ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Reserpine, Rescinnamine and Deserpidine, Content of, in Rauwolfia Roots. D. Banes, A. E. H. Houk and J. Wolff. (*J. Amer. pharm. Ass., Sci. Ed.*, 1958, **47**, 625.) Alkaloids of the reserpine-rescinnamine group were extracted from a sample of rauwolfia root by the method previously described (*J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 708). The chloroform extract was mixed with ethanol and evaporated to dryness at 70° *in vacuo*. The residue was incorporated in a silica column and fractionated chromatographically by the method of A. L. Hayden and colleagues (*J. Amer. pharm. Ass., Sci. Ed.*, 1958, **47**, 157). The fractions containing deserpidine, reserpine and rescinnamine were mixed and diluted as necessary, and the content of the alkaloids determined spectrophotometrically. The results of analyses of powdered root of *Rauwolfia serpentina* by this method were generally in agreement with those obtained by the nitrite colorimetric method, but discrepancies were observed with some samples. The proposed method is believed to be more reliable than the colorimetric method, since the active alkaloids are isolated individually and determined by direct measurement. G. B.

ANALYTICAL

Antibiotics, Paper Chromatographic Method for the Determination of Suitable pH Values for the Extraction of. V. Betina. (*Nature, Lond.*, 1958, **182**, 796.) pH Chromatograms are determined only when cultures of antibiotically active micro-organisms are available, before even crude substances have been isolated. The solubility of the antibiotic in ten solvents (distilled water, 3 per cent w/v ammonium chloride, methanol, acetone, ethyl acetate saturated with water, *n*-butanol saturated with water, chloroform, benzene, light petroleum, and ether saturated with water) is determined as follows. Agar plugs cut from colonies of the micro-organism are placed in contact with filter paper strips for 15–50 minutes. The latter are dried, and chromatographed by assending technique with each of the above solvents, and the spots detected bioautographically. pH Chromatograms are then run with the most suitable solvent on a series of paper strips buffered with McIlvain citrate-phosphate buffers of pH 2·2, 3, 4, 5, 6, 7 and 8, and with phosphate buffers of pH 9 and 10 respectively, using the same technique, and allowing the solvent front to advance 15 cm. in each case. The pattern of spots obtained is a characteristic of each antibiotic. Acidic substances advance further with acid buffers, whilst basic substances advance further with alkaline buffers. The movement of neutral antibiotics is unaffected by pH. R_F values are dependent on the partition coefficient of the antibiotic between the organic and aqueous phases, and extraction with an organic solvent will be most successful at the pH value which gives the highest R_F value. Conversely re-extraction into aqueous media will be most effective at that pH at which the R_F has a low value. J. B. S.

Glycosidal Alkaloids of the Solanine Complex, separation of, by Paper Electrophoresis and Chromatography and their Colorimetric Determination. J. Šerák and M. Kutáček. (*Československ. Farm.*, 1958, **7**, 322.) A sample of the mixture (80 to 500 μg.) is subjected to electrophoresis on a sheet (30 × 45 cm.) of Whatman No. 1 paper for 18 hours at a voltage of 140 V., 2·5 M acetic acid being used as electrolyte. The paper is dried at 60 to 80° and then used for paper chromatography in a direction perpendicular to that for the electrophoresis; a mixture of ethyl acetate, glacial acetic acid and water (11 : 1 : 2) is used as solvent system. The spots are located by a satured solution of antimony trichloride in chloroform. For the quantitative determination of the glycosidal alkaloids a standard chromatogram is run at the same time as the test and the spots on both are eluted with a 1 per cent solution of acetic acid. To the eluate (3 ml.) 5 ml. of conc. sulphuric acid and 2·5 ml. of a 1 per cent solution of formaldehyde are added, the sample being cooled during the addition. The maximum colour develops in 70 minutes and remains stable for 3 hours. The average recovery is 94·3 per cent; the loss can be allowed for by the use of a calibration curve constructed from results obtained on standards. E. H.

Meprobamate, Identification of, by Adsorption Chromatography on Chromatoplates. A. Fiori and M. Marigo. (*Nature, Lond.*, 1958, **182**, 943.) Chromatoplates are prepared by pouring a paste, prepared from silicic acid, rice starch and water onto glass plates, and spreading to form a homogeneous layer. After drying at room temperature the plates are activated by heating in an oven for 30 minutes at 100–105°. Meprobamate is isolated from urine by making alkaline, extracting with ether, and evaporating the latter solution; it is applied (in ethanol) to the starting line of the chromatoplate in a stream of hot air. Plates are developed in *cyclo*hexane and ethanol for 2 hours, when the solvent front moves about 10–11 cm., dried at room temperature and sprayed with concentrated sulphuric acid. Yellow spots appear after the plates are heated at 110–115° for 2–3 minutes, sprayed lightly with distilled water and re-heated. Meprobamate has R_F 0·30, and unidentified impurities migrate with the solvent front, and become violet under sulphuric acid treatment. Quantitative determination can be made by cutting out the spots, adding to water, treating with hydroxyquinone in sulphuric acid, heating at 100° for 20 minutes, and measuring the yellow colour at 420 mμ. J. B. S.

Morphine, New Method for the Determination of Small Quantities of. G. Nadeau and G. Sobolewski. (*Canad. J. Biochem. Physiol.*, 1958, **36**, 625.) The method depends upon the development of a blue fluorescence when morphine is warmed with concentrated sulphuric acid, and ammonia added. The solution containing morphine is evaporated to dryness, the last traces of moisture being removed under reduced pressure, and the residue is heated with 0·5 ml. of sulphuric acid in a water bath at 50° for 8 minutes, after which 5 ml. of water and 6 ml. of strong solution of ammonia are added. The liquid is allowed to stand at 50° for 2 hours, and then cooled. The fluorescent product is extracted by shaking with 10 ml. of *iso*butanol, and the fluorescence of the extract is determined in a fluorimeter. The solution may be centrifuged if necessary to remove turbidity. The intensity of fluorescence is proportional to the concentration of morphine for samples containing 0·02–20μg. Quenching occurs with higher concentrations, but the method can be used without serious error up to 100μg. The method is sensitive, and as the fluorescence is not given by other alkaloids of opium, morphine can be estimated in opium preparations without a preliminary separation. Diamorphine gives a fluorescence about one-third the intensity of that given by morphine. G. B.

Piperitone, Colorimetric Estimation of, with 3 : 5-Dinitrobenzoic Acid. D. H. E. Tattje. (*Pharm. Weekbl.*, 1958, **93**, 694.) To 4 ml. of an ethanolic solution of (—)-piperitone containing up to 1·6 mg. is added 5 ml. of a 4 per cent solution of 3 : 5-dinitrobenzoic acid in ethanol and 2 ml. of 3N sodium hydroxide. The colour intensity at 5375Å is measured in a 0·5 cm. cell 40 minutes after the addition of the sodium hydroxide. The blank consists of a solution identical in all respects except for the omission of the (—)-piperitone, and the temperature of measurement is 20°. A calibration curve is essential since the relationship of intensity of colour to concentration deviates appreciably from linearity. Cineole and 1-α-phellandrene do not interfere. Results obtained on mixtures of piperitone and oil of *Eucalyptus globulus* were in fair agreement with the theoretical values, whereas two oils of the *Eucalyptus dives* type gave results about 10 per cent lower than those obtained by the neutral sulphite method. This may be due to the greater specificity of the colorimetric method D. B. C.

Salicylic Acid, An Inverse Isotope Dilution Analysis of. H. A. Swartz and J. E. Christian. (*J. Amer. pharm. Ass., Sci. Ed.*, 1958, **47**, 701.) Samples of salicylic acid are dissolved in glacial acetic acid and allowed to react with the appropriate quantity of iodine monochloride reagent labelled with iodine-131. The reaction is completed by heating in a water bath at 70°–80° for 20 minutes, shaking repeatedly. 3 : 5-Di-iodosalicylic acid in hot glacial acetic acid solution is added as carrier, and the solution set aside to cool and crystallise. After purification, the specific activity of the 3 : 5-di-iodosalicylic acid is determined, and from the activity of the labelled iodine monochloride reagent, the quantity of salicylic acid is calculated. The method of standardising the reagent is described. The method was verified using quantities of 0·2 to 20 mg. of salicylic acid, and should be applicable to smaller quantities. Acetylsalicylic acid may be determined by the same method, after quantitative hydrolysis to salicylic acid. G. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Adrenaline and Noradrenaline, Association of, with Blood Platelets. H. Weil-Malherbe and A. D. Bone. (*Biochem. J.*, 1958, **70**, 14.) Blood platelets, in addition to their role in blood coagulation, act as carriers of pharmacologically active amines. In this paper the distribution of adrenaline and noradrenaline between plasma and blood platelets is studied by a fluorimetric method. It was found that in human plasma the platelets contained approximately 50 per cent of the total catechol amines present in platelet-rich plasma. The mean amounts contained in 10^9 platelets were 2 ng. of adrenaline and 7 ng. of noradrenaline. The concentration of catechol amines is about 125 times higher in platelets than in plasma. No amines passed into the serum from the platelets during clotting, whether spontaneous or induced. Lysis of platelets by freezing and thawing or by treatment with a surface-active agent resulted in a partial release. No uptake of adrenaline by platelets was observed in heparinized platelet-rich plasma at an adrenaline concentration of about 10 μg./1. An uptake, resulting in a final concentration of platelet-bound adrenaline of about three times the initial concentration, was found in citrated platelet-rich plasma at an adrenaline concentration of 80–200 μg./1. An increase of adrenaline was found in platelet-rich plasma after electroconvulsive treatment in mental patients. M. B.

Intrinsic Factor and Pernicious Anaemia, Studies on. H. Berlin, R. Berlin, G. Brante and S-G. Sjoberg. (*Scand. J. clin. and Lab. Invest.*, 1958, **10**, 278.) A study has been made of the relationship between intrinsic factor (IF) activity and vitamin B_{12}-binding capacity for hog pylorus concentrates. The investigations were carried out in well established pernicious anaemia (p.a.) patients. The B_{12}-binding capacity was determined by the method of Hoff-Jørgensen and others (*Nord. Med.*, 1952, **48**, 1754) and 1,000 C.U. defined as the amount of the IF preparation capable of making 1 μg. vitamin B_{12} unavailable to *E. coli*. The IF activity was determined by measurement of the urinary radioactive B_{12}–^{60}Co excretion according to Schilling. The results showed that a significant increase in B_{12} uptake was obtained by IF doses higher than 500 C.U. A dose of 1,000 C.U., which was "equivalent" to the B_{12} dose given in terms of binding capacity, was not sufficient for maximal response. The response increased most sharply in the range of approximately 1,000 to 3,000 C.U. Maximal response occurred when an IF dose of 10,000 C.U. was given. The individual response to the same IF dose was highly significant between patients, which may have been due to cases being included at different stages of development of the p.a. Some patients appeared to become refractory to oral treatment with B_{12} and IF.

G. F. S.

Radiovitamin B_{12} Bound in Pig Liver, Intestinal Absorption of. W. Nyberg and P. Reizenstein. (*Lancet*, 1958, **2**, 832.) Support is given to the theory of Heathcote and Mooney (this Journal, 1958, **10**, 593) that the absorption of vitamin B_{12} is enhanced by binding with peptides. Liver containing native radiovitamin B_{12} was obtained by injecting a pig with radiovitamin B_{12} daily for one month. The liver contained approximately 6 mg. vitamin B_{12}–^{60}Co and approximately 560 mg. non-labelled B_{12} per g. of wet tissue. Of the vitamin in the liver approximately 88 $\pm$ 3 per cent was non dialysable. Samples of this liver were fed to five healthy volunteers and to four patients with untreated pernicious anaemia. Faeces were collected for seven days and the excreted radioactivity measured. Eight of the patients then received comparable, though somewhat larger doses of crystalline radioactive B_{12} as a control test. Absorption of liver B_{12} was not significantly higher in the controls than in the cases of pernicious anaemia, and more of the bound B_{12} than of the crystalline vitamin appeared to be absorbed. This may indicate that B_{12} in the form in which it occurs in the liver is more easily absorbed than crystalline B_{12}.

G. F. S.

Sorbitol, Failure of, to Replace Intrinsic Factor in the Gastrectomised Rat. B. A. Cooper. (*Nature, Lond.*, 1958, **182**, 647.) In a series of controlled experiments on gastrectomised rats, the oral administration of a preparation containing D-sorbitol was shown to be without effect on the absorption of a small dose (0·015 μg.) of labelled vitamin B_{12}. On the other hand the administration of rat gastric juice or of homogenised rat stomach promoted absorption to an extent comparable with that in the normal rat. These results showed that D-sorbitol did not take the place of rat intrinsic factor. It has been demonstrated that in pernicious anaemia two mechanisms seem to exist for the absorption of vitamin B_{12}. Rapid absorption, independent of, or even inhibited by, intrinsic factor, follows the administration of a large dose of the vitamin. On the other hand a slower physiological mechanism involving the intrinsic factor, is required for the absorption of small amounts. From these results and those of other workers, the authors conclude that D-sorbitol affects only the absorption of large doses of vitamin B_{12}, that is, the mechanism which is independent of intrinsic factor activity.

W. C. B.

BIOCHEMICAL ANALYSIS

Ethanol, Optimum Conditions for Determination of, in Body Fluids; using the Acid Dichromate Method. L. Wilkinson. (*Analyst*, 1958, **83**, 390.) The rate of reduction of potassium dichromate at room temperature by ethanol was studied in concentrations of sulphuric acid ranging from 5–37N, the optimum lying between 18·5 and 23N and the rate also rising with increase in dichromate concentration. Loss by volatilisation and the effect of light were found to be negligible. In the method described, ether, acetone, ethyl chloride, chloroform and methylamine do not interfere and formaldehyde can be polymerised by treatment with potassium carbonate. Conway diffusion can be used in the routine determination. D. B. C.

Glucocorticoids, A Simple and Rapid Method for the Biological Assay of. B. P. Block and P. F. D'Arcy. (*Nature, Lond.*, 1958, **182**, 181.) It is well known that adrenocortical steroids have a pronounced effect on carbohydrate metabolism and in particular on the liver glycogen level. Various methods for the assay of adrenocorticoids, based on the deposition of liver glycogen in adrenalectomised rats and mice, have been reported but in all instances they are too complex and tedious for rapid screening procedures. The authors then give a detailed description of their simple and rapid, yet highly sensitive, method of assaying glucocorticoids, based on the liver glycogen method of Venning, Kazmin and Bell. The results are expressed in terms of mg. of liver glycogen equivalent per 100 g. of mouse. Using this method it was found that doses of either 10 or 20 mg. of glucose per mouse, incorporated into the injection solution, greatly increased the response to a given dose of cortisone acetate, without themselves having any pronounced effect on the liver glycogen level. Doses greater than 20 mg. per mouse caused glycogen deposition. The only disadvantage of the method is that, because the livers are bulked for assay, no estimate of the scatter of the individual responses within each group can be obtained. However, the method is most suitable for the routine screening of new compounds for glucocortical activity. M. B.

Tranquillisers and Sedatives, Bioassay of Potential, Against Audiogenic Seizures in Mice. N. P. Plotnikoff. (*Arch. int. pharmacodyn.*, 1958, **116**, 130.) The bioassay consists of selecting mice which are sensitive to audiogenic seizures and placing them in groups of five. The responses of mice susceptible to the auditory stress (ringing of two door-bells for 90 sec.) consist of running, jumping, circling, convulsing and passing into catalepsy. Each type of response is graded one point if present so that any one mouse might have a maximum of 5 points. Each group acts as a control one day and is drug tested the next, all agents being administered orally one hour prior to testing. Percentage protection is calculated on the basis of the control level and is plotted against log dose. Chlorpromazine, reserpine and meprobamate inhibited both running and convulsive seizures in small doses. High doses of chlorpromazine (200 mg./kg.) and reserpine (300 to 400 mg./kg.) potentiated convulsive seizures. Sodium phenobarbitone, methylpentynol and chloral hydrate inhibited convulsive seizures but not running seizures even at high doses. It is suggested that new agents may be classified as potential tranquillisers if they inhibit running seizures. W. C. B.

PHARMACY

Ammonium Alginate Wool as a Filter for Collecting Micro-organisms from large Volumes of Air. E. C. Hammond. (*J. gen. Microbiol.*, 1958, **19**, 267.) Filters containing ammonium alginate wool were sterilised by autoclaving at 10 lb. wt./sq. in. for 30 minutes. Air containing the test organism (*Bacillus subtilis*), either in the form of an aerosol or of a dry dust prepared from sifted soil was blown through the filters at speeds varying from 1 to 5 cu. ft./min., any organisms which had passed through the alginate wool being collected on membrane filters. The alginate wool from each filter was dissolved in a sterile 0·5 per cent solution of dipotassium hydrogen phosphate and samples plated out with glucose nutrient agar and incubated at 30° for 3 days. The membrane filters were shaken with 0·5 per cent sodium chloride solution and glass beads and the solution plated out with nutrient agar. In these experiments, ammonium alginate wool retained the dry spores of *B. subtilis* with an efficiency of 99·13 to 99·96 per cent. It appeared to be 100 per cent efficient for micro-organisms of diameter greater than 2μ, including the majority of yeasts and moulds. G. B.

Enteric Coated Tablets, Correlation of *In Vivo* with *In Vitro* Disintegration Times. J. G. Wagner, W. Veldkamp and S. Long. (*J. Amer. pharm. Ass., Sci. Ed.*, 1958, **47**, 681.) Tablets of barium sulphate (3 grains) were undercoated, and then enteric-coated, using five different enteric-coating preparations. The 5 batches of enteric-coated tablets were submitted to the action of simulated gastric fluid (U.S.P.) for 2 hours at 37°. The tablets, which were unaffected by this treatment, were placed in artificial gastric fluid at 37° in the U.S.P. disintegration test apparatus. The tablets with undercoating only were also tested in artificial intestinal fluid, and by subtracting the disintegration time for these from that for the enteric-coated tablets, an estimate of the disintegration time of the enteric coating was obtained. Dogs were given 4 enteric-coated tablets and 50 ml. of 0·1N hydrochloric acid after an overnight fast. X-ray photographs were taken after 45 minutes, and subsequently at intervals of 15 minutes. In some experiments to determine the stability of the enteric coating in the stomach, the dogs were fed 30 minutes before receiving the 4 tablets. Graphs are presented showing the relationship between the *in vitro* and *in vivo* disintegration times. The methods appear to be useful for investigating enteric coatings and the effect of storage conditions on them, but it is suggested that similar experiments, together with blood level determinations, should be carried out in human subjects before attempting to lay down standards for the disintegration of enteric-coated tablets. G. B.

6-Fluorothymol, The Synthesis and Antifungal Properties of. C. A. Discher, J. M. Cross, P. F. Smith and M. Iannarone. (*J. Amer. pharm. Ass., Sci. Ed.*, 1958, **47**, 689.) 6-Aminothymol fluoroborate was diazotised and, after purification, the diazonium salt was distilled with benzene and toluene to remove the gaseous products of decomposition. 6-Fluorothymol was extracted from the reaction mixture with sodium hydroxide solution and subsequently purified. The antifungal activity of this compound was compared with that of other 6-halothymols and thymol, using the serum-agar cup-plate method, with 8 test organisms (*Candida, Microsporon, Trichophyton* spp.). At a concentration of 0·05 per cent in propylene glycol solution, 6-iodothymol was the most effective antifungal agent, followed by 6-bromothymol, 6-chlorothymol, 6-fluorothymol and thymol. G. B.

Mould-inhibiting Compounds, The Effect of pH on the Efficiency of. F. J. Bandelin. (*J. Amer. pharm. Ass., Sci. Ed.*, 1958, **47**, 691.) A modified Sabouraud agar, enriched with yeast extract was buffered to pH 3, 5, 7 and 9 with the aid of citrate-phosphate buffer solutions. The compounds under test were incorporated in various concentrations in the warm fluid medium, and sterilised slopes prepared. These were inoculated with standard suspensions of spores of the test organisms, *Aspergillus niger*, *Penicillium citrinum*, *Alternaria solani* and *Chaetomium globosum*. The slopes were incubated at 30° for 14 days, the criterion of inhibition being lack of growth at the end of the incubation period. Benzoic, salicylic, propionic and sorbic acids were effective at pH 3–5, but lost their activity at higher pH values. Dehydroacetic acid (3-acetyl-6-methyl-1:2-pyran-2:4(3H)-dione) was effective at concentrations of 0·001–0·005 per cent in acid media, but less effective in alkaline media. Esters of *p*-hydroxybenzoic acid were most effective in acid media, but less affected by pH changes than the other compounds. Vanillin and ethylvanillin were effective in concentrations up to 0·2 per cent, and relatively little affected by pH changes. Kojic acid, an antifungal substance isolated from *Aspergillus* sp., was the least effective substance examined. G. B.

Pyrogens, Bacterial, Determination of the Components of, by Chromatography. K. Macek and J. Hacaperková. (*Československ. Farm.*, 1958, **7**, 300.) A sample of pyrogen (200 mg.) is hydrolysed by heating with dilute sulphuric acid and the purines are precipitated by silver sulphate. Sugars are detected by chromatography on Whatman No. 1 or No. 3 paper with *n*-butanol:pyridine:water (6:4:3) as solvent system; the spots are located by spraying the paper with a reagent comprising 4 per cent ethanolic aniline, 4 per cent ethanolic diphenylamine and 85 per cent phosphoric acid (5:5:1). For the detection of glucose, mannose and galactose the chromatogram is moistened with yeast suspension, incubated for 2 hours at 38°, and then treated with diphenylamine reagent. In the separation of purines, water-saturated *n*-butanol containing 5 per cent of formamide is used as solvent. For amino acids the solvent system *n*-butanol:acetic acid:water (4:1:5) is used. Results are given for pyrogens produced by *S. typhimurium*, *E. typhi*, *P. vulgaris* (Westphal), *P. vulgaris* (Dare) and *S. abortus equi*. E. H.

PHARMACOGNOSY

Digitalis Glycosides, Enzymatic Decomposition of. O. Gisvol. (*J. Amer. Pharm. Ass.*, 1958, **47**, 594.) Glycosides were extracted from the leaves of several species of *Digitalis* using two different procedures. (i) Fresh leaves were disintegrated in the presence of water and dilute methanol, heated for a short time and then filtered. The filtrate was extracted successively with a mixture of ether and methylene chloride (3:1) and with ethyl acetate and the extracts examined chromatographically. By this procedure the terminal glucose of the primary glycoside was removed and certain of the resulting desglucoglycosides could be obtained crystalline. (ii) Fresh leaves were immersed in boiling phosphate buffer (pH 7) and then disintegrated in the medium, filtered, and the filtrate treated by suitable solvent solvent extraction and examined chromatographically. This second procedure resulted in the production of primary glycosides as the preliminary heat treatment evidently inactivated the carbohydrases responsible for splitting off the terminal glucose. It is claimed that this simple procedure is more satisfactory than that originally used by Stoll.

Fresh leaves which had been dried in an incubator at 60° were examined in a similar manner: it was shown that considerable decomposition of the primary glycosides had taken place. Two solvent systems were used in the paper-chromatographic work; the first consisted of methyl *iso*butyl ketone and *iso*-propyl ether (100:25) saturated with formamide. The stationary phase, formamide, was applied to the paper as a 30 per cent solution in acetone. The second system was prepared from methyl*iso*butyl ketone, *iso*propyl ether, tetrahydrofuran and formamide (40:10:15:15). The stationary phase was the same as in the first solvent system. J. W. F.

Digitalis Glycosides, Enzymatic Decomposition of. O. Gisvol. (*J. Amer. pharm. Ass.*, 1958, **47**, 600.) Fresh leaves of *D. purpurea* were carefully dried below 60° and examined by the methods already described. The results showed that only a small proportion of the primary glycosides had been decomposed. Similar results were obtained with a commercial sample of dried leaf and with U.S.P. Digitalis Reference Standard 1942. In contrast, fresh leaves dried at 100° showed almost complete decomposition of the primary glycosides into desglucoglycosides. Extraction of carefully dried leaf with 66 per cent methanol (or stronger) inhibited enzyme action. Since the primary extract of Digitalis Reference Standard used in assay work is made with strong ethanol the primary glycosides will be the chief reference standard. With unknown samples of leaf, the amount of primary glycosides present will depend on the care with which the leaf has been dried and stored. J. W. F.

PHARMACOLOGY AND THERAPEUTICS

Adrenaline and Noradrenaline, Replacement of, in the Innervated and Denervated Adrenal Gland of the Rat, following Depletion with Reserpine. B. A. Callingham and M. Mann. (*Nature, Lond.*, 1958, **182**, 1020.) It has already been shown that reserpine causes a similar percentage loss of adrenaline and noradrenaline, followed by slow recovery, in the normal rat adrenal gland. Experiments have now been performed to show whether this action is central or peripheral. The left adrenal gland was denervated by cutting the splanchnic fibres. Subsequently the rats were given reserpine and then killed at suitable time intervals in order to study both the depletion and the replacement of adrenaline and noradrenaline in the innervated and denervated glands. It was found that at three days there was a similar degree of depletion of both amines in the innervated and the denervated glands. The adrenaline then slowly recovered in both groups, while the noradrenaline content rose well above its normal level of 10 per cent before returning to normal. Complete recovery of the two amines had taken place in both the groups by three weeks. Thus no appreciable difference, in either the depletion or the replacement of adrenaline and noradrenaline could be found between innervated and denervated adrenal glands of the rat. It may thus be concluded that this action of reserpine is peripheral. M. B.

Bis-Quaternary Ammonium Compounds, the Pharmacology of Some. Z. P. Horovitz, E. C. Reif and J. P. Buckley. (*J. Amer. pharm. Ass., Sci. Ed.*, 1958, **47**, 718.) Tests were carried out to compare the pharmacological effects of 4 experimental hypotensive agents with chlorisondamine dimethochloride. The compounds examined were 1:6-bis-*NN*-dimethylamino-2-hexyne dimethobromide (JB-520), 1:6-bis-*NN*-morpholino-2-hexyne dimethobromide (JB-540),

β-(3-dimethylamino)propylamino-*N*-methylpiperidine dimethobromide (JB-549) and β-dimethylaminoethyl-*N*-methylpipecolinate dimethobromide (JB-591). All these compounds blocked the transmission in the superior cervical ganglion of the cat, blocked the hypertensive activity of 1:1-dimethyl-4-phenylpiperazinium iodide and dilated the mesenteric blood vessels in the rat. JB-520 and chlorisondamine depressed the auricular musculature of the perfused turtle heart, and JB-520 produced a negative inotropic effect on the isolated rabbit heart. All of the compounds except JB-591 increased the activity of the isolated rabbit ileum, and none of the compounds produced alterations in the blood pressure of the pithed rat. G. B.

Dihydrocodeine and Morphine in Man, Comparison of the Analgesic and Respiratory Effects of. J. C. Seed, S. L. Wallenstein, R. W. Houde and J. W. Bellville. (*Arch. int. pharmacodyn.*, 1958, **116**, 293.) In a double-blind, random-sequence, cross over study inpatients suffering from chronic pain due to cancer, 68 mg. of dihydrocodeine was found to be equivalent in analgesic potency to 10 mg. of morphine. The respiratory effects of the two drugs were compared by determining the displacement of the alveolar ventilation-alveolar partial pressure of carbon dioxide gas response curves in normal volunteers and in patients. When given in doses which were equi-analgesic with those of morphine, dihydrocodeine was found to produce an equal amount of respiratory depression. It appeared that the effects of the two drugs on the response curve were primarily due to an effect on the respiratory control mechanism and not to changes in cerebral circulation, cerebral metabolism, airway resistance, or physiological dead space. W. C. B.

Dioscorine and Dioscine, Pharmacological Properties of. J. L. Broadbent and H. Schnieden. (*Brit. J. Pharmacol.*, 1958, **13**, 213.) These alkaloids are present in certain varieties of "yams", ingestion of which may cause convulsions. In both rats and mice these drugs caused clonic followed by tonic convulsions. The convulsions resemble those produced by picrotoxin and were antagonised by sodium pentobarbitone. The LD50 values in mice were 60 mg./kg. for dioscorine and 100 mg./kg. for dioscine. The toxicity of dioscine solutions rapidly decreased on storage even when stored in the refrigerator. Both drugs had an analeptic action in anaesthetised rats but the effective dose was close to the convulsant dose. Solutions of both drugs showed a local anaesthetic action when injected intradermally into the guinea pig but were inactive on the cornea. In the anaesthetised cat neither drug affected the blood pressure in doses up to 20 mg./kg. but the hypotensive action of acetylcholine was reduced and the hypertensive action of adrenaline was increased. On the guinea pig isolated ileum the responses to acetylcholine and histamine were reduced. On the isolated heart doses of 2 mg. had no effect but the response to acetylcholine was reduced. Both drugs had an antidiuretic action. G. F. S.

Ganglion-blocking Agent. (189c56) an Orally Effective. S. Locket. (*Brit. med. J.*, 1958, **2**, 74.) This ganglion-blocking agent is chemically related to pentacynium methylsulphate. Compared with pentacynium, about twice the dose of the active cation of 189c56 by subcutaneous or intravenous injection produces the same fall in blood pressure. It produces hydrodynamic changes and effects on renal function comparable to those of pentacynium. Its valuable features are (1) that it is invariably effective when taken by mouth, (2) that when given daily before breakfast it consistently produces the expected degree

of hypotension, (3) that its duration of action is such that a single daily dose treatment of hypertension becomes possible in some patients, (4) that dosage is highly critical (a small alteration in the controlling dose of 5–12 per cent causes a marked effect on the extent and duration of the hypotension), (5) constipation is absent or minimal, even with large doses, and ileus does not occur, and (6) disturbances of bladder emptying do not occur. The initial dose employed is usually 200 mg. This is increased by 50 mg. each day until an appreciable hypotensive effect is obtained. This dose is then given daily. In a few days the patient becomes accommodated to the hypotension and the dose is then increased by 25–50 mg. every few days. The degree and duration of hypotension achieved show little daily variation once a satisfactory drug level is obtained. After several weeks of daily administration of this dose it may be necessary to reduce it because of increased severity and duration of the fall in blood pressure. Dryness of the mouth occurs with the larger doses, and disturbance of vision occurs as with other ganglion-blocking agents. This treatment was given to 11 patients. S. L. W.

Glycyrrhizin-induced Inhibition of the Pituitary Adrenal Response. S. D. Kraus. (*J. exp. Med.*, 1958, **108**, 325.) Glycyrrhizin has been shown to have an action like deoxycortone, reducing the resistance of mice to a cold stress and decreasing the ability of rats deprived of food to mobilize glucose. The average survival time of normal mice, weighing from 8 to 10 g., at 5° was 6·5 hours. Mice which had received 0·4 per cent ammoniated glycyrrhizin, in place of drinking water, for four days previously only survived for 2·5 hours. Rats fasted for 24 hours showed hypoglycaemia, but after 48 hours mobilized sufficient glucose to bring the blood sugar level up to the normal value. Rats pretreated for seven days with ammoniated glycyrrhizin in their drinking water showed much less mobilization. It is concluded that glycyrrhizin, like deoxycortone, depressed the output of ACTH by the pituitary gland. G. F. S.

5-Hydroxytryptamine and Cooling, Effect of, on the Peristaltic Reflex. D. Beleslin and V. Varagić. (*Brit. J. Pharmacol.*, 1958, **13**, 266.) The effect of 5-HT and cooling has been studied on the peristaltic reflex of the guinea pig isolated intestine to obtain more information about the physiological role of 5-HT in peristalsis. A modification of the method of Trendelenburg was used so that the drug could be introduced directly into the lumen. The results showed that lowering the temperature to 19–26° temporarily abolished the emptying phase of the reflex, while cooling to 5° for 3 to 8 hours caused this to be permanent. While 5-HT added to the bath at 37° depressed or abolished the reflex, in the cooled preparation at 19° introduction of 10 to 400 ng. into the lumen easily restored the reflex. This effect may be due to sensitizing receptors in the mucosa which trigger the reflex, the facilitation of transmission at the synapse or to both actions, according to the site of application. The effects of 5-HT were prevented by 2-bromolysergic acid diethylamide. G. F. S.

Isoetharine, the Pharmacology of. A. M. Lands, F. P. Luduena, J. O. Hoppe and I. H. Oyen. (*J. Amer. pharm. Ass., Sci. Ed.*, 1958, **47**, 744.) Isoetharine (Dilabron, Win3046), a bronchodilator drug, is the hydrochloride of 1-(3 : 4-dihydroxyphenyl)-2-*iso*propylaminobutan-1-ol, the *N-iso*propyl derivative of ethylnoradrenaline. Using intact anaesthetised guinea pigs it was shown that isoetharine is an effective antagonist to bronchostriction induced by histamine. The drugs were given in the form of an aerosol and the inclusion of thenyldiamine hydrochloride in the aerosol enhanced the action of isoetharine. Subcutaneous injection of isoetharine with phenylephrine was found to increase

the protective action of thenyldiamine against toxic doses of histamine administered intravenously to dogs. The increase in pulse-rate following subcutaneous administration of isoetharine to dogs was decreased by the addition of 25 per cent of phenylephrine and 10 per cent of thenyldiamine to the injection. Isoetharine is rapidly absorbed following oral administration, and usually gives rise to tachycardia; experiments in dogs showed that the absorption of the drug may be suitably delayed by giving it in the form of enteric-coated tablets. Isoetharine, alone or mixed with phenylephrine and thenyldiamine has a low acute toxicity for mice when given by intravenous injection. G. B.

Laminarin Sulphate (LM 46), Effect of, on Bone Growth. S. S. Adams, H. M. Thorpe and L. E. Glynn. (*Lancet*, 1958, **2**, 618.) LM 46 was administered subcutaneously or intravenously to rabbits (15, 30, and 60 mg./kg.), rats (15 and 30 mg./kg.) and guinea pigs (15 mg./kg.) on five days of each week for 2–9 weeks. Control animals received a dose of heparin equivalent in anticoagulant activity to the laminarin sulphate. In all animals there was a pronounced loss of weight after prolonged dosing and the rabbits and guinea pigs had a white discharge in the eyes. Bone lesions developed which appeared to depend primarily on disturbance of the endochondrial bone formation that normally takes place on the diaphyseal side of the epiphyseal cartilage. In rats and guinea pigs the normal sequence of maturation of the cartilage cells was impaired and this was associated with virtually complete absence of the vascular invasion that normally precedes the actual deposition of bone on the newly formed tongues of calcified cartilage. In rabbits it was apparently this deposition of bone which was impaired. The structural similarity of laminarin sulphate to chondroitin sulphate suggested that it might be acting by interfering with the normal metabolism of chondroitin sulphate. In addition to the bone lesions all the animals showed accumulation of metachromatic material in the reticuloendothelial system and in the proximal convoluted tubules of the kidneys. W. C. B.

Metamidium: a New Trypanocidal Drug. W. R. Wragg, K. Washbourn, K. N. Brown and J. Hill. (*Nature, Lond.*, 1958, **182**, 1005.) This paper describes a new trypanocide which possesses considerable potentiality, both as a curative and as a prophylactic drug. Metamidium chloride hydrochloride (M & B 4404) was examined for activity against three strains of *Trypanosoma congolense* which gave an acute infection in mice. A single subcutaneous injection was given to mice with an infection in the peripheral blood stream of from 1–10 trypanosomes per high power field. Ten mice were used for each dose and five untreated mice which died within 5–7 days were used as controls in each experiment. Homidium chloride was used as the standard. Wet blood smears were examined three times a week for four weeks and the number of animals cleared of trypanosomes for that period was noted. The subcutaneous LD50 for mice was also determined. The results show that metamidium was more active than homidium against all three strains. Prophylactic experiments in mice were also carried out and it was found that metamidium completely protected mice for up to sixteen weeks at about one-ninth of the LD50. This is of importance because neither the parent compound homidium chloride (2:7-diamino-10-ethyl-9-phenylphenanthridinium chloride) nor Berenil, with which metamidium shares some structural similarities, has any appreciable prophylactic activity at one-third of the LD50. Of the two isomers of metamidium, the red was more active than the purple, both therapeutically and prophylactically, but there was little difference in their toxicities. M. B.

Pain in Spasmodic Dysmenorrhoea, Relief of, by Bromelain. C. A. Simmons. (*Lancet*, 1958, **2**, 827.) Bromelain is obtained by extraction from the stem of the pineapple. It contains an unstable proteolytic enzyme and is freeze dried for storage. The intravaginal administration of a fresh solution has been shown to rapidly relieve the cramping pain of spasmodic dysmenorrhoea. Its mode of action is unknown, but is possibly due to relaxation of the smooth muscle of the cervix. Ovulation is not inhibited. G. F. S.

Pempidine, Pharmacological Properties of. S. J. Corne and N. D. Edge. (*Brit. J. Pharmacol.*, 1958, **13**, 339.) Pempidine (1:2:2:6:6:-pentamethylpiperidine) is an orally effective, long acting ganglion-blocking agent. In the anaesthetised cat an intravenous injection of pempidine caused, like mecamylamine, a relaxation of the preganglionically stimulated nictitating membrane. The relaxation was slower than with hexamethonium and recovery very prolonged. Pempidine acted specifically at the ganglion and not on the nictitating membrane itself. Large doses did not reduce the output of acetylcholine from the perfused ganglion. In both the anaesthetised cat and the normal mouse pempidine caused mydriasis through its effect on the ciliary ganglion. Doses of 0·05 to 1·0 mg./kg. caused a fall in the cat blood pressure, which developed more slowly than with hexamethonium. Doses up to 10 mg./kg. did not affect the depressor actions of acetylcholine or histamine, but like other ganglionic blocking agents it potentiated the response to adrenaline. A dose of 0·1 mg./kg. abolished the pressor action of nicotine. There was no evidence of histamine release in the atropinised cat, but high doses caused a pressor action, which was not due to adrenaline release. A dose of 0·4 mg./kg. abolished the effect of peripheral vagal stimulation, but not injected acetylcholine. On the isolated rabbit heart a dose of 16 mg. was required to cause cardiac arrest. Smaller doses caused a slowing and decreased amplitude of the beat. Coronary flow increased. In the perfused hind limb of the dog intra-arterial doses of 8 mg. were without effect. Larger doses caused vasodilatation. On the isolated ileum of the guinea pig 800 μg./ml. had little direct effect in most preparations, in others 8 μg./ml. caused a contraction which was abolished by atropine. After 0·8 μg./ml., responses to histamine, pilocarpine and acetylcholine were normal; but the response to nicotine was abolished. Doses of 80 μg./ml. depressed the responses to histamine, pilocarpine, acetylcholine and 5-HT. Pempidine inhibited the peristaltic reflex in doses of 1 μg./ml. On skeletal muscle doses of 10 to 40 mg./kg. were required to block the response of the indirectly stimulated tibialis muscle of the cat, the block being curare-like. The anticholinesterase activity *in vitro* was 10^6 times less than neostigmine. Nicotine induced convulsions in mice were prevented by 0·09 mg./kg. G. F. S.

Perphenazine, A Potent and Effective Antiemetic. S. C. Wang. (*J. Pharmacol.*, 1958, **123**, 306.) Perphenazine (Trilafon), a phenothiazine compound, has been shown to have a potent antiemetic activity. In dogs treated with 0·5 mg./kg. of apomorphine, 0·1 mg./kg. i.v. prevented vomiting and 0·035 mg./kg. was about the 50 per cent effective dose. It was therefore about forty-eight times as effective as chlorpromazine. Similarly good protection was given against vomiting induced by morphine sulphate, Hydergine, and also against Lanatoside C where chlorpromazine was ineffective. Against oral emetic doses of 40 mg./kg. of copper sulphate, 0·1 mg./kg. of perphenazine gave 27 per cent protection, comparable with 2·0 mg./kg. of chlorpromazine. It is concluded that the increased potency of perphenazine is related to its greater affinity for the receptors in the trigger zone located in the area postrema. G. F. S.

Serotonin Antagonism of Noradrenaline *In Vivo*. P. Gordon, F. J. Haddy and M. A. Lipton. (*Science*, 1958, **128**, 531.) It has been reported previously that pretreatment with serotonin (5-HT) reduces the mortality of mice given bacterial endotoxin. Since the administration of the endotoxin is followed by the secretion of and hypersensitivity to adrenaline and noradrenaline, it is possible that 5-HT has antiadrenergic properties. That 5-HT does antagonise noradrenaline has been shown for the following *in vivo* systems: inhibition of the toxicity of noradrenaline in mice, suppression of the pilomotor response in mice, lysis of small blood vessel tone, and inhibition of noradrenaline-induced bradycardia. 5-HT pretreatment of mice considerably reduced the mortality caused by noradrenaline and it also abolished the concomitant pulmonary oedema. The pilomotor response in mice was elicited either by injection of noradrenaline or by exposure to cold or by the injection of reserpine. Prior injection of 5-HT completely suppressed the piloerection when caused by any one of the above methods. Dibenzyline also blocked this response. The effect of 5-HT on the vascular resistance was studied in the dog forelimb. Pressures were measured simultaneously in the brachial artery, a small vein in the footpad, a small vein in the paw and the cephalic vein. Large artery, small vessel (mainly arteriolar) and large vein resistances were calculated separately. The administration of 5-HT decreased the small vessel resistance at all levels of tone, the decrease being proportional to the initial level of tone. The effect of noradrenaline on these blood vessels was also antagonised. Again in dogs, noradrenaline given after 5-HT caused tachycardia instead of the usual bradycardia. Also the tachycardia induced by decreasing the intraluminal pressure of the carotid sinus was abolished during 5-HT infusion. Since noradrenaline-induced bradycardia is dependent on the carotid sinus reflex and since the denervated heart responds to noradrenaline with tachycardia, these results suggest that 5-HT antagonises the action of noradrenaline on the carotid sinus. These results from the different preparations suggest that the biological role of 5-HT lies in its interaction with noradrenaline. M. B.

APPLIED BACTERIOLOGY

Essential Oils, *In Vitro* Antifungal Activity of. J. C. Maruzzella and L. Liguori. (*J. Amer. pharm. Ass., Sci. Ed.*, 1958, **47**, 250.) Essential oils were tested by the filter paper disk method, in which disks ¼ inch in diameter were moistened with the oil and placed on plates of Sabauraud's maltose agar, previously seeded with a test organism. After incubation zones of inhibition were measured, and part of the clear zone was incubated in Sabauraud's maltose broth to obtain an indication whether the oil was fungicidal or fungistatic under the conditions of the test. Of 92 volatile oils examined, 90 showed activity against at least one of the test organisms. The most effective were red origanum, lemon grass, red thyme, sweet birch, savory select, coriander, sassafras, cinnamon, distilled laurel leaf and chenopodium oils. Chenopodium, red origanum and terpeneless dill oils were shown to be very effective against *Ustilago avenae*, chenopodium and red origanum against *Trichophyton mentagrophytes*, and cinnamon against *Epidermophyton interdigitale*. *Streptomyces venezuelae* was the most susceptible of the test organisms, and *Candida krusei* the most resistant. Of 12 terpeneless oils examined, cinnamon, caraway, dill and anise showed the greatest activity. Castor, olive and white mineral oils were shown to be devoid of antifungal action. G. B.

Essential Oils and Oil Combinations, *In Vitro* Antibacterial Activity of. J. C. Maruzzella and P. A. Henry. (*J. Amer. pharm. Ass., Sci. Ed.*, 1958, **47**, 294.) Thirty-five volatile oils were examined for antibacterial activity, using the filter paper disk method with 5 test organisms. The most effective oils, giving the greatest sum of diameters of zones of inhibition of the test organisms, were eucalyptus, cinnamon and red origanum. Volatile oils were found to be more effective against Gram-positive than Gram-negative organisms. Castor, cod-liver, olive and white mineral oils, infused oils of asafoetida, burdock, henbane, lobelia and mullein, vitamin K_1, and volatile oils of cedar wood and myrrh were shown to be devoid of antibacterial activity. Mixtures of two or three of the volatile oils examined were usually less effective than the individual oils, but enhancement of effect was observed with mixtures of equal quantities of eucalyptus, cinnamon and dwarf pine needle oils, eucalyptus, cinnamon and juniper berry oils, and eucalyptus, cinnamon and niaouli oils. The activity of volatile oils was markedly diminished by the addition of fixed or infused oils. G. B.

Novobiocin Sodium in Selected Ointment Bases, Antibacterial Activity and Stability of. E. Stempel, L. Greenberg and A. Urdang. (*Amer. J. Pharm.*, 1958, **130**, 116.) Ointments of novobiocin were prepared in a number of bases of different types, and their antibacterial activity against *Micrococcus pyogenes* var. *aureus* determined by the agar cup-plate method. Hydrophilic ointment U.S.P.XV appeared to be the most suitable of the water-miscible bases, as the antibiotic was found to be unstable in polyethylene glycol ointment U.S.P.XV. If a washable absorption base is preferred, the following formula is recommended: cetyl alcohol 12, stearyl alcohol 16, Ethofat '60/60' 8, propylene glycol 21, white soft paraffin 43. Of the greasy absorption bases examined, Plastibase hydrophilic-water (4:1), Falba-water (2:1) or Hydrosorb-water (2:1) were found to be satisfactory. Greasy bases did not release the antibiotic so readily as other types. Ointments prepared with petrolatum rose water ointment U.S.P.XV showed the greatest antibacterial activity after storage for one week, but ointments prepared with Plastibase were more stable.

G. B.

Ophthalmic Ointments, Bacteriological Study of. R. W. V. Wyk and A. E. Granston. (*J. Amer. pharm. Ass., Sci. Ed.*, 1958, **47**, 193.) Commercial opthalmic ointments were examined by the following method. The content of an unused tube of ointment was transferred aseptically to a flask containing 25 ml. of sterile water and a few glass beads. The flask was warmed to 45° and the melted ointment dispersed by placing the flask on a shaker for an hour. 1 ml. of the liquid was mixed with blood agar and a plate poured. Counts were carried out after incubation at 37° for 24 hours. Of the ointments examined 14·5 per cent were sterile, and most had counts of less than 50 organisms per g. For experimental purposes an ophthalmic ointment base consisting of white wax 1, cetyl alcohol 1 and *iso*propyl myristate (Deltyl extra) 4 was inoculated with *Micrococcus pyogenes* var. *aureus*. Various substances were examined for their efficiency in killing or inhibiting growth of the organism, and it was shown that benzyl alcohol (0·5 per cent) is more effective than benzalkonium chloride (0·02 per cent) or chlorbutol (0·25 per cent). A series of ointments prepared extemporaneously with the addition of 0·5 per cent of benzyl alcohol showed a reduction of 97 per cent in their content of bacteria, as compared with similar commercial ointments prepared without benzyl alcohol. G. B.