ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Barbiturates in Pharmaceuticals, Determination of. F. A. Rotondaro. (*J. Assoc. off. agric. Chem., Wash.*, 1955, **38**, 809.) An examination is made of the different extraction procedures available and one extraction method using chloroform is recommended. Samples containing 100–150 mg. of the barbiturate if a gravimetric or a volumetric determination is to be made, or 15–30 mg. if the determination is to be made spectrophotometrically are dissolved in water, acidified with hydrochloric acid and extracted with chloroform. The chloroform layer is then passed successively through four separating funnels; the first contains sodium bicarbonate solution, the second and third 0·1N sodium hydroxide, and the fourth, water. Acidification of the bicarbonate solution in the first funnel yields any acidic decomposition products and acidic materials such as saccharin, aspirin, salicylic acid, and benzoic acid. The pure barbituric acid derivative is retained in the alkali and water washes. These are bulked and analysed spectrophotometrically, titrimetrically with silver, bromine or alkali, gravimetrically, or chromatographically. Results are given for the analysis of typical barbiturate samples. R. E. S.

Calciferol, Colorimetric Determination of. J. Büchi and H. Schneider. (*Medd. Norsk farm. Selsk.*, 1955, **17**, 87.) A detailed study of the Schaltegger method (*Helv. chim. acta*, 1946, **29**, 285) for the colorimetric determination of calciferol led to the following recommendation for an improved method: 1·00 ml. of a solution of 100 μg. of calciferol in thiophen-free benzene is placed in a 20-ml. test tube with a ground-in air condenser, and treated with 2 ml. of freshly prepared (0·15 per cent.) cumin aldehyde solution in benzene. The mixture is diluted with 4 ml. of benzene, and treated with 3 drops of perchloric acid reagent. The solution is heated on the water bath for 1½ minutes in the dark, then kept for 7 minutes in the dark, allowed to cool, and treated with 3 ml. of glacial acetic acid: 9 minutes after the heating the colour is determined in a 1 cm. cell, using filter No. 12 (550 mμ). The colour intensity is somewhat decreased by the presence of hydroquinone, but not by pyrogallol. The perchloric acid reagent used is prepared by adding 0·6 ml. of perchloric acid (60 per cent.) to a mixture of 2ml. of acetic anhydride and 2·5 ml. of glacial acetic acid. The solution is protected from moisture by a calcium chloride tube, and warmed at 95° to 100° C. for 30 minutes. G. M.

Cyanide, Thiocyanate and α-Hydroxynitriles, Determination of. R. B. Bruce, J. W. Howard and R. F. Hanzal. (*Analyt. Chem.*, 1955, **27**, 1346.) A method is described, based on the conversion of cyanide and thiocyanate into cyanogen bromide which is subsequently allowed to react with benzidine in pyridine to give an intense red colour. Air is drawn rapidly through a mixture of the sample and 20 per cent. trichloroacetic acid and into a receiving tube containing 0·1N sodium hydroxide. One drop of saturated bromine water is added to the contents of the receiving tube followed by 0·20 ml. of arsenous acid solution to remove the excess bromine. The vapours of bromine above the solution are blown off with a stream of air, 3·6 ml. of a pyridine-benzidine

mixture is then added and the red colour is allowed to develop for 15 minutes before being measured spectrophotometrically at 532 mμ; the concentration is determined from a previously prepared calibration curve. Thiocyanate is determined in the residual mixture in the aeration tube after removal of the cyanide, the contents being filtered and treated as for cyanide. α-Hydroxynitriles under alkaline conditions liberate hydrocyanic acid and this is used as a basis for the analytical procedure. In the presence of free cyanide and thiocyanate, the cyanide is determined as described above. After removal of cyanide by aeration, 10 per cent. sodium hydroxide is added to the sample in trichloroacetic acid and it is allowed to stand for 5 minutes to hydrolyse the α-hydroxynitrile. The mixture is then acidified again by the addition of trichloroacetic acid the liberated cyanide being determined as before. In experiments on cyanide and thiocyanate added to plasma 98 to 102 per cent. were recovered; 89 to 91 per cent. of cyanide was obtained from glycolonitrile and lactonitrile; recovery results of the same order were also obtained for lactonitrile added to plasma in the presence of added cyanide and thiocyanate. R. E. S.

Glucose in Invert Sugar, Iodimetric Determination of. S. O. Ericksson. (*Farm. Revy*, 1955, **54**, 441, 456.) The oxidation of glucose by alkaline iodine is dependant on the dilution, temperature, alkalinity and excess of iodine in the solution. Larger reaction volume and smaller amounts of sodium hydroxide than those previously recommended substantially reduce the oxidation of lævulose during the time necessary for quantitative oxidation of the glucose. Changes in the lævulose resulting from the action of sodium hydroxide and of bicarbonate-hydroxide buffer affect the determination, and have been studied. In the assay of invert sugar solutions changes in the lævulose are produced during the inversion of sucrose and influence the rotation of the solution and, to some extent, the determination of the glucose. The method recommended for the determination of glucose in invert sugar is as follows: 10 ml. of a 2 per cent. solution of invert sugar is treated with 30 ml. of 0·1N iodine solution and 75 ml. of 0·05M sodium hydroxide solution; 20 minutes after the addition of the alkali, the mixture is acidified with 10 ml. of 5N hydrochloric acid, and the excess of iodine is titrated back with 0·1N thiosulphate. A blank experiment is carried out similarly with 10 ml. of water. G. M.

Morphine, Polarographic Determination of. J. Holubek. (*Pharm. Zentralh.*, 1955, **94**, 347.) A method used for the determination of morphine in poppy capsules is based on the polarographic determination of 2-nitrosomorphine. Under the conditions used, with large excess of nitrous acid, the nitrosomorphine is partially converted to chinitrol and morphic acid, so that it is essential to standardise the conditions of the assay, and to standardise the method using the same conditions. For the assay, 3 g. of the dried and powdered material is extracted with 100 ml. of N hydrochloric acid, the mixture is filtered, and the extraction is repeated with a further 100 ml. of hydrochloric acid. The combined filtrates are made up to 250 ml, and to 5 ml. of this solution is added 2 ml. of N sodium nitrite solution. After exactly 5 minutes 3 ml. of potassium hydroxide (20 per cent.) is added and 7 drops of gelatin solution (0·5 per cent.). The morphine is then determined by polarograph. Results agree well with those obtained colorimetrically. G. M.

(—)-Noradrenaline and (—)-Adrenaline, Polarographic Measurement of. J. Henderson and A. Stone Freedberg. (*Analyt. Chem.*, 1955, **27**, 1064.) (—)-Adrenaline and (—)-noradrenaline were converted by iodate oxidation to

iodoadrenochrome or iodonoradrenochrome and aliquots of the reaction solutions were measured polarographically. Half-wave potentials for iodoadrenochrome and iodonoradrenochrome were $E_{1/2} = +0{\cdot}03$ volt and $E_{1/2} = +0{\cdot}02$ volt, respectively, in 0·1M acetic acid-acetate buffer, pH 4·52, 0·01 per cent. in gelatin. Polarographic measurements on aliquots containing the equivalent of about 20 μg. of amine gave diffusion currents from which the amount of adrenaline or noradrenaline could be calculated by referring to standard curves. A linear relation between concentration and diffusion current was found for 1 to 50 μg. quantities of adrenaline or noradrenaline; the error of the method was 5 to 10 per cent. Adrenaline and noradrenaline could not be measured individually in the same solution, but by using a paper chromatographic method for separation prior to polarographic analysis, eluates containing these amines were collected, converted to the iodo derivatives in the same test tubes and analysed polarographically. Coloured or fluorescent substances normally found troublesome in other methods for the measurement of catechol amines, did not interfere. R. E. S.

Opium, Assay of, by Paper Chromatography. A. B. Svendsen, E. D. Aarnes and A. Paulsen. (*Medd. Norsk farm. Selsk.*, 1955, **17**, 116.) 2·5 g. of opium is rubbed down with 5 ml. of concentrated acetic acid and the mixture filtered through sintered glass (3G3) and the filter is then washed with 5 per cent. acetic acid to a total volume of 50 ml. 25 ml. of this solution is made alkaline with ammonia and shaken out repeatedly with chloroform-*iso*propanol (3 + 1). The solvent is evaporated off, and the residue is dissolved in 5 per cent. acetic acid to a volume of 25 ml. For the chromatography a mixture of butanol, toluene, acetic acid and water (20:10:3:9) is used, the R_F value for morphine being about 0·15. A number of spots with differing quantities of morphine are applied to the paper, and one strip of the chromatogram is cut out and developed with Folin and Ciocalteu reagent. The corresponding areas of the other strips are then cut out for the determination, each spot being treated with 5 ml. of diluted reagent (1·5 ml. of Folin and Ciocalteu reagent diluted to 25 ml. with water). After 2 hours 5 ml.of sodium hydroxide solution (10 per cent.) is added and after a further hour the extinction is measured at 700 mμ. There is good agreement between the results obtained and assays by the Swiss official method. G. M.

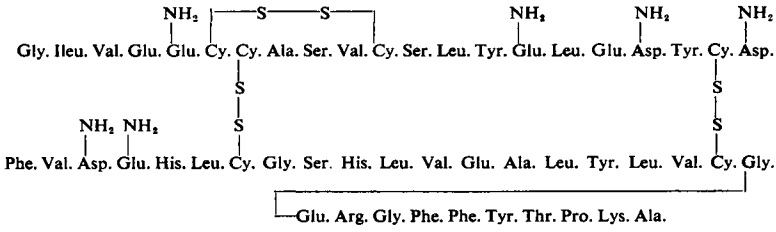
***Rhamnus frangula*, Glycoside Content of.** H. Mühlemann and H. Schmid. (*Pharm. Acta Helvet.*, 1955, **30**, 363.) A method of isolation of a mixture of reduced genuine glycosides, in acetylated form, from *Rhamnus frangula* bark is described. This mixture consists of varying amounts of glucofrangulinanthranol acetate and a bimolecular glucofrangulindihydrodianthranol acetate, resembling the sennosides described by Stoll. On storage of the drug, the bimolecular form is converted into glucofrangulinanthrone and finally into glucofrangulin. A simultaneous hydrolysis to frangulin, its reduced forms and corresponding aglucones is probable, but could not be confirmed. The bioside content of the drug decreases on storage. G. M.

Silicon, Colorimetric Determination of. E. J. King and B. D. Stacy. (*Analyst*, 1955, **80**, 441.) The removal of phosphate by basic ferric acetate precipitation prior to the determination of silica has been critically investigated; it was found that the method could give inaccurate results unless strict control of the pH were observed owing to (*a*) incomplete removal of phosphate, or (*b*) removal of

silica with phosphate. Both phosphate and silica were found to couple with molybdate in weak acid, but only the silicomolybdate complex was reduced in strongly acid solutions (Milton, *J. Appl. Chem.*, 1951, **1**, Suppl. Issue No. 2126); following this principle a new procedure for the determination of silica in biological material was developed, in which phosphate was left in solution. Iron, in amounts greater than those occurring in tissue, did not interfere. Two methods were evolved to overcome interference in urines with high phosphate concentrations by precipitation of ammonium phosphomolybdate; either phosphate was partly or completely removed by precipitation with calcium hydroxide, or ammonia and urea were removed with nitrous acid and sodium molybdate was used to couple with silica. The new colorimetric procedure gave good agreement with gravimetric analyses, and good recoveries of added silica were obtained with tissue, blood and urine. The procedure likewise gave results in good agreement with gravimetric methods when applied to small samples of mineral dusts. Details of the method are given together with tables of comparative results for silica analyses by different methods. R. E. S.

ORGANIC CHEMISTRY

Insulin, Disulphide Bonds, and Structure of. A. P. Ryle, F. Sanger, L. F. Smith and R. Kitai. (*Biochem. J.*, 1955, **60**, 541.) Experiments are described which were undertaken to determine the specific linking of the various half-cysteine residues of polypeptide fractions A and B (obtained by the action of performic acid) into the disulphide bonds which join the two fractions together in the intact molecule. Insulin was subjected to partial hydrolysis with chymotrypsin, with a crude pancreatic extract and with acid under conditions such that the disulphide bonds remained intact. This was followed by fractionation of cysteine peptides, oxidation of the latter to cysteic acid peptides, and finally identification of the resulting amino-acids after fractionation by paper ionophoresis. The structure of the cysteine peptides obtained in this way indicated the distribution of the dipeptide bonds in insulin, giving the complete structure for insulin.



J. B. S.

Insulins of Pig and Sheep, Structure of. H. Brown, F. Sanger and R. Kitai. (*Biochem. J.*, 1955, **60**, 556.) The structures of pig and sheep insulins have been determined by oxidative degradation into the respective glycyl (fraction A) and phenylalanyl (fraction B) chains. Each of these fractions was treated with proteolytic enzymes and the resulting large peptides were separated by paper ionophoresis, and finally subjected to complete hydrolysis. Certain of the large peptides were also partially hydrolysed to smaller peptides which were readily separated and identified by chromatography on paper, and which also were representative of all the residues in the insulin chains. In this way it was shown

that the phenylalanine chains have the same amino-acid sequence in pig, sheep and cattle insulins. The glycyl chains show a small variation in the three insulins, the three residues in the 8, 9 and 10 positions being Ala. Ser. Val. in cattle insulin, Thr. Ser. Ileu. in pig insulin, and Ala. Gly. Val. in sheep insulin.

J. B. S.

BIOCHEMISTRY

BIOCHEMICAL ANALYSIS

Glycyrrhetinic Acid in Urine, Estimation of. V. M. van Katwijk and L. G. Huis in 'T Veld. (*Rec. Trav. chim. Pays-Bas*, 1955, **74**, 889.) Using the method described, 20 mg. of glycyrrhetinic acid can be estimated quantitatively in a 24 hour sample of human urine. For the extraction, take 400 ml. of the sample, add 40 ml. of 25 per cent. hydrochloric acid, 200 ml. of chloroform and reflux for 10 minutes to hydrolyse any readily saponifiable esters. Cool, remove the chloroform extract and re-extract the aqueous layer twice by shaking with 150 ml. of chloroform in a separating funnel. Evaporate the combined chloroform extracts under reduced pressure and dissolve the residue in a measured volume (10 or 20 ml.) of 96 per cent. ethanol. The extract is purified chromatographically. A quantity of the extract corresponding to 1/5th of the 24 hour sample is taken, the solvent is evaporated and 50 ml. of benzene is added to the residue. 7 g. of floridine earth are brought into a chromatographic column 15 mm. in diameter and moistened with benzene. The benzene solution of the urinary extract is poured over the column, and elution carried out as follows.

3 × 50 ml. benzene (eluates 2–4).
2 × 50 ml. benzene + 0·1 per cent. ethanol (eluates 5–6).
10 × 50 ml. benzene + 0·5 per cent. ethanol (eluates 6–16).
4 × 50 ml. benzene + 1·0 per cent. ethanol (eluates 17–20).
6 × 50 ml. benzene + 2·0 per cent. ethanol (eluates 21–26).

For the spectrophotometric estimation 5 ml. of each eluate is pipetted into a 10 ml. flask, the solvent is carefully evaporated and the residues are dissolved in 96 per cent. ethanol. The extinctions of these solutions are determined at 248 mμ. No glycyrrhetinic acid could be demonstrated in the urine of patients who received orally 1·33, 2·28 and 2·5 g. from which it is concluded that less than 2 per cent. of orally administered doses are excreted as such or in the form of a salt or an easily saponifiable ester.

G. F. S.

α-Keto-acids, 1 : 2-Diamino-4-nitrobenzene as a Reagent for. K. W. Taylor and M. J. H. Smith. (*Analyst*, 1955, **80**, 607.) A paper-chromatographic method is given for the separation and detection of α-keto-acids in blood and urine, based on the use of 1 : 2-diamino-4-nitrobenzene as a reagent for α-keto-acids. Blood was added as quickly as possible to freshly prepared 5 per cent. w/v metaphosphoric acid, set aside for 10 minutes at room temperature, centrifuged and the supernantant liquid removed; 3 ml. of 0·2 per cent. w/v 1 : 2-diamino-4-nitrobenzene in 0·66N hydrochloric acid was then added and the mixture allowed to stand for 12 to 16 hours. After extraction with ethyl acetate the nitroquinoxalinols were extracted into 5 per cent. w/v sodium carbonate solution together with a small amount of unused reagent which was removed by washing with ether. The carbonate phase was then adjusted to pH 4 with 10N hydrochloric acid and re-extracted with ethyl acetate. The combined ethyl acetate extract was evaporated to dryness at a temperature not exceeding 40° C., the dry residue dissolved in acetone and chromatographed

for about 8 hours using descending chromatography with a solvent mixture consisting of 5 parts of ethanol, 8 parts of *n*-pentanol and 6 parts of ammonia. The nitroquinoxalinols appeared as yellow spots which faded rapidly on removal from the solvent system; the spots were eluted with 30 per cent. aqueous ethanol and optical densities after filtration measured at 280 mμ. Results are given for R_F values and ultra-violet absorption spectra of the nitroquinoxalinols of pyruvic acid and α-ketoglutaric acid. A number of compounds normally present in blood and urine were examined for possible interference with the method but this did not occur; α-ketoglutaric and pyruvic acids were detected in 15 blood and urine specimens. Results are given for normal blood levels of pyruvic and α-ketoglutaric acids and for recovery experiments using known amounts of keto-acids added to blood; from 67 to 77 per cent. of added pyruvic acid and from 77 to 100 per cent. of added α-ketoglutaric acids were recovered. R. E. S.

Lead in Urine, Determination of. W. M. McCord and J. W. Zemp. (*Analyt. Chem.*, 1955, **27**, 1171.) Lead, in an acid solution and in the presence of excess potassium iodide, is extracted quantitatively by methyl *iso*propyl ketone, followed by extraction from the ketone into an aqueous sodium hydroxide solution and development of colour with dithizone using the method of Snyder (*Analyt. Chem.*, 1947, **19**, 684). A first extraction is performed in the presence of sodium or potassium cyanide at pH 9·5 to 10·0 and interfering ions are limited to stannous tin, thallium, and bismuth; stannous tin and thallium were ignored, the bismuth being removed by an acid chloroform-dithizone extraction into a buffer solution at a pH of 3·4. An aqueous solution of the lead was then adjusted to pH 11·5 with ammoniacal cyanide solution and extracted with dithizone solution, the lead as lead dithizonate being quantitatively transferred to the chloroform layer. A low, but constant, concentration of unreacted dithizone in the chloroform layer improved the accuracy and reliability of the results as compared with lower pH methods; the high pH extraction also permitted the use of a standard dithizone solution and eliminated the necessity for titrimetric extraction. It was found that the range of sensitivity of the method, 0 to 70 μg. of lead using a 22 mm. cell was sufficient for the determination of lead in mild chronic lead poisoning. Satisfactory recoveries were obtained for quantities of lead up to 35 μg. added to urine. R. E. S.

Magnesium in Serum, Estimation of. A. J. Smith. (*Biochem. J.*, 1955, **60**, 522.) A colorimetric method is described for the routine estimation of magnesium in serum. Calcium can also be estimated in the same sample of serum. To 2 ml. of serum add 1 ml. of saturated ammonium oxalate. Mix, allow to stand for 30 minutes, centrifuge, add 2 ml. of the supernatant to 8 ml. of distilled water and add 10 ml. of 10 per cent. trichloroacetic acid solution. Mix, and after 10 minutes filter through Whatman No. 42 filter paper. Take 10 ml. of the filtrate and add 3 ml. of 8 per cent. v/v ammonia and 5 ml. of buffer (prepared by dissolving 6·75 g. of ammonium chloride A.R. in distilled water, adding 57 ml. concentrated ammonia A.R. and making up to 1000 ml. with distilled water giving a pH of 10·1). Mix and add 2 ml. of a dye solution (0·1 per cent. eriochrome black T in absolute methanol). Determine within 5 minutes the extinction at 520 mμ in a spectrophotometer and compare with a parallel series of standard solutions of magnesium acetate containing 0, 4, 8, and 16 μg. Mg to which are added 0·4 ml. of saturated ammonium oxalate, 10 ml. of 10 per cent. trichloroacetic acid, the volume made up to 20 ml., filtered and 10 ml. of the filtrate treated in the same way as the serum filtrate. The method is accurate to ±5 per cent. G. F. S.

PHARMACY

NOTES AND FORMULÆ

Ascorbic Acid, Decomposition of Solutions. F. F. Jensen. (*Dansk Tidsskr. Farm.*, 1955, **29**, 125.) Aqueous solutions of ascorbic acid (2 per cent.) were prepared with varying concentrations of dissolved oxygen, i.e., oxygen-free, in equilibrium with air (6·20 ml. per litre), and an intermediate concentration. After heating for prolonged periods at 90° and 100° C., determination of the ascorbic acid showed that there was no difference between the three series. Similar results were obtained at other pH values ranging from 2·55 to 9·90. The less acid solutions showed a considerable drop in pH. Decomposition was at its slowest at pH 6·30. When, however, similar solutions were heated in ampoules containing a considerable volume of air, the decomposition was more rapid at pH 6·30 than at 2·55. Catalysts are not of significance in the oxygen-free reaction. It was not found possible to demonstrate the presence of 2-ketogulonic acid as the result of this reaction. It was observed, however, that after heating solutions of ascorbic acid at pH 1·10, considerable pressure was produced in the ampoules, due to the formation of carbon dioxide. The reaction involved has not yet been elucidated. G. M.

Morphine Injection, Stability of. E. Gundersen and J. Mørch. (*Dansk Tidsskr. Farm.*, 1955, **29**, 181). The decomposition of morphine to oxydimorphine in solution was followed by means of the vanillin reaction, as used by Foster, MacDonald and Whittet (*J. Pharm. Pharmacol.*, 1950, **2**, 673). The absorption maximum however appears to be at 640 mμ, not at 600 mμ as previously reported. It is important to use pure vanillin. Discolouration of the morphine solution was measured at 500 mμ, as these solutions show no maximum or minimum. The presence of glycerol does not interfere. The results confirm the conclusions of previous workers, and show that there is no appreciable decomposition after sterilisation for 20 minutes at 120° C. (pH = 4·1), although there is a slight discolouration, which may be prevented by the addition of sulphite. Various samples of 2 per cent. morphine injection, having an age of 10 years, showed from 1·6 to 4·4 per cent. decomposition.
G. M.

PHARMACOLOGY AND THERAPEUTICS

Adrenaline and Noradrenaline in Tissues. K. Montagu. (*Nature, Lond.*, 1955, **176**, 555.) A study of the distribution of noradrenaline in rats does not support the hypothesis that its concentration can be correlated with the density of adrenergic nerve fibres. Two and a half hours after the subcutaneous injection of insulin there is a significant increase in the adrenaline catechol ratio in the heart, kidney, liver, diaphragm and leg muscles. There are significant increases in the absolute concentration of adrenaline in heart and kidney, and a significant increase in noradrenaline in the heart. Six weeks after bilateral demedullation, and 3 to 4 weeks after bilateral adrenalectomy, there is a reduction in the adrenaline concentrations of heart and kidney. In the kidney, noradrenaline is reduced, but in the heart it is increased. In demedullated and adrenalectomised rats, insulin does not increase significantly the adrenaline concentration of either heart or kidney. The altered effect of insulin on the concentration of adrenaline and noradrenaline, brought about by operation, is very significant

for heart muscle. The changes seem to depend on medullary secretion, while the presence of high concentrations of adrenaline and noradrenaline in heart and kidney, some weeks after adrenalectomy, suggests an ability of the rat to synthesise, or store the amines, in these organs. G. F. S.

Butylamine, A Substituted, (L1935), Release of Histamine by: Comparison with Compound 48/80. W. Feldberg and J. Lecomte. (*Brit. J. Pharmacol.*, 1955, **10**, 254.) L 1935, an equimolecular mixture of 3-(4′-hydroxyphenyl)-3-(4″-hydroxy-3″-methylphenyl)-1-methylpropylamine and its dehydro form, caused a release of histamine from perfused skin flaps of the cat's hind legs and from the perfused hind quarters of the rat. Weight for weight it was 10 to 12 times less active than compound 48/80. Injected intravenously into cats, the drug caused a fall in blood pressure after a latent period of 20 to 40 seconds, characteristic of the histamine liberators. Tachyphylaxis resulted from repeated injections. On the isolated guinea-pig ileum L 1935, added to the bath in high concentration, caused a transient contraction and after washing out, the ileum showed increased spontaneous activity and decreased sensitivity to histamine and acetylcholine. Similar effects were obtained with 48/80; the two substances were equiactive on the ileum. G. P.

Capsaicin and Analogues, Pharmacological Actions of. C. C. Toh, T. S. Lee and A. K. Kiang. (*Brit. J. Pharmacol.*, 1955, **10**, 175.) Intravenous injection of capsaicin into cats under chloralose anæsthesia caused apnœa, which was either abolished or greatly reduced by cooling the vagi to 9 to 10° C. Any residual apnœa disappeared with further cooling to 2 to 3° C. The site of action was traced to receptors in the lungs, probably pulmonary stretch receptors. The drug also caused a fall in blood pressure and heart rate, abolished by cutting the vagi; receptors in the coronary circulation were probably involved. With injections of capsaicin into the carotid sinus circulation, apnœa and a fall in blood pressure were again observed and were greatly reduced by cutting the sinus nerve. Sensitisation of the baroreceptors of the carotid sinus was suggested as causing the vasodepressor action. After injection into the splanchnic circulation a rise in blood pressure was obtained, abolished by removal of the superior and inferior mesenteric plexuses. The drug acted on undetermined receptors in the skeletal muscles causing hyperpnœa and variable effects on the blood pressure when injected close-arterially into the muscle circulation. These effects were absent after section of the nerve supplying the muscle. Capsaicin contracted the isolated guinea-pig ileum, which rapidly became tachyphylactic to it, but the drug had no effect on the rat uterus or perfused rabbit ear vessels. The analogues of capsaicin, vanillylamine and vanillyl acetamide had little or no pharmacological activity. Vanillyl-*n*-decoylamide, on the other hand, had similar activity to capsaicin. G. P.

Carboline Unsymmetrical Bis-quaternary Hypotensive Agents, Pharmacology of. T. B. O'Dell, C. Luna and M. D. Napoli. (*J. Pharmacol.*, 1955, **114**, 306.) Study of a group of carboline unsymmetrical bis-quaternary derivatives has shown many of them to be potent hypotensive agents having primarily a "central" site of action with varying degrees of peripheral effects. The hypotensive action in the anæsthetised dog was biphasic, an initial rapid fall with recovery in twenty minutes, followed by a more sustained secondary fall. Only the initial fall was prevented by atropine or vagotomy. In the series, increase of the methylene chain to more than three decreased the duration of action. Variations in the small or the large (carboline) cationic heads caused pronounced

differences in hypotensive action. The more potent compounds were effective by intramuscular injection or following injection into the small intestine. In the series there was very little correlation between hypotensive activity and sympathetic ganglion blockade, tested on the nictitating membrane of the cat. The most potent ganglionic blocking compound was the *n*-methyl tetrahydroharman derivative. Toxic doses of the compounds caused death from repiratory depression and increase in the length of the methylene chain increased toxicity *In vitro*, on the isolated rabbit and guinea-pig ileum, the compounds showed varying degrees of blockade of acetylcholine, but they did not antagonise the vasodepressor action of acetylcholine in the dog. The vasopressor action of adrenaline was not blocked, but was potentiated by compounds causing ganglionic blockade. Some of the compounds caused dilatation and fixation of the pupils in male cats and humans. Several of the compounds blocked nicotine convulsions in mice. In unanæsthetised dogs large intravenous doses caused transient staggering, shivering and sometimes collapse. Mice and monkeys showed ptosis and became sleepy. G. F. S.

Catechol Amines Injected in Man, Amine Oxidase in the Inactivation of. U. S. von Euler and B. Zetterström. (*Acta physiol. scand.*, 1955, **33**, Suppl. 118, 26.) There was no significant difference between the percentage of adrenaline, noradrenaline and corbasil (3 : 4-dihydroxynorephedrine) excreted in the free form in the urine during the two hour period following their subcutaneous injection into healthy adults. Very little of any of the amines was excreted in the conjugated form. Since corbasil, although being unaffected by amine oxidase, is inactivated to about the same extent as adrenaline and noradrenaline, it may be inferred that some other enzyme system must play a significant part in the destruction of circulating catechol amines. M. M.

Cortisone and ACTH; Treatment of Blood Disorders. (*Brit. med. J.*, 1955, **2**, 455.) This is the Third Report to the M.R.C. by the Panel on the Hæmatological Application of ACTH and Cortisone, previous reports having been submitted in 1952 and 1953. Dosage tended to be higher in this than in the two previous series, most patients receiving at least 200 mg. of cortisone a day in their initial course of treatment. With the increased availability of cortisone tablets for oral administration the number of patients treated with cortisone rather than ACTH steadily increased. Twenty-eight further cases of acquired hæmolytic anæmia were treated, making a total of 48 cases treated since 1951. Of this number partial or complete response was obtained in 39 (81 per cent.). When a favourable response occurred this was always apparent within 2 weeks of starting an adequate dosage of cortisone. Eight patients were given continuous treatment with cortisone for at least 6 months, the dose requirement varying from 50 to 200 mg. a day. In all these patients a relapse could be induced promptly by stopping cortisone or reducing the dosage. In 7 of the patients there were no apparent toxic effects from the treatment; the eighth patient developed a gastric ulcer. Of 26 new cases of idiopathic thrombocytopenic purpura complete responses were obtained in 12 and partial responses in a further 10; these results were more favourable than those reported in 1952 (partial or complete responses in 11 out of 16) or in 1951 (partial or complete responses in 8 out of 14). This was probably due to the use of larger doses. Of 8 cases of non-thrombocytopenic purpura 6 failed to respond in spite of adequate dosage. No favourable response to cortisone was noted in 16 patients with aplastic anæmia or in 2 with refractory anæmia. In acute leukæmia the

administration of ACTH or cortisone induced temporary remissions in approximately half the patients treated (34 remissions in 49 children, and 22 remissions in 52 adults) and compared favourably with other forms of treatment. Remissions, however, were not of long duration, and few patients survived for more than a year. S. L. W.

Cortisone and Aspirin in the Treatment of Rheumatoid Arthritis. (*Brit. med. J.*, 1955, **2**, 695.) This is the second Report by the Joint Committee of the Medical Research Council and Nuffield Foundation on Clinical Trials of Cortisone, ACTH, and Other Therapeutic Measures in Chronic Rheumatic Diseases. The report relates to the second year of treatment of the 58 adult patients who completed the first year's treatment as described in the committee's first report. Dosage was determined by the physician in charge in accordance with each patient's need. The doses of cortisone in use at the end of the year were from 25 to 125 mg. per day, 17 of the 26 patients receiving either 75 or 100 mg. per day. The doses of aspirin were from 2 to 6·7 g. per day, 13 of the 20 patients receiving either 4 or 5 g. per day. Treatment of one patient in each group had to be discontinued during the year because of severe side-effects and 10 patients were not receiving treatment at the end of the year. Appraisal was based on joint tenderness, range of movement in the wrist, strength of grip, manual dexterity, hæmoglobin level, blood sedimentation rate, X-ray studies of the hands and feet, and clinical assessment. The results showed remarkable similarity between the two treatment groups, and in some cases they were even closer than at the end of the first year; in particular, the previously reported advantage of the cortisone group in respect of hæmoglobin level and sedimentation rate had disappeared. The X-ray investigation, by three independent observers, was a new measure of assessment; the aspirin group showed more erosion but the difference was not statistically significant. Side effects occurred in 19 patients of the cortisone group and 12 of the aspirin group. In the cortisone group the most frequent were œdema of the ankles, moon-face, depression, euphoria and obesity, and in the aspirin group, were nausea, dyspepsia or anorexia, tinnitus and œdema of the ankles.

H. T. B.

Demecolcine (Desacetylmethylcolcine) Intravenously in Acute Gout. W. C. Kuzell, R. W. Schaffarzick and W. E. Naugler. (*Arch. intern. Med.*, 1955, **96**, 153.) Demecolcine (Colcemid), an alkaloid from the meadow saffron, differing from colchicine structurally in the replacement of the acetyl by a methyl radical, was given intravenously to a series of 20 patients with acute gout. The patients ranged in age from 42 to 83 years and the known duration of gout from 1 to 45 years. Only acute attacks were treated. Of the 20 patients, 15 enjoyed complete remission within 48 hours after the intravenous administration of 1 to 4 mg. of demecolcine, and 4 others obtained partial amelioration of symptoms. The only undesirable side reaction was the occurrence of diarrhœa in 2 patients. In contrast with intravenous colchicine, there was a marked absence of venous irritation. S. L. W.

β-Dimethylaminoethyl-2-methylbenzhydryl Hydrochloride (B.S. 5930) in the Treatment of Parkinsonism. R. O. Gillhespy and A. H. Ratcliffe. (*Brit. med. J.*, 1955, **2**, 352.) This compound, belongs to the class of substance which antagonises both histamine and acetylcholine, of which the best known example is diphenhydramine hydrochloride. It has about the same toxicity in mice as

diphenhydramine. The compound was tried in the treatment of 67 patients suffering from Parkinsonism, and a clinical improvement was noted in 39 cases. The results were considered good or excellent in 31 cases (46 per cent.). Patients were given 150 mg. of the compound daily divided into three 50-mg. doses. In some cases the amount was increased to 250 mg. daily. Above that dose signs of intolerance developed, including restlessness, slight blurring of vision and nausea, but there were few unpleasant side-effects if the daily dose was kept below 250 mg. The authors conclude that B.S. 5930 compares favourably with other drugs for the treatment of the Parkinson syndrome and is a most useful addition to the range at present available. A new method of carrying out clinical trials of this type is described and its advantages discussed.

S. L. W.

p-Hydroxybenzoic Esters, Action of, Against *Candida albicans*. T. Sabalitschka, H. Marx and U. Scholz. (*Arzneimitt.-Forsch.*, 1955, **5**, 259.) Esters of *p*-hydroxybenzoic acid have already been used with some success in cases of monialisis, and the authors now report the results of experiments *in vitro* on the action against the causative organism—*Candida albicans*, Robin. Six strains of the organism were used. The most effective compounds were the butyl esters, preferably in admixture. The action of the methyl ester was less marked, and the difference was more noticeable at low temperatures (20° C.) than at 37° C. At 37° C. the effect of the esters is only slightly decreased by the presence of bouillon.

G. M.

Iron by Intramuscular Injection in Infancy. W. Gaisford and R. F. Jennison. (*Brit. med. J.*, 1955, **2**, 700.) More than 100 infants with various types of anæmia were treated with intramuscular injections of Imferon, an iron-dextran complex containing 50 mg. of iron per ml. The injections were given deeply, into the upper and outer quadrant of the buttock, either daily, or alternate days or weekly, the dosage being calculated from the formula

Fe required in mg. $= 1{\cdot}31 \times (19 - \text{Hb mg.}) \times \text{wt. in lb.}$

There was marked and rapid hæmatological and clinical improvement, the average rate of response being from 1·4 to 1·5 per cent. of hæmoglobin per day. Provided the dosage calculated to be equivalent to the iron deficit is not exceeded, no unpleasant reactions are produced and discomfort is of short duration. Although not a substitute for oral iron as a routine the preparation is valuable for the treatment of severely anæmic infants or those who cannot tolerate, or are resistant to, iron by mouth.

H. T. B.

Magnesia and Alkaline Carminatives in Infancy. R. D. G. Creery. (*Brit. med. J.*, 1955, **2**, 178.) Of 200 apparently healthy infants aged from 6 months to 3 years, 184 (92 per cent.) had at some time been given alkaline mixtures by their mothers. Magnesia had been given to 158 (79 per cent.), generally for regulation of the bowels, and 133 (66·5 per cent.) had received carminatives, usually for "wind". Both magnesia and carminatives had been given to 107 (53·5 per cent.). Often only an occasional teaspoonful had been given but in a considerable number of cases the mixtures had apparently been persisted with for prolonged periods. Although not actively harmful (alkali reserve levels were always within normal limits), this habit of dosing infants with alkalis is meddlesome and unnecessary and should be discouraged. The medicaments are often given for symptoms which exist only in the imagination of the mother. The frequent resort to magnesia is due to the obsession that infants should

defæcate daily. The crying and fretfulness often attributed to "wind" may well be due to unsatisfied hunger. One of the most popular of the proprietary carminative mixtures contains about 4 per cent. of ethanol which, acting as a mild soporific, may possibly serve to allay hunger and induce sleep. S. L. W.

Methiodides of Dimethylaminobenzoylhydroxamic Acids, Antagonists of Dyflos. A. Funke, G. Benoit and J. Jacob. (*C. R. Acad. Sci., Paris*, 1955, **240**, 2575.) The isomeric dimethylaminobenzoylhydroxamic acids, *o*, *m* and *p*, were obtained in the form of their sodium salts by the action of hydroxylamine on the corresponding esters. The hydrochlorides were prepared by acidifying with hydrochloric acid and the quaternary ammonium compounds by the action of an excess of methyl iodide on the sodium salts. The effect of these compounds was investigated in mice. In a series of protective tests in which the quaternary salts were administered 15 minutes before an injection of 6 mg./kg. of dyflos in water, the coefficient—dose killing 50 per cent./dose protecting 50 per cent.—was better than for nicotine hydroxamic acid methyl iodide. The administration of atropine with the *m*-compound enabled almost complete protection to be obtained. In the curative tests the compounds merely retarded onset of death, although a certain proportion of the animals lived when given atropine as well. Again the best results were obtained with the *m*-isomer. G. B.

Morphine, Mechanism of Pituitary-Adrenal Activation by. R. George and E. L. Way. (*Brit. J. Pharmacol.*, 1955, **10**, 260.) Morphine and (+)- and (−)-methadone all caused a significant fall in the ascorbic acid content of the adrenal glands of rats in a dose of 1/5 LD50, but not with a dose of 1/15 LD50. In hypophysectomised animals these doses had no effect on the adrenal ascorbic acid. After bilateral adrenal demedullation, on the other hand, much lower doses of the three analgesics caused an appreciable depletion. Pretreatment with cortisone did not prevent depletion of ascorbic acid by morphine, aspirin or adrenaline, but reduced the severity of depletion. Nalorphine inhibited depletion by morphine and by (−)-methadone, but not by (+)-methadone or aspirin. This may suggest that different receptors or sites are involved in the mediation of the depleting action, possibly in the hypothalamus. G. P.

Nitrofurantoin; Clinical and Laboratory Evaluation. B. A. Waisbren and W. Crowley. (*Arch. intern. Med.*, 1955, **95**, 653.) *In vitro* studies of the antibacterial activity of nitrofurantoin show that it has a wide antibacterial spectrum, is bactericidal, and does not readily invoke resistant mutants. It was found particularly effective against *Micrococcus pyogenes* and *Proteus* and ineffective against *Pseudomonas æruginosa*. Sixty patients with infections of the urinary tract were treated with nitrofurantoin, in an average daily dose of 300 to 400 mg. The results were satisfactory in 27 cases, unsatisfactory in 12 cases and indeterminate in 21 cases; 14 of the 27 cases in whom treatment was effective had received prior unsuccessful therapy with antibiotics. Nine of the 60 patients had difficulty in tolerating nitrofurantoin owing to nausea and vomiting, but in only 1 case was it necessary to discontinue treatment. In none of the patients was there any evidence of rectal irritation or of depression of hæmopoiesis due to nitrofurantoin, nor were skin rashes or other manifestations of hypersensitivity encountered. Nitrofurantoin did not cause diarrhœa in any of the patients. Judged as a whole the clinical results obtained were satisfactory and equal to those obtainable with any other single drug. S. L. W.

Noradrenaline and Adrenaline Content in Cat Organs, Effect of Increased Adrenergic Nerve Activity on. U. S. von Euler and S. Hellner-Björkman. (*Acta physiol. scand.*, 1955, **33**, Suppl. 118, 17.) Electric stimulation of the splenic nerves of the cat caused no significant change in the adrenaline and noradrenaline content of the stimulated portion of the spleen, when compared with the non-stimulated portion. Increased reflex adrenergic activity, elicited by occlusion of the carotid artery or by denervation of the carotid sinus, also caused no significant change in the noradrenaline content of the spleen, heart and liver. These facts suggest that the release of adrenergic transmitter is normally followed by rapid resynthesis, thus allowing the store of amine to be maintained at an approximately constant level. M. M.

***iso*Quinoline Unsymmetrical Bis-quaternary Hypotensive Agents, Pharmacology of.** T. B. O'Dell, C. Luna and M. D. Napoli. (*J. Pharmacol.*, 1955, **114**, 317.) A series of unsymmetric bis-quaternary derivatives of *iso*quinoline and related compounds have been studied for hypotensive activity. Many of them are potent hypotensive agents with primarily a central site of action, showing a biphasic fall in blood pressure in the anæsthetised dog. The bis-quaternary salt seemed essential for overall hypotensive activity. Increasing the methylene chain showed a chain length of three to provide maximum activity. Variations in the small cationic head resulted in very pronounced differences in activity, while substitution on the large cationic (*iso*quinoline) head caused considerable variations in hypotensive activity. Derivatives of the benz*iso*quinolines were generally more active and more toxic than the simple *iso*quinoline analogues. Most interesting were the tetrahydro derivatives (tetrahydro*iso*quinoline and benztetrahydro*iso*quinoline) all of which were more active than the analogous *iso*quinoline derivative. There was no correlation between hypotensive activity and sympathetic ganglion blockade. Toxic doses caused respiratory paralysis. *In vitro* the compounds showed varying degrees of blockade to acetylcholine but they did not block the vasodepressor action of acetylcholine in the dog. The vasopressor action of adrenaline was not blocked or depressed by any of the compounds. G. F. S.

Roter Tablets in the Treatment of Peptic Ulcer. R. R. Hamilton. (*Brit. med. J.*, 1955, **2**, 827). Seventy-nine cases of peptic ulcer and 19 of presumptive peptic ulcer were treated with Roter tablets. Immediate clinical response was satisfactory in 90 per cent. of patients (81 per cent. became symptom-free within 2 weeks), with a relapse rate of 57 per cent. in the first year. A reduction in the average number of relapses was recorded in 15 cases kept on a maintenance dose for 1 year. Roter tablets contain heavy magnesium carbonate 400 mg., sodium bicarbonate 200 mg., bismuth subnitrate 350 mg., calamus 25 mg., an aromatic bitter, and frangula. The patients (all of whom were ambulant) were instructed to take 2 tablets three times a day after food for 3 weeks; thereafter 1 tablet three times a day for 2 months. Fifteen patients with a long history of frequent relapses continued on a maintenance dose of 2 tablets daily for a year. The patients were advised to take an average diet but to avoid fried foods. In 75 per cent. of the cases the patients were of the opinion that the tablets were superior to alkaline powders, and they found they were able to take foods they had avoided for years. No serious toxic effects were observed in any of the patients treated. Though the author admits that a study of the individual drugs contained in these tablets yields no solution to their mode of action, he considers that the treatment is of value for general practice, where its simplicity appeals to both doctor and patient. S. L. W.

Serotonin Release as a Possible Mechanism of Reserpine Action. A. Pletscher, P. A. Shore and B. P. Brodie. (*Science*, 1955, **122**, 374.) Reserpine (5 mg./kg.), injected intraperitoneally in rabbits, effected the release of up to 85 per cent. of total serotonin in intestinal tissue. The serotonin content of the small intestine decreased progressively for about 16 hours after administration of the reserpine and, after remaining constant for a further 16 hours, regained normal levels after 5 days from injection. Serotonin depletion in the intestine was demonstrable with doses down to 0·25 mg./kg. Phenobarbitone and barbitone in doses inducing deep narcosis had no effect on intestinal serotonin content. The results were consistent with the view that reserpine owes some of central sedative actions to the release of serotonin.

G. P.

Steroid Anæsthesia in Surgery. F. J. Murphy, N. P. Guadagni and F. DeBon. (*J. Amer. med. Ass.*, 1955, **158**, 1412.) This is a report on the use of the steroid Viadril (21-hydroxypregnanedione sodium succinate) as a basal anæsthetic on 125 patients in a variety of surgical procedures. Viadril is a white, crystalline substance, soluble in water and having an alkaline reaction (pH 8·5 to 9·8). It is administered intravenously in a freshly prepared 2·5 per cent. solution in either distilled water or isotonic sodium chloride solution. The Viadril solution is introduced via the intravenous tubing into an intravenous drip of 5 per cent. glucose in distilled water, so that mixing and dilution of the two solutions takes place in the tubing before the needle is reached. Approximately 5 minutes is required to give 1500 mg. and an additional 5 minutes is allowed before the patient is considered ready for further manipulation. In this series pre-medication usually consisted of administration of 50 to 75 mg. of pethidine hydrochloride, and hyoscine or atropine in the appropriate dosage. In the lightly pre-medicated patient Viadril produces sleep in 5 to 10 minutes, without an excitement phase. The authors conclude that Viadril is a true anæsthetic agent, as evidenced by its ability to control pain, obtund reflexes, produce relaxation, and produce sleep, without depression of vital functions. Most satisfactory results have been obtained with administering the compound with nitrous oxide 75 per cent. and oxygen 25 per cent. In many cases it has been found necessary or advantageous to add pethidine hydrochloride, other agents, or relaxants, but in these cases the dosages required have been considerably smaller than would have been employed had the patient been receiving thiopentone sodium-nitrous oxide-oxygen. No complications were observed following the use of Viadril except for three cases of thrombophlebitis in the injected vein: in each of these cases the patient had widespread vascular disease and was receiving the anæsthetic for an aortogram. S. L. W.

Synthalin A as Selective Mitotic Poison Acting on Alpha-Cells of Islets of Langerhans. H. Ferner and W. Runge. (*Science*, 1955, **122**, 420.) Synthalin A (decamethylene diguanidine dichlorhydrate), decreased the mitotic rate of the alpha-cells of the islets of Langerhans (considered to be the producers of glucagon) in one- to five-day-old rats. The drug was given in a single subcutaneous injection of 10 mg./kg. and the rats were killed 12 to 18 hours later. In none of the animals were there any signs of lesion of the alpha-cells, although lesions and alpha-cell destruction often occur in adult rats after Synthalin A administration. Beta-cell mitosis was not affected by the drug.

G. P.

BACTERIOLOGY AND CLINICAL TESTS

Mumps Virus, Reproduction in the Chorio-allantoic Membrane. H. L. Wolff. (*Nature, Lond.*, 1955, **176**, 604.) The cultivation of mumps virus is described in the chorio-allantoic membrane of the incubated hen's egg. Using "Enders" mumps strain, compliment fixing antigen could not be detected until 18½ hours after inoculation, and hæmagglutin after 19½ hours. The infection titre decreased from 2½ hours after inoculation, reaching a minimum after 5 hours and remaining low until 14½ hours when it slowly rose again. Experiments were also carried out using the chorio-allantoic membrane of 9 day old chicks cut into small pieces and placed with the virus into a petri dish. It is concluded that mumps virus is reproduced in the living chorio-allantoic cell in 14 to 17 hours. G. F. S.

Mustard Oils, Bacteriostatic Action of. P. Klesse and P. Lukoschek (*Arzneimitt.-Forsch.*, 1955, **5**, 505.) A strong bacteriostatic action was observed with a number of synthetic mustard oils: methyl, ethyl, allyl, phenyl, benzyl and phenylethyl mustard oil. Effective concentrations ranged between 1:60,000 and 1:2,000,000 against *Staph. aureus*, *E. coli* and *Sarcina lutea*. The action is also observed in the urine—after the administration of 10 mg. of benzyl mustard oil the urine was found to be bacteriostatic for more than 10 hours. In plate tests the action is decreased by a factor of from 3 to 10 by the presence of blood or serum. By subcutaneous injection into mice, the toxicity showed a variation between LD50 = 0·05 g. per kg. for methyl mustard oil, and LD50 = 0·25 g. per kg. for phenylethyl mustard oil. G. M.

Usnic Acid, Antibacterial Action of. J. R. Möse. (*Arzneimitt.-Forsch.*, 1955, **5**, 510.) Tests were carried out with pure usnic acid against a large variety or micro-organisms, using the plate method. With the exception of meningococci and *Neisseria flava*, only Gram-positive bacteria were affected. No action was observed on moulds or viruses. The minimum concentration required (for *Staph. aureus* and *N. flava*) was 1:64,000. Thirty different strains of *Clostridium tetani* showed particularly fine bacteriostatic zones of 10 to 12 mm., which is considerably greater than with penicillin and streptomycin. Although there were differences between the many different strains of *Staph. aureus* tested, only one proved resistant. The effect differed according to the solvent used and the pH value of the solution, and was also modified by various additions such as powder and ointment bases. Usnic acid is not affected by the action of heat or of ultra-violet light. Its toxicity appears to be low. The oral administration of 2 g. of the pure acid to a guine-pig of 4·08 kg. weight showed no permanent ill effects. In this case the bacterostatic action of the urine was nil. G. M.

Water-soluble Filter for Trapping Airborne Micro-organisms. M. Richards. (*Nature, Lond.*, 1955, **176**, 559.) The preparation of a water soluble "wool" of sodium alginate to trap spores is described. Calcium alginate yarn is cut into short lengths and stirred into a large volume of water to untwist the filaments. After draining on a Buchner funnel the mass of calcium alginate wool is soaked in N hydrochloric acid to leech out the calcium, drained, suspended in 50 per cent. ethanol and neutralised with sodium hydroxide. The sodium alginate yarn produced is soluble in water. It is washed with 50 per cent., then absolute ethanol and dried. The wool is used to trap micro-organisms when packed as a plug in a special brass tube. It is very useful for sampling airborne fungi and bacteria for culture. G. F. S.