcium chloride solution is used to reduce the tendency for the resolved spots to spread when treated with the enzyme solution, so that phosphate subsequently liberated on the paper at an alkaline *p*H is immediately fixed as insoluble calcium phosphate. It is claimed that the use of alkaline phosphatase provides a method which is more certain and more specific than purely chemical methods previously employed. Two dimensional chromatograms run on acid-washed Whatman No. 1 paper using conventional techniques were developed as follows:—after thorough air drying at room temperature the papers were mounted on glass sheets and sprayed with 5 per cent. $CaCl_2.6H_2O$ in 80 per cent. ethanol (10 ml.), and the solvent allowed to evaporate at room temperature. The papers were then sprayed with 10 ml. of the enzyme solution prepared in 0·1 M glycine buffer at *p*H 9, covered with a second glass sheet and incubated at 37° C. for 4 hours. The papers, still damp and attached to the glass, were sprayed with 5 per cent. ammonium molybdate in 20 per cent. hydrochloric acid (3 ml.), when yellow phosphomolybdate spots rapidly appeared wherever orthophosphate had been liberated in amounts exceeding 1 μg. After 2 minutes the papers were further sprayed with benzidine hydrochloride in dilute acetic acid, and finally inverted over a trough of dilute ammonia. Bright blue spots formed within one minute and were indicative of as little as 0·5 μg. of phosphorus. J. B. S.

ORGANIC CHEMISTRY

Alcohols, Reaction of Diazonium Salts with. N. Kornblum and A. E. Kelley. (*Science*, 1953, **117**, 379.) References are cited to show that the "well-known" reaction between alcohols and diazonium salts is completely misunderstood in the interpretation expressed by the following equation

$$\text{Aryl-N}_2^{+}\text{X}' + \text{CH}_3\text{CH}_2\text{OH} \longrightarrow \text{Aryl-H} + \text{CH}_3\text{CHO} + \text{N}_2 + \text{HX}$$

When benzene diazonium chloride or sulphate is treated with absolute ethanol, phenetole is the main product, i.e.,

$$\underset{}{\text{C}_6\text{H}_5\text{-N}_2^{+}\text{Cl}'} + \text{CH}_3\text{CH}_2\text{OH} \longrightarrow \underset{(61\text{ per cent.})}{\text{C}_6\text{H}_5\text{OCH}_2\text{CH}_3} + \underset{(5\text{ per cent.})}{\text{C}_6\text{H}_6}$$

When benzene diazonium salts are treated with methanol the methyl ether (anisole) is formed in 70 per cent. yield and there was no evidence of benzene formation. Two points are emphasised: (1) ethanol is not, in general, dependable for replacing diazonium groups by hydrogen; (2) reduction with hypophosphorous acid is reliable for this purpose. A. H. B.

1-Alkoxy-2 : 2-bis(4-hydroxycoumarinyl-3) ethanes as Synthetic Anticoagulants. A. Veldstra, P. W. Wiardi and G. Alberda. (*Rec. Trav. chim. Pays-Bas*, 1953, **72**, 358.) On the assumption that the favourable clinical properties of ethyl biscoumacetate with respect to resorption and excretion were connected with its structure as an ester (lipophilic) of a rather water-soluble substituted dicoumarol, a number of new compounds have been synthesised. A series of ethers of 2 : 2-bis(4-hydroxycoumarinyl-3)ethanol have been obtained by condensation of 4-hydroxycoumarin with acetals of alkoxyacetaldehydes in aqueous solution. Diacetals are readily available, and are readily hydrolysed during the reaction to give the free aldehydes, which condense with two molecules of hydroxycoumarin. A number of the lower members of the series of compounds obtained show activities as high as that of dicoumarol.

J. B. S.

Cinchocaine, Stability of. J. Mørch. (*Dansk Tidsskr. Farm.*, 1953, **27**, 173.) When cinchocaine is hydrolysed by heating in alkaline solution, the amido group is removed with the formation of 2-butoxyquinoline-4-carboxylic acid, whereas in acid solutions, removal of the 2-butoxy group occurs with formation of 2-hydroxyquinoline-4-carboxylic acid and its diethylaminoethyl amide. In aqueous solutions at *p*H 5·45 (the *p*H of a 1 per cent. solution of the pure substance) all the cinchocaine hydrochloride is hydrolysed to 2-hydroxyquinoline-4-carboxylic acid diethylaminoethyl amide, before removal of the amido group occurs. Decomposition in a 1 per cent. solution at 90 to 100° C. is a monomolecular process with a temperature coefficient of 2·54/10° C., in agreement with the results of storage experiments at 10, 20 and 30° C. The minimum decomposition rate on autoclaving occurs at *p*H 5, and it is concluded that aqueous solutions are sufficiently stable to be sterilised by autoclaving at 120° C. for 20 minutes, at *p*H 4·5 to 6. In these experiments a spectrophotometric assay was found to be unsuitable because decomposition products have absorption spectra similar to cinchocaine. The method of precipitation of the base with sodium hydroxide solution, drying and weighing the residue was used, since all known decomposition products are soluble in sodium hydroxide and do not interfere with the results. G. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Corticotrophin-B, Purification of, by Countercurrent Distribution. F. A. Kuehl, Jr., M. A. P. Meisinger, N. G. Brink and K. Folkers. (*J. Amer. chem. Soc.*, 1953, **75**, 1955.) The procedure for the isolation of corticotrophin-B from its concentrates by the countercurrent distribution technique is described. The distribution system is prepared by equilibrating equal volumes of *s*-butanol and 0·5 per cent. aqueous trichloroacetic acid solution at 25° C. Corticotrophin-B has a favourable distribution coefficient ($K = C_{org}/C_{aq} = 0{\cdot}5$ to 0·6 in this system) and both solvent phases are transparent to ultra-violet light in the 277·5 mμ region where both corticotrophin-B (and corticotrophin) and its peptidic impurities absorb, permitting facile analysis of the fractions. This solvent system showed no tendency to emulsify. The component of high adrenocorticotrophic activity behaved as a pure substance on countercurrent distributions of 200 to 450 transfers. The presence of two other components in all corticotrophin-B concentrates subjected to this technique was observed. One of these appeared to have a low order of adrenocorticotrophic activity.

A. H. B.

Dihydrostreptomycin, Complex Salts of. V. V. Bogert and I. A. Solomons. (*J. Amer. chem. Soc.*, 1953, **75**, 2355.) It was found that dihydrostreptomycin formed crystalline complexes or mixed acid salts. Addition of a water-soluble iodide to an aqueous solution of dihydrostreptomycin sulphate precipitated crystalline dihydrostreptomycin iodide sulphate. In a similar manner a number of new crystalline dihydrostreptomycin salts, each containing two different negative ions, were isolated and characterised. The triacidic base, dihydrostreptomycin, can combine with one dibasic and one monobasic acid or with two similar and one dissimilar monobasic acids. Also, two molecules of the antibiotic may react with two similar and one dissimilar dibasic acids. Dihydrostreptomycin iodide sulphate is of particular interest because of the specificity of salt formation and its relatively low solubility in water. The solubility is dependent upon the anions present in the supernatant solutions. These solubility data were utilised in designing a new process for isolating dihydrostreptomycin from solutions of relatively crude streptomycin. Dihydrostreptomycin iodide sulphate has a bacterial spectrum, resistance pattern, acute and chronic toxicity comparable with crystalline dihydrostreptomycin sulphate.

A. H. B.

4 : 6-Dinitro-*o*-cresol, Urinary Metabolites of, in the Rabbit. J. N. Smith, R. H. Smithies and R. T. Williams. (*Biochem. J.*, 1953, **54**, 225.) 4 : 6-Dinitro-*o*-cresol was administered orally to a group of chinchilla rabbits, the urine was collected and various extracts prepared therefrom. Unchanged 4 : 6-dinitro-*o*-cresol amounting to about 6 per cent. was isolated by ether extraction from the acid urine (*p*H 2 to 5). Dinitro-*o*-cresol was separated chromatographically and estimated spectrophotometrically. The most important metabolic product is 6-acetamido-4-nitro-*o*-cresol which is mainly in an *O*-conjugated form, and represents about 12 per cent. of the administered dinitro-*o*-cresol. This metabolic product has apparently one-twentieth the toxicity of dinitro-*o*-cresol. When 6-acetylamino-4-nitro-*o*-cresol is fed to rabbits it is excreted in the urine as a glucuronide. J. B. S.

Œstrogens, Paper Chromatography of. P. H. Jellinek. (*Nature, Lond.*, 1953, **171**, 750.) Existing methods used for the paper chromatography of adrenal cortical steroids have been used for chromatography of non-steroid œstrogens of the hexœstrol series and also for ethinyl œstradiol. Toluene/propylene glycol was used for development and a mixture of 1 per cent. ferric chloride and 1 per cent. potassium ferricyanide (Barton, Evans and Gardner, *Nature, Lond.*, 1952, **170**, 249) gave a blue spot with quantities less than 2 μg./sq. cm. of synthetic œstrogen. The presence of trienœstrol in the urine and fæces, but not in blood extracts of animals injected with this substance, has been demonstrated using this method, followed by a bioassay technique. Trienœstrol added to blood was readily detected. Animals injected with ethinyl œstradiol showed positive reaction for unchanged œstrogen, but no œstrone or β-œstradiol in the fæces. The method is uncertain for the detection of œstriol in urine and fæces. J. B. S.

Vitamins A and E, Paper Chromatography of. J. A. Brown. (*Analyt. Chem.*, 1953, **25**, 774.) A procedure is given by which the common forms of vitamin A can be separated from each other and from vitamin E using silicone-impregnated paper strips and acetonitrile–water solvent systems. Strips of 0·5-inch filter paper were immersed in a suspension of silicone grease in methylene chloride and dried in an oven at 50° C.; ascending chromatographic technique was used and the developing solvent was a mixture of acetonitrile and distilled water, the relative amounts of each varying according to the nature of the material under test. The vitamins were dissolved in chloroform, *n*-hexane or *iso*propanol before transferring to the paper strip. After drying in carbon dioxide the strips were examined for their ultra-violet absorption, quantitative as well as qualitative estimations being possible. Results are given for numerous mixtures of vitamins A and E. The vitamins could be separated from each other, from impurities in the vehicle, and from oxidised vitamin A; an automatic spectrophotometric arrangement, providing a graph of band position *vs.* strip length could be used for locating the substances on the strip. Quantitative results were obtained by measuring the zonal areas on the graph and comparing with standards. R. E. S.

BIOCHEMICAL ANALYSIS

Isoniazid in Blood, Microdetermination of. M. B. Jacobs. (*Science*, 1953, **118**, 142.) A simple and rapid method depends upon the reduction of potassium ferricyanide in acid solution and colorimetric estimation of the blue colour produced. To 1 ml. of blood plasma is added 5 ml. of water, 1 ml. of a 10 per cent. solution of sodium tungstate and 1 ml. of 2/3 N sulphuric acid and the mixture heated in a water bath at 80 to 85° C. to coagulate the precipitate. After cooling and filtering 4 ml. of the clear filtrate is placed in a colorimeter tube and 0·5 ml. of 3 N acetic acid and 0·5 ml. of a 0·4 per cent. solution of potassium ferricyanide are added. The solution is heated at 80° C. for 15 minutes after which it is cooled and the colour measured after allowing to stand for 25 minutes. The quantity of isoniazid is read from a standard curve prepared in a series of experiments in which known quantities of isoniazid solution are mixed with 1 ml. of normal horse serum and treated in the same manner. The method is applicable to *iso*nicotinyl hydrazide (isoniazid) and its 2-*iso*propyl derivative (iproniazid) and experiments in which known quantities were added to blood plasma indicate that the method may be employed for amounts of 4 to 5 μg. upwards. The effect of varying the concentration of acid and the time of heating and cooling is discussed. As the method is

empirical, the procedure should be followed exactly or standard curves prepared under the action conditions of the test. Glucose and other normal blood constituents do not reduce ferricyanide solution under the conditions described. Attempts to adapt the method to the determination of isoniazid in urine were unsuccessful. G. B.

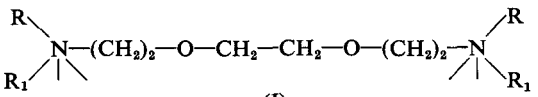
Œstrone, Œstradiol and Œstriol, Chromatographic Separation of. J. Bitman and J. F. Sykes. (*Science*, 1953, **117**, 356.) A micromethod has been developed as a modification of existing methods for the separation of œstrone and œstradiol by partition chromatography. This allows of the further separation of œstriol in the one operation. The method is based on the partition of œstrone and œstradiol using sodium hydroxide adsorbed on celite as the stationary phase and benzene as the mobile phase. Œstriol because of its more hydrophilic characters is not eluted by benzene from NaOH-celite. Columns are set up and run with benzene to elute œstrone and œstradiol in the accepted manner. Gaseous carbon dioxide is then bubbled up through the column to effect a reduction in the alkalinity of the stationary phase, forming a $NaHCO_3$-celite phase, and the elution then continued with benzene when œstriol is separated. A detailed account of the experimental technique is given. The separate fractions are finally estimated fluorimetrically. The entire process can be completed within 3 to 4 hours, and the method gives a quantitative separation of 2 to 10 μg. amounts of œstrogens. J. B. S.

Steroid Alcohols, Separation of, by Chromatography of their Benzoates. R. V. Brooks, W. Klyne and E. Miller. (*Biochem. J.*, 1953, **54**, 212.) Epimeric 20-hydroxysteroids, which are almost impossible to separate by chromatography as alcohols and difficult to separate as acetate, can be separated quantitatively as benzoates on a single chromatogram. The behaviour of synthetic mixtures of a number of pairs of isomeric steroid benzoates of types likely to occur in work on urines has been examined. In many cases separation as benzoates is as good as for the acetates and in the above instance better. 3-Epimeric hydroxysteroids can be sharply separated by chromatography either as acetates or benzoates, and the method has certain advantages over corresponding separations depending upon the precipitation of the β-isomer with digitonin. Separation of 5-epimeric steroids, which is poor by other methods, is little better with the benzoates. There appears to be little correlation between the conformation of aryloxyl groups in the steroid skeleton and the order in which the epimeric compounds were eluted from alumina (either as acetates or benzoates). J. B. S.

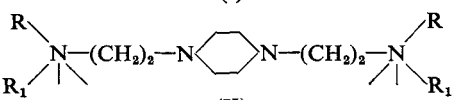
Vitamin B_1 and Choline, Chromatography of. A. Heyndrickx (*J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 315.) The moisture content of a sample of starch powder was determined, a quantity equivalent to 13·4 g. of the dried material was suspended in 25 ml. of anhydrous butanol and water equivalent to 30 per cent. of the weight of dried starch was added. The mixture was stirred, poured into columns and allowed to settle. Columns for the separation of aneurine and choline were washed with a mixture of 2 parts of propanol and 1 of 0·1N hydrochloric acid, the sample, dissolved in the same solvent, being placed on the column and eluted with the propanol-hydrochloric acid mixture. Using columns 15 cm. high and employing suction to adjust the rate of flow to 25 ml./hour, separation was satisfactorily achieved provided that fractions of 0·5 ml. were collected at the separation point. Samples containing 0·5 g. of choline and 0·1 mg. of aneurine gave results correct to within 3 per cent. for choline and 5 per cent. for aneurine. G. B.

CHEMOTHERAPY

Quaternary Ammonium Salts of Bis (di-alkylaminoethoxy) ethylene, Curarising Activity of. R. Hazard, J. Cheymol, P. Chabrier, E. Corteggiani, P. Muller and Y. Gay. (*Arch. int. Pharmacodyn.*, 1953, **94**, 1.) Compounds of the series I were prepared by the action of the alkyl halide on the corresponding tertiary diamine in ethanol, acetone or ether.



(I)



(II)

The effect of different terminal groups (R,R_1) and of the alkyl halide used in quaternisation were studied. The compounds were tested against a *d*-tubocurarine standard for toxicity in mice, head-drop, respiratory paralysis and cardiac arrest in rabbits, turning reflex in frogs, antiacetylcholinergic action on the isolated *rectus abdominis* in frogs, arterial pressure in dogs and skin reactions by intradermal injection in rabbits. The most powerful compound was substituted on the nitrogen atoms with diethyl, followed by the piperidyl, dimethyl and morpholinyl compounds. Quaternisation with an ethyl halide enhanced the activity compared with methyl, and the benzyl derivative was even more active. Chlorides were more active than bromides, iodides or camphosulphonates. In comparison with corresponding members of the isosteric series II, 7 compounds of series I were the more active and 2 of series II. In one case activity was of the same order. 4 of the compounds exhibited curarising action with a minimum of secondary reactions harmful in therapeutic use. G. B.

PHARMACY

GALENICAL PHARMACY

Cyanocobalamin, Stability of Solutions of. E. Hoff-Jørgensen, K. Ilver, O. I. Johansen and F. Reimers. (*Dansk Tidsskr. Farm.*, 1953, **27**, 117.) The loss in microbiological activity of a solution of cyanocobalamin, at a *p*H of 4·5, after autoclaving for 20 minutes at 120° C., is small and does not exceed 5 per cent., while the effect of 1 hour at 100° C. is negligible. Autoclaving is therefore permissible. As buffer monosodium phosphate (0·02M) is recommended. G. M.

***Polygonum hydropiper*, Investigation of Glycoside-containing Extracts prepared from.** I. R. Gnidets. (*Aptechnoe Delo*, 1953, **2**, No. 2, 41.) Extracts were prepared by percolating the dried ground herb with ethanol (20 per cent.) and ethanol (40 per cent.) followed by chloroform water, inert matter being removed from the concentrated percolate by treatment with lead acetate. Estimation of the dry residue, ash, reducing substances (calculated as glucose), acid number, ester number and saponification number showed no difference whether the herb was collected in July, August or September. Passing the extracts through columns

of alumina or magnesium oxide failed to remove the glycosides or to make material changes in the characteristics. When an extract after treatment with lead acetate was passed successively through cationite and anionite columns, a colourless neutral liquid was obtained which on analysis showed: dry residue 0·8, ash 0·11, reducing substances (as glucose) 0·57 per cent., ester number 4·25. The sugar component of the glycoside in this liquid was identified as glucose.

E. H.

NOTES AND FORMULÆ

Arsthinol (Balarsen). (*New and Nonofficial Remedies*: *J. Amer. med. Ass.*, 1953, **152**, 531.) Arsthinol is 2-(3′-acetamido-4′-hydroxyphenyl)-1 : 3-dithia-2-arsa*cyclo*pentane-4-methanol. It occurs as a white odourless microcrystalline powder, m.pt. 163° to 166° C., very slightly soluble in ether and water, and soluble in about 37 parts of ethanol. When dissolved in sodium hydroxide solution and warmed on a water bath with sodium hydrosulphite, a yellow precipitate is produced which dissolves in an excess of sodium hydroxide. The addition of ferric chloride to a dilute solution in ethanol produces a light green colour which does not change on dilution with water (distinction from the *O*-acetyl derivative, in which the green colour changes through purple to brown on the addition of water). When sodium nitroprusside solution is added to a suspension in sodium bicarbonate solution, no red or violet colour appears (absence of free sulphhydryl group); on the subsequent addition of sodium hydroxide solution, a violet colour is formed (presence of bound sulphhydryl group). When stirred with hydrochloric acid and filtered, the filtrate yields no red or brown colour with potassium dichromate (absence of aminohydroxyphenylarsonic acid), and no precipitate with magnesia mixture (absence of inorganic arsenates). Arsthinol loses not more than 1·0 per cent. of its weight when dried at 105° C. for 4 hours, and contains 97·5 to 102·5 per cent. of arsthinol. It is assayed by dissolving in propylene glycol, adding sulphuric acid and iodine, allowing to stand in the dark for 2 hours with occasional stirring and titrating the excess of iodine. Arsthinol is a trivalent arsenical effective against intestinal amœbiasis and yaws.

G. R. K.

Nalorphine Hydrochloride (Nalline Hydrochloride). (*New and Nonofficial Remedies: J. Amer. med. Ass.*, 1953, **152**, 45.) Nalorphine hydrochloride is a white, odourless, crystalline powder, m.pt. 265° to 270° C. completely soluble in water (a 0·5 per cent. solution has *p*H 4·4 to 5·5), soluble in about 16 parts of ethanol, very slightly soluble in chloroform, and practically insoluble in ether. It yields an intense purple colour with sulphuric acid containing molybdic acid, and an intense purple colour which gradually becomes blue with sulphuric acid containing formaldehyde. An aqueous solution becomes deep blue on the addition of a mixture of potassium ferricyanide and ferric chloride solutions, and when a solution in sulphuric acid is warmed with ferric chloride, a blue colour forms, which changes to dark red brown on the addition of nitric acid. When warmed with a saturated solution of sodium bicarbonate, a precipitate of the base is obtained which melts at 205° to 208° C. A 0·5 per cent. solution in methanol has specific rotation −115° to −120°, and a 0·01 per cent. solution in water exhibits ultra-violet absorption maximum 285 mμ ($E^{1\ \text{per cent.}}_{1\ \text{cm.}}$, about 44) and minimum about 260 mμ. Nalorphine hydrochloride yields not more than 0·1 per cent. of sulphated ash and loses not more than 0·5 per cent. in weight when dried at 105° for 4 hours. It contains 95·0 to 105·0 per cent. of nalorphine hydrochloride, when assayed by measuring

the absorption at 285 mμ of a 0·01 per cent. solution in water. It also contains 87·3 to 91·8 per cent. of nalorphine, the determination being made in glacial acetic acid by treatment with mercuric acetate and titration with perchloric acid, using crystal violet as indicator. Nalorphine hydrochloride is used as an antidote to morphine, pethidine, and methadone. G. R. K.

PHARMACOLOGY AND THERAPEUTICS

***d*-Amphetamine, Amobarbital, Acetylsalicylic Acid and Phenacetin, "Analgesic" Action of.** S. C. Harris and R. C. Worley. (*Proc. Soc. exp. Biol. N.Y.*, 1953, **83**, 515.) Results are reported on the effectiveness of mixtures of (*a*) *d*-amphetamine sulphate 5 mg., amobarbital 32 mg., acetylsalicylic acid 162 mg. and phenacetin 162 mg. ("Daprisal"); (*b*) acetylsalicylic acid 162 mg. and phenacetin 162 mg., in raising the pain threshold of human volunteers. Pain thresholds were measured by electrical stimulation of the tooth pulp through dental fillings before medication and then at 20 minute intervals afterwards. Comparisons were made with results giving placebo tablets and with no drug treatment at all, neither the volunteer nor the operator making the pain threshold measurements knowing which treatment was given. The pain threshold was raised significantly by the amphetamine mixture, but not by treatment with the aspirin-phenacetin mixture, placebo or no treatment at all. G. F. S.

Coanesin, a New Hæmostatic. J. L. Leitch, B. M. Rhodes, P. Lenney and T. J. Haley. (*Arch. int. Pharmacodyn.*, 1953, **94**, 35.) Coanesin is a mixture of 32 per cent. of 3-(2′-methylphenoxy) propan-1 : 2-diol(mephenesin) and 68 per cent. of 3-(2′-methoxyphenoxy)propan-1 : 2-diol, reported to have a high solubility in water and a pronounced hæmostatic action. In this study the substance had no effect upon the coagulation of rabbit plasma *in vitro*. In heparinised rabbit plasma it inhibited heparin activity at concentrations of 100 to 200 μg./ml., but at higher concentrations it had an anticoagulant effect. *In vivo* tests indicated that the substance did not decrease the coagulation time in rabbits. Coanesin was relatively nontoxic, the LD50 being 290 mg./kg. in mice and 715 mg./kg. in guinea-pigs, toxicity being mainly due to the methyl component. It appears that rabbits do not respond to the drug in the same way as men and dogs, so far as blood coagulation is concerned. G. B.

Ethopropazine Hydrochloride in Cerebral Palsy. R. G. Mitchell. (*Brit. med. J.*, 1953, **1**, 1313.) In tests on 19 children, the starting dose was 20 to 30 mg. daily increased to a maximum of 100 to 150 mg. daily for the first half of the trial. In the second half of the trial, the starting dose of 30 to 50 mg. daily was increased to 200 to 250 mg. Progress was assessed by a number of tests designed to measure different activities, such as ability to relax, balance, active movement, manual dexterity and rolling. An improvement of 23·3 per cent. was observed in the children treated with ethopropazine, but a similar improvement occurred in the group treated with lactose instead. It is concluded that ethopropazine is of no value in the treatment of cerebral palsy. G. B.

Glucose, Active Absorption of, from the Intestine. S. Hestrin-Lerner and B. Shapiro. (*Nature, Lond.*, 1953, **171**, 745.) Glucose disappeared from the lumen of an isolated loop of rat intestine, immersed in Tyrode solution at 37° C. at a rate (during the first 15 to 30 minutes) which was of the same order of magnitude as that occurring during resorption *in vivo* from similar solutions. Phlorrhizin inhibited absorption of glucose *in vitro* and *in vivo*. The outer

fluid showed no increase of glucose content. With radioactive glucose in the lumen, between 30 and 60 per cent. of the radioactivity lost from the lumen was recovered in the outer fluid. The recovered radioactive substance was not glucose, was non-reducing and unfermented by yeast; it was not converted into glucose by acid hydrolysis. Much of the glucose which had disappeared from the lumen was recovered from the lumen as a substance apparently similar to the one appearing in the outer solution. Glucose concentration in the portal vein and artery were compared during resorption of glucose from a 400 mg. per cent. solution. In one experiment 18 mg. disappeared in 12 minutes; on this basis the glucose concentration of the vena porta was calculated to exceed that of the artery by 37 mg. per cent., assuming that it was transported as glucose. Very much lower glucose concentrations were present in the vena porta than in the artery, indicating that glucose undergoes some change during the absorption process. This conclusion is supported by other *in vitro* experiments. The possibility of glucose consumption by the intestinal tissues has been eliminated by measurements of oxygen consumption. It is concluded that glucose is therefore absorbed in the form of some intermediate metabolite to be reconverted to glucose at a later stage presumably in the liver.

J. B. S.

Isoniazid, Streptomycin and *p*-Aminosalicylic Acid, Determination of Sensitivity of Tubercle Bacilli to. Laboratory Subcommittee of the Tuberculosis Chemotherapy Trials Committee, Medical Research Council. (*Lancet*, 1953, **265**, 213.) The methods of determining sensitivity used in the M.R.C. isoniazid trial are described in detail. For the determination of sensitivity to isoniazid, a primary culture is prepared from a specimen and a loopful, representative of all parts of the growth suspended in 0·3 to 0·5 ml. of water. The opacity of the suspension is not standardised, although this may be advisable in future work. A 3 mm. loopful is spread over Lowenstein-Jensen slopes containing concentrations of 0, 0·2, 1, 5, 10 and 50 μg. of isoniazid/ml., and observed after 14 and 28 days at 37° C. Resistant strains are defined as those which grow (i.e. 20 or more colonies) after 4 weeks' incubation on a concentration of 1 μg. of isoniazid/ml. and doubtfully resistant strains as those which grow (20 or more colonies) on 0·2 μg./ml. but not on 1 μg./ml., provided that strain H37Rv shows a lesser degree of growth on 0·2 μg./ml. Sensitive strains are those which do not grow (i.e. less than 20 colonies) on 0·2 μg./ml. and those which grow on 0·2 μg./ml. but not on 1 μg./ml. provided that H37Rv shows the same or a greater degree of growth on 0·2 μg./ml. "Doubtfully resistant strains" in a patient who has never received isoniazid may be regarded as sensitive, but those in a person under treatment with isoniazid are frequently strains with a low degree of resistance. Similar methods and definitions are given for sensitivity to streptomycin and sodium *p*-aminosalicylate and results may be expressed as resistance ratios. Great care is necessary to avoid infection of the laboratory workers making sensitivity tests. G. B.

Isoniazid, Streptomycin plus *p*-Aminosalicylic Acid, and Streptomycin plus Isoniazid, Emergence of Bacterial Resistance in Pulmonary Tuberculosis under Treatment with. Laboratory Sub-committee of the Tuberculosis Chemotherapy Trials Committee, Medical Research Council. (*Lancet*, 1953, **265**, 217.) Patients were divided into groups treated with isoniazid alone, streptomycin with isoniazid, and streptomycin with *p*-aminosalicylic acid. The doses used were isoniazid, 100 mg. twice daily, streptomycin, 1 g. daily and *p*-aminosalicylic acid, 5 g. 4 times a day. After 3 months' treatment, the dose of streptomycin

was reduced to 1 g./week. Bacterial resistance to isoniazid, administered alone, developed rapidly, 64 per cent. of positive cultures representing 35 per cent. of patients yielding resistant strains after 3 months and 95 per cent. of positive cultures (58 per cent. of patients) after 6 months. Combined treatment with streptomycin and isoniazid largely prevented the development of isoniazid resistance in patients whose organisms were sensitive to streptomycin, 11 per cent. of positive cultures showing resistance after 3 months' treatment. There was no evidence of reversion of resistant strains to sensitivity in the 3 months following treatment with isoniazid. The use of isoniazid or *p*-aminosalicylic acid in combination with streptomycin was effective in preventing the emergence of strains resistant to streptomycin. In the streptomycin-isoniazid group, 8 per cent. of positive cultures at 3 months and none at 6 months were resistant to streptomycin, and in the streptomycin-*p*-aminosalicylic acid group the figures were 6 and 29 per cent. In this latter group streptomycin resistance developed more rapidly when the organisms were initially resistant to *p*-aminosalicylic acid. G. B.

***Magnolia grandiflora*, Liquid Extract of, as a New Therapeutic Agent.** O. I. Belova and Ya. Kh. Nolle. (*Aptechnoe Delo*, 1953, **2**, No. 2, 65.) A 1:1-extract prepared by the method of repercolation with ethanol (70 per cent.) consisted of a brownish green aromatic liquid with a bitter spicy taste; sp.gr. at 20° C. 0·974, extractive 23 per cent. Administration of 0·3 to 0·5 ml. (after the ethanol had been removed and replaced by an equal volume of water) subcutaneously and orally to white mice produced no toxic effects. When given intravenously to urethanised cats in doses of 2, 3 and 5 ml. it produced a rapid fall in the blood pressure: this amounted to 30 to 50 per cent. of the initial level and lasted 25, 30 or 50 minutes according to the dose. There was no weakening of the heart's action, but the depth and frequency of respiratory movements were markedly increased. These doses produced no toxic effects on the experimental animals. E. H.

Mipafox, an Organic Phosphorus Insecticide, Poisoning by. P. L. Bidstrup, J. A. Bonnell and A. G. Beckett. (*Brit. med. J.*, 1953, **1**, 1068.) Two cases are described of paralysis occurring after acute organic phosphorus poisoning in workers occupied in the production of the insecticide *NN'*-di*iso*propylphosphorodiamidic fluoride (mipafox). Both patients had a history of previous mild poisoning by organic phosphorus compounds, and in both cases paralysis developed in the third week after poisoning, and resembled "ginger paralysis" caused by tri-*o*-cresyl phosphate. In a third patient who had no previous history of poisoning by organic phosphorus compounds the symptoms were relatively mild and recovery was rapid and complete. The acute gastrointestinal symptoms of poisoning in each patient were controlled by the intramuscular administration of atropine. Some mental disturbance occurred, but this was attributed to overdosage with atropine and it disappeared on cessation of treatment. Both patients were discharged from hospital apparently well, but had to be readmitted within a few days with weakness and loss of tone of the leg and foot muscles. In one case this rapidly increased, and included the arms and hands, and recovery took place only slowly and incompletely over a period of 12 months. It has been shown that mipafox is an inhibitor of cholinesterase, and the authors discuss these cases in the light of recent work showing that certain organic phosphorus compounds which have the property of inhibiting

cholinesterase, may cause paralysis in man. Details are given of the cholinesterase activity in both patients, and of the rate of regeneration of plasma cholinesterase compared with the rate of regeneration following poisoning by di*iso*propyl phosphorofluoridate. It is suggested that substances capable of inhibiting cholinesterase should not be recommended as insecticides until both acute and chronic toxicities have been studied in several species of animals. The blood cholinesterase activity of workers exposed to organic phosphorus compounds should be estimated frequently, and no person in whom it is found to be reduced should be subjected to the risk of absorbing even small additional amounts of any cholinesterase inhibitor. H. T. B.

Nickel, Toxicity of. Ya. M. Grushko, V. A. Donskov and V. S. Kolesnik. (*Farmakologiya i Toksikologiya*, 1953, **16**, No. 2, 47.) 5 one-and-a-half-month old rabbits were given daily doses (0·0001, 0·001, 0·01, 0·10 and 0·54 mg./kg.) of nickel sulphate in their drinking-water over a period of 160 days. During the course of the experiment all the animals remained healthy, showing no signs of intoxication and their body weights were not different from those of 2 control rabbits. At the end of the experiment the animals were killed. Macroscopic examination showed no changes in the organs or tissues. However, microscopic examination showed pathological changes in the kidneys, liver and spleen of the animal receiving 0·001 mg./kg. and upwards, the effects being very marked in the one which received 0·54 mg./kg. E. H.

Primidone in the Treatment of Epilepsy. A. J. M. Butter. (*Lancet*, 1953, **264**, 1024.) The response to primidone (mysoline, 5-phenyl-5-ethyl-hexahydropyrimidine-4 : 6-dione) in the treatment of epilepsy was observed over a period of 15 months in 58 males ranging in age from 20 to 66, whose fits had not been satisfactorily controlled by phenobarbitone, soluble phenytoin, methoin, phenylmethylmalonylurea, bromides, amphetamine sulphate, or combinations of these. The change of treatment was effected slowly, the reduction of previous medication being effected in stages as the dosage was increased to 1 to 2 g. daily in divided doses. Most patients complained of slight drowsiness for the first day or two, and in 8 cases drowsiness continued, necessitating withdrawal of the drug after trial of smaller doses. There was 1 complaint of nausea, 2 patients became slightly ataxic and 1 had slurred speech, but no other toxic signs or symptoms were observed. 50 per cent. of cases derived benefit although in 15 per cent. the fits were not reduced but the patients felt better than when taking other drugs. Petit mal attacks were reduced by half in 23 per cent. of the patients and 1 patient with psychomotor attacks showed great improvement. The cases selected were the worst possible from the point of view of treatment and less severe cases might well respond more satisfactorily. H. T. B.

Primidone in Treatment of Epileptic and Non-epileptic Psychiatric Patients. A. Bonkalo and R. G. S. Arthurs. (*Canad. med. Ass. J.*, 1953, **68**, 570.) The effects of primidone (mysoline) were investigated in patients with epileptic states with social-psychiatric implications and also in patients with psychiatric conditions with epileptic implications. Three lines of inquiry were followed: (1) its usefulness in epileptic patients in whom a non-convulsive attack would manifest itself as a psychiatric condition ("psychomotor epilepsy", etc.); (2) its possible role in treating non-epileptic psychiatric patients with episodic manifestations in whom

electroencephalographic abnormalities were found; and (3) the psycho-physiological factors relevant to treatment of patients with this drug. 18 patients were studied, of whom 10 had clinical epilepsy and 8 had not, and observations ranged from 3 months to a year. The drug provided effective control in all types of epilepsy studied, major, minor and psychomotor, the usual effective dose being 1 to 1·5 gm. daily. In 2 cases additional drugs were required, and hydantoins were found preferable to barbiturates. No serious toxic effects were noted. Initial nausea, dizziness and, occasionally, vomiting usually ceased in a few days; these effects were minimised by commencing with small doses and increasing gradually. 2 non-epileptics could not tolerate the drug and treatment was terminated. 5 of the epileptic patients showed improvement of psychic and motor functions, and were better adjusted socially. No beneficial effect was observed in any of the 8 non-epileptic patients. H. T. B.

Procaine Penicillin with Aluminium Monostearate; Injections for Children. S. A. Doxiadis and K. S. Holt. (*Lancet*, 1953, **265**, 376.) The duration of therapeutic serum-penicillin levels (0·06 or more units/ml.) was studied in 36 children given single doses of procaine penicillin with aluminium monostearate intramuscularly. The dosages were as follows: infants (5), 450,000 units; children 1 to 4 years (14), 600,000 units; children 5 to 10 years (8), 900,000 units; children 11 to 14 years (9), 1,200,000 units. With these dosages effective serum-penicillin levels were maintained in infants for at least 84 hours; in children aged 1 to 4 for at least 72 hours; in children aged 5 to 10 for at least 60 hours; and in children aged 11 to 14 for at least 48 hours. In the older age-groups such large doses are not justified since similar results have been achieved by other workers with smaller doses. There were no general reactions to these injections. Locally, there was a small lump for a few hours but in all cases these lumps were absorbed without any sequelæ. This type of therapy should be substituted for oral penicillin therapy in children. S. L. W.

Pyrimethamine (Daraprim) in the Treatment of Vivax Malaria. G. Covell, P. G. Shute and M. Maryon. (*Brit. med. J.*, 1953, **2**, 258.) 12 patients suffering from primary attacks of malaria due to *P. vivax*, Madagascar strain, induced by mosquitoes were treated with 50 mg. of pyrimethamine daily for 5 days. The clinical response was slow, fever persisting for 3 to 7 days. Pyrimethamine alone is therefore not considered satisfactory for treatment of vivax malaria in non-immune subjects. 4 of the patients relapsed between the 55th and 99th day after the treatment. Subsequently a dose of 25 mg. of pyrimethamine weekly was administered for 8 weeks and all subjects relapsed between the 8th and 18th weeks after this treatment. There was no indication that pyrimethamine was of value in preventing relapse, but possibly the attacks were somewhat delayed by the administration of the drug. G. B.

Superfusion, Technique of. J. H. Gaddum. (*Brit. J. Pharmacol.*, 1953, **8**, 321.) A technique, superfusion, is described for applying drugs to tissues which are suspended in air and having a stream of a suitable solution run over the surface. The preparation is particularly sensitive, the effective concentration of the drug being the same as if it was placed in a 0·2 ml. bath. The value of the preparation has been confirmed in a simple assay of histamine. It is particularly valuable with slow acting substances, such as trypsin, where the effect of the drug becomes apparent after the drug has been washed off the tissue, thus avoiding prolonged contact. It has been used for the analysis of the

properties of a substance "R" which comes from the rats intestine when superfused and causes a slow contraction of the rat's uterus. This substance has been shown to be undialysable through cellophane, to be precipitated with 80 per cent. cold acetone and to not be destroyed by trypsin. Its properties were distinguishable from those of histamine, acetylcholine, 5-hydroxytryptamine, oxytocin and substance P. While its properties resembled those of some proteolytic enzymes its identity has not been established. G. F. S.

Suxamethonium (Succinylcholine), Effect of Pseudo-cholinesterase on Action of, in Man. F. T. Evans, P. W. S. Gray, H. Lehmann and E. Silk. (*Brit. med. J.*, 1953, **1**, 136.) In some patients suxamethonium has a prolonged effect which is related to a low pseudo-cholinesterase. This paper reports that the injection of a concentrated solution of human pseudo-cholinesterase (cholose) reduced the duration of apnœa in 4 patients anæsthetised with thiopentone and given suxamethonium, and in one human unanæsthetised volunteer. Large doses of pseudo-cholinesterase are required for a prolonged protection since it readily "leaks" into the tissues. G. F. S.

***Veratrum viride*, Assay of Preparations of.** G. Chen and D. Russell. (*J. Amer. pharm. Ass., Sci. Ed.*, 1953, **42**, 373.) In determining the potency of extracts of *Veratrum viride* by measurements of the hypotensive response in anæsthetised dogs, the range of doses which may be used is very small, the maximal hypotensive dose being only 30 per cent. higher than the minimal effective dose. Variation between experiments may be kept to a minimum by keeping the depth of anæsthesia constant and operating at the minimum effective dose. In assaying a veratrum preparation of unknown potency, the MED may first be determined by extrapolation from the logarithmic-linear relationship between dose and response, using 3 dogs and 4 dose levels. This is compared with the MED of a standard preparation in a cross-matching test in 6 animals anæsthetised with pentobarbitone sodium. G. B.

BACTERIOLOGY AND CLINICAL TESTS

Common-Cold Virus in Tissue Cultures. C. H. Andrewes, D. M. Chapron-iere, A. E. H. Gompels, H. G. Pereira and A. T. Roden. (*Lancet*, 1953, **265**, 546.) A successful cultivation of the human common-cold virus in tissue cultures and its propagation through ten serial dilutions has been achieved. Cultures of human embryonic lung tissue were placed in plasma-lined tubes and a nutrient fluid of bovine amniotic fluid, bovine embryo extract and normal horse serum was added. Penicillin, streptomycin and a soya-bean trypsin inhibitor were also added. The tubes were incubated at 37° C. in a revolving drum. Initial innoculation was with unfiltered nasal washings from a person with a typical afebrile cold. Passages were effected by grinding the total contents of the cultures in an homogeniser, centrifuging lightly and transmitting the diluted supernatant to other cultures. The cultures were tested in human volunteers and colds were induced up to and including the tenth serial culture when the original virus had survived 35 days at 37° C. After the tenth culture no further colds could be obtained, which may have been due to loss of the virus or to attenuation. Demonstration of the activity of the virus in cultures may soon be possible without the use of human volunteers. G. F. S.