ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

***Aspidosperma olivaceum* M. Arg., Alkaloids of.** J. Schmutz and F. Hunziker. (*Pharm. Acta Helvet.*, 1958, **33**, 341.) The isolation of uleine, $C_{18}H_{22}N_2$, and of a newly discovered alkaloid olivacine, $C_{17}H_{14}N_2$ from the root- and stem-barks and leaves of *Aspidosperma olivaceum* M Arg. is described. Uleine had been previously shown by the authors to be a tetracyclic, oxygen-free, dihydrocarbazol alkaloid. The new alkaloid, olivacine, crystallizes from dilute methanol in yellow needles, m.p. 318°–324°, is optically inactive, has an intense blue fluorescence in very dilute alcoholic solution and shows an absence of *N*-methyl and *C*-alkyl. On chromic acid oxidation it yields a trace of acetic acid and another acid of unknown structure already obtained from the alkaloids or related species. The ultra-violet absorption spectrum indicates a polycyclic aromatic system. Addition of acid causes a bathochromic shift and a loss of fine structure, indicating an aromatically bound nitrogen atom. The alkaloid is a relatively strong base. The infra-red spectrum is very similar to that of u-alkaloid D, $C_{17}H_{16}N_2$ from *Aspidosperma ulei* Mgf., for which the authors assigned a carbazole structure. These two alkaloids also have a great many similar colour reactions which are given. D. B. C.

Holarrhidine, A New Alkaloid from *Holarrhena antidysenterica* Wall. L. Lábler and V. Černý. (*Coll. Czech. Chem. Comm.*, 1959, **24**, 370.) Cold extraction of the bark with ethanol-aqueous ammonia is reported to yield the total alkaloids in 1·39 per cent yield, which can be separated with light petroleum into insoluble (0·31) and soluble fractions (1·07 per cent). Holarrhimine (0·028 per cent) was isolated from the petrol-insoluble matter, which contains at least four alkaloids, by precipitation as the sparingly-soluble *p*-nitrobenzylidene derivative. Treatment of the mother liquors with cinnamic acid precipitated the sparingly-soluble holarrhidine cinnamate. Holarrhidine, m.p. 181 to 182°, $[\alpha]_D^{20}$–23° has the molecular formula, $C_{21}H_{36}ON_2$. Methoxyl and *N*-methyl groups are absent, and the molecule contains one ethylenic bond. The petrol-soluble fraction contains at least six alkaloids, including connessine, which on methylation with formic acid-formaldehyde yielded small amounts of *NNN'N'*-tetramethylholarrhimine. J. B. S.

***Lobelia cardinalis*, Chromatographic Investigation of the Basic Fraction from.** F. Kaczmarek and E. Steinegger. (*Pharm. Acta Helvet.*, 1958, **33**, 852.) Since it was thought that *L. cardinalis* contained only one alkaloid, lobinaline, some plants were grown from seed, harvested in bloom, and the crude alkaloidal content determined in the flowers, leaves and stalks. The average content was 0·46 per cent, and was largest in the flowers, smaller in the leaves and much smaller in the stems. Paper chromatography of the crude alkaloids revealed two spots, with R_F values respectively 0·5 and 0·86, which gave a positive reaction with Dragendorff's reagent. Both of these substances were isolated by fractional crystallization procedures, and the substance with $R_F = 0{\cdot}5$ was identical with lobinaline. The second substance of $R_F = 0{\cdot}86$ was a base and was designated Cardinalis-alkaloid 2. Its structure will be investigated. D. B. C.

ANALYTICAL

Barbituric Acids, Substituted, Non-aqueous Assay for. S. W. Goldstein and D. F. Dodgen. (*Drug Standards*, 1958, **26**, 113.) Samples of barbitone, amylobarbitone, aprobarbitone, methylphenobarbitone, phenobarbitone and vinbarbitone were assayed by non-aqueous titration against potassium hydroxide in anhydrous methanol, or lithium methoxide. Potassium hydroxide in methanol was readily prepared and relatively stable, but gave somewhat lower results than lithium methoxide. A method of titration with 0·1N lithium methoxide using dimethylformamide as solvent and thymol blue as indicator is recommended. When titrating with potassium hydroxide in methanol using thymol blue, a more definite end point was obtained in dimethylformamide than in chloroform-methanol. G. B.

Camphor, Gravimetric Estimation of, in Pharmaceutical Preparations. K. K. Kaistha. (*Drug Standards*, 1958, **26**, 83.) Liniment of camphor (1·5 g.), liniment of mustard (2 ml.) or acetic liniment of turpentine (2 ml. of oily distillate) was mixed with 2·5 ml. of aldehyde-free ethanol (80 per cent), 2 g. of semicarbazide hydrochloride and 1·5 g. of freshly fused potassium acetate and heated at 70° for 3 hours under reflux, shaking occasionally. Water was added, and after stirring and cooling, the precipitate was collected on a filter, washed with cold water and light petroleum, dried, and weighed, each g. of residue being equivalent to 0·727 g. of camphor. With suitable modifications the method was applied to the determination of camphor in liniments of aconite, belladonna and chloroform, concentrated camphor water, spirit of camphor, concentrated camphorated tincture of opium, and compound chloral pigment. Recoveries of camphor ranged from 96·9 to 102·75 per cent. G. B.

(±)-Canadine, Resolution of, by Paper Chromatography. O. F. Uffelie and M. M. Nijland. (*Pharm. Weekbl.*, 1958, **93**, 1045.) In this method ordinary Whatman No. 1 paper is used without special preparation, and the mobile phase is a 2 per cent aqueous solution of potassium dihydrogen phosphate in freshly boiled and cooled distilled water and other buffer solutions at pH 5. Quantities of 1 to 4 μg. of the racemic mixture can be separated. Canadine isolated from hydrastis gave one spot with the same R_F value as that of (−)-canadine. D. B. C.

Novobiocin, Carbamate-Ammonia Assay for. F. A. Bacher, G. V. Downing, Jr. and J. S. Wood, Jr. (*Analyt. Chem.*, 1958, **30**, 1993.) This method depends upon the release of ammonia from the carbamyl group in novobiocin by alkaline hydrolysis, and subsequent titration of the ammonia after its recovery in an ammonia distillation apparatus. The reaction is not quantitative on alkaline hydrolysis alone since a certain amount of carbamate ion may form which is stable in alkaline solution. To overcome this difficulty the solution is made acid with sulphuric acid to hydrolyse the carbamate ion to carbon dioxide and ammonia, and then alkaline again to recover the final traces of ammonia. About 50 mg. of novobiocin is required for each assay and the error is ± 1 per cent. A blank determination is performed and a correction procedure is described for ammonium ion and non-extractable amides and amines in the sample, but it was rarely found necessary to apply any correction. As little as 5 mg. of sample could be assayed, but only with considerable loss in precision. D. B. C.

Ointments, A Note on the Assay of, in Non-aqueous Solution. S. M. Wang, H. W. Starr and R. J. Hoffman. (*Drug Standards*, 1958, **26**, 116.) Ointments were treated with a solvent capable of dissolving the bases and providing solutions miscible with glacial acetic acid. The active ingredients were then determined by titration with perchloric-acetic acid, using methyl violet as indicator. Ointments prepared with paraffin, macrogol and hydrophilic ointment bases were successfully assayed using a mixture of five parts of chlorobenzene and one part of chloroform as solvent, and the colour of the bases did not affect the accuracy of the titration. Results are reported for ammoniated mercury, benzocaine and butamben picrate ointments. G. B.

Reserpine, A Rapid and Simple Colorimetric Determination of, in Pharmaceutical Preparations. A. W. M. Indemans, I. M. Jakovljevic, J. J. A. M. van der Langerijt. (*Pharm. Weekbl.*, 1959, **94**, 1.) This method is based on an orange colour reaction obtained by the extraction of reserpine with acetic acid. A reserpine solution in glacial acetic acid equal to 50 to 300 μg. is diluted to 6 ml. with glacial acetic acid, and 0·4 ml. of a 2 per cent solution of sodium nitrite is added. This is then heated on a boiling water bath for 5 minutes. After cooling the contents are transferred to a separating funnel, and 40 ml. of water is added. It is then shaken with 10 ml. of chloroform for 1 minute. The organic phase is separated and filtered, and absorption measured at 465 mμ. Readings are linear up to 300 μg. of reserpine per 10 ml. of chloroform. Reserpinic acid and methyl reserpinate also react to give a coloured compound but these are not removed from the aqueous phase by chloroform. B. R.

ORGANIC CHEMISTRY

1 : 6-Di-(2-bromoethylamino)-1 : 6-dideoxy-D-mannitol Dihydrobromide: a new Cytostatic Agent. L. Vargha and T. Horváth. (*Nature, Lond.*, 1959, **183**, 394.) The analogue of mannomustine (Degranol), 1 : 6-di-(2-bromoethylamino)-1 : 6-dideoxy-D-mannitol dihydrobromide has been synthesised from 1 : 6-diethylenimino-1 : 6-dideoxy-3 : 4-isopropylidene-D-mannitol and concentrated aqueous hydrobromic acid. It was obtained as a crystalline solid, m.p. 204–205°, easily soluble in water, and shows cytostatic activity in smaller doses than the chloric analogue, mannomustine. J. B. S.

BIOCHEMISTRY

BIOCHEMICAL ANALYSIS

Adrenaline and Noradrenaline in Urine, Differential Fluorimetric Estimation of. A. F. De Schaepdryver. (*Arch. int. Pharmacodyn.*, 1958, **115**, 233.) A modified method, based on the technique of von Euler and Floding, for differential fluorimetric estimation of adrenaline and noradrenaline in urine is described. W. C. B.

Adrenaline, Noradrenaline, and 5-Hydroxytryptamine Subjected to Various Simple Treatments, Relative Stability of. D. Joyce. (*Nature, Lond.*, 1958, **182**, 463.) Adrenaline and noradrenaline, which are often present in tissue extracts containing 5-hydroxytryptamine (5-HT) and interfere with the bioassay of the latter, may be destroyed without loss of 5-HT by the action of alkali. 5-HT was assayed upon the rat stomach preparation, and adrenaline and noradrenaline in

concentrations 50–200 times those which were found to abolish the response of the tissue to the stated amounts of 5-HT were inactivated at pH 11 after 30–60 minutes (room temperature). At 98°, 5 minutes at pH 7·8 was equally effective. Heating under more alkaline conditions leads to a loss of 5-HT activity. Similar results were obtained with human serum containing adrenaline, noradrenaline and 5-HT. J. B. S.

Calcium, Determination of, in Biological Material. A. A. Henly and R. A. Saunders. (*Analyst*, 1958, **83**, 584.) The calcium is precipitated at pH 5.0 (to avoid the co-precipitation of magnesium) by an ammonium oxalate-oxalic acid buffer, centrifuged and the calcium oxalate dissolved in 0·2N hydrochloric acid. A known amount of a standard EDTA-ethanolamine reagent and an indicator solution containing eriochrome black T are added, and the excess EDTA is back-titrated with a standard magnesium solution until the colour changes from blue to red. Faeces, tissues or foods are firstly ashed, and the ash dissolved in hydrochloric acid, and the pH adjusted to 5 before precipitation. The method is applicable directly to serum, urine or cerebrospinal fluid, using 1 ml. samples. The accuracy of the method was proved by adding known amounts of calcium to standardised body fluids and reassaying. The standard deviation of 50 analyses was $\pm$ 1 per cent of the mean value. D. B. C.

Catechol Amines, Urinary, A Rapid Quantitative Method for Chemical Estimation of, in the Diagnosis of Phaeochromocytoma. J. T. Wright. (*Lancet*, 1958, **2**, 1155.) The usual method for the extraction and preliminary purification of urinary catechols is to adsorb them on to alumina. The main disadvantages of this method is that the alumina is difficult to standardise and that the adjustments of pH throughout the procedure are critical. A new method is described where the catechol amines are extracted from solution as boric acid complexes by means of anion exchange resins. The method is simple, quick and economical to operate. Using four Dowex anion exchange columns, it is possible to screen four unknown urines for the presence of excess catechol amines or to obtain an accurate estimate of the catechol amine content of one urine in about 2 hours. Urine samples from 69 hypertensive patients have been examined by this method. A high urinary catechol amine content, indicating the presence of a phaeochromocytoma, was found in six cases, the diagnosis being confirmed at operation in each case. M. B.

Dopamine (3-Hydroxytyramine), Fluorimetric Method for the Determination of. A. Carlsson and B. Waldeck. (*Acta physiol. scand.*, 1958, **44**, 293.) Dopamine, a probable intermediate in the biosynthesis of adrenaline and noradrenaline, has been detected in many tissues and in urine. Only fluorimetric methods are sensitive enough for the estimation of this substance. However, in the ethylenediamine condensation method of Weil-Malherbe and Bone the fluorescent products of dopamine and adrenaline have almost the same characteristics. The method described in this paper is similar to the trihydroxyindole method for the estimation of adrenaline and noradrenaline. In this method the catechol amines are first oxidised to red indole derivatives, which are then rearranged in alkali to strongly fluorescent trihydroxyindoles. Utilising differences in the fluorescence characteristics at pH 5·3, micro quantities of dopamine can be detected and estimated in the presence of at least equal amounts of adrenaline or noradrenaline. M. B.

Glucose, Determination of, in Biological Fluids with Ethylenediaminetetra-acetic Acid. H. V. Street. (*Analyst*, 1958, **83**, 628.) This method depends upon the determination of the unreduced bivalent copper in the presence of cuprous oxide, after boiling the sample with excess alkaline cupric tartrate reagent, by titration with ethylenediaminetetra-acetic acid at pH 10. A freshly prepared saturated aqueous solution of murexide (ammonium purpurate) is used as indicator. This changes from yellow to reddish violet. With blood and cerebrospinal fluid a sodium tungstate reagent is used to precipitate protein and does not affect the titration. For blood, cerebrospinal fluid and urine the amount of sample required is 0·2 ml. Recovery experiments showed that the precision of the assay on blood was ± 5 per cent and on urine ± 2 per cent.

D. B. C.

PHARMACOLOGY AND THERAPEUTICS

Acetazolamide, Renal Colic and Anuria from. J. G. Yates-Bell. (*Brit. med. J.*, 1958, **2**, 1392). This is a report of a case of renal colic and anuria in a man of 54 who had been treated with acetazolamide (2 tablets daily for a week) for glaucoma. The diagnosis lay between bilateral non-opaque ureteric calculi and crystalluria. The obstruction to the ureteric catheter closely resembled the blockage experienced in sulphonamide crystalluria, but the diagnosis was not clinched until it was appreciated that acetazolamide was a sulphonamide derivative. The condition responded to the treatment for sulphonamide crystalluria. Previous reports of anuria and ureteric colic following acetazolamide administration are reported, though in no case have sulphonamide crystals been detected, nor has an expected phosphaturia been confirmed. S. L. W.

Anileridine Hydrochloride as an Analgesic and Sedative. R. C. Therien, L. W. Lee, E. M. Malashock and N. B. Davis. (*J. Amer. med. Ass.*, 1958, **168**, 2098.) Anileridine hydrochloride is a substituted pethidine, chemically known as ethyl-1-(4-aminophenethyl)-4-phenyl*iso*nipecotate dihydrochloride. It is an analgesic agent with a potency approaching that of morphine and greater than that of pethidine. It has a prompt onset of action (15 to 30 minutes) and a long duration of analgesia (5 to 6 hours). The side-effects such as general depression, depression of respiration, and lowering of blood pressure, are considerably milder than those produced by morphine and somewhat milder than those of pethidine. The clinical effects of anileridine were studied in 2,500 administrations given to more than 600 patients. It was used both as premedication for general anaesthesia in surgery, in doses averaging 50 mg. orally or subcutaneously, and as a post-operative sedative and analgesic, in doses varying from 25 to 75 mg. Its effects resemble those of morphine and pethidine, but euphoria was uncommon and no evidence of addiction was seen, even in a patient who received 552 doses during the course of treatment of metastatic carcinoma of the colon. Nausea occurred in 8 patients and sickness in 6. Respiratory depression caused by excessive doses in a few patients was easily counteracted by levallorphan in doses of 1 mg. for every 25 mg. of anileridine hydrochloride. S. L. W.

Atropine Eye-drops, Toxic Psychosis Following. J. P. Baker and J. D. Farley. (*Brit. med. J.*, 1958, **2**, 1390.) This is a report of a case of acute confusional psychosis in a woman of 33 following routine daily instillations of 1 per cent atropine sulphate drops over a period of 3½ weeks in the treatment of retinal

detachment. The psychosis was accompanied by myocardial changes. Other peripheral signs of atropine intoxication were absent. A remission of the confusional state followed discontinuation of the eye-drops and a short course of electroplexy; paranoid trends and emotional lability persisted for a further few days. The psychotic symptoms and myocardial change were later reproduced in the same patient following a small test dose of atropine given by injection. The origin of states of minor emotional disturbance in patients undergoing eye surgery with prolonged atropinisation warrant closer investigation. E.C.G. changes appear to be useful in establishing a diagnosis of atropine sensitivity where other peripheral signs of atropine intoxication are lacking.

S. L. W.

Bephenium Hydroxynaphthoate in the Treatment of Hookworm. L. G. Goodwin, L. G. Jayewardene and O. D. Standen. (*Brit. med. J.*, 1958, **2**, 1572.) Bephenium (benzyldimethyl-2-phenoxyethylammonium) was compared with tetrachlorethylene in the treatment of 284 cases of hookworm infection in Ceylon. Of the three salts employed—bromide, embonate and hydroxynaphthoate—the hydroxynaphthoate was selected as the most suitable, administered as tablets or as a sweetened and flavoured suspension in water. The drug has a bitter taste but was accepted by both adults and children. A single dose of 2 or 3 g. of base (1·73 g. of hydroxynaphthoate equals 1 g. of bephenium base), or multiple doses given on successive days or three times on the same day, compared favourably in effectiveness with tetrachlorethylene. The bephenium was given early in the morning on an empty stomach and no food allowed for 2 hours; if given in three doses on one day, the second and third doses were spaced between meals. No purge was given after the dose. Bephenium hydroxynaphthoate was found particularly suitable for the treatment of patients with advanced anaemia, diarrhoea, and heavy hookworm infections because of its low toxicity, even with large doses, and because no purge is necessary. In view of its bitter flavour, however, it is desirable to withhold the treatment from patients with severe vomiting and dehydration until the fluid balance has been restored by intravenous infusion. Bephenium hydroxynaphthoate was also effective against roundworm, which was present as a concurrent infection in 85 of the patients treated with this drug; tetrachlorethylene was ineffective against roundworm in 16 patients treated. S. L. W.

Bephenium Salts; Excretion in Urine. E. W. Rogers (*Brit. med. J.*, 1958, **2**, 1576.) From a study conducted on 18 healthy volunteers in Britain, given doses of bephenium bromide, embonate and hydroxynaphthoate, it was shown that the urinary excretion of bephenium and its metabolites in man is low. The percentage of dose recovered in 24 hours following administration of hydroxynaphthoate, 2·5 g. of base daily for 3 or 4 successive days, varied between 0·05 and 0·27. Side-effects from the hydroxynaphthoate were slight; mild diarrhoea sometimes occurred and there were some complaints of borborygmi. Side-effects ceased completely 24 hours after the dose. A technique for the determination of bephenium in urine by means of a modification of the methyl orange dye-lake procedure is described. S. L. W.

Cyanocobalamin in Pernicious Anaemia: Intramuscular or Oral? E. H. Hemsted and J. Mills. (*Lancet*, 1958, **2**, 1302.) Over a period of 2 years a group of 71 patients suffering from pernicious anaemia, taking a daily dose of 100 μg. of cyanocobalamin by mouth, were maintained at least as well as a

group of 84 patients receiving a monthly intramuscular injection of 100 μg. The oral preparation was given as an elixir containing 50 μg. per teaspoonful, and each patient was instructed to take two teaspoonfuls each morning on awakening and to take no food or drink for one hour afterwards. It had previously been shown that during a period of 5 years no relapse occurred in any of 43 patients treated throughout with 100 μg. of cyanocobalamin by intramuscular injection once a month, and that no advantage was gained by increasing the dose beyond that amount. S. L. W.

1 : 6-Di-(2-bromoethylamino)-1 : 6-dideoxy-D-mannitol Dihydrobromide, Effect of on Tumours of Laboratory Animals. J. Baló, G. Kendrey, J. Juhász and I. Beszynyák. (*Nature, Lond.*, 1959, **183**, 395.) The effects of 1 : 6-di-(2-bromoethylamino)-1 : 6-dideoxy-D-mannitol dihydrobromide and mannomustine have been compared on different tumours in rat and mouse. The compounds were administered once daily intraperitoneally after the appearance of palpable inoculated tumours. The bromo compound inhibits the growth of both rat and mouse tumours to a greater extent than mannomustine. Effective dose levels are 3 to 5 mg./kg. in mice and 2 to 3 mg./kg. in rats for the bromo compound compared with 20 mg./kg. and 15 mg./kg. for the chloro compound (mannomustine) in mice and rats respectively. Changes in the bone marrow and the blood are described. J. B. S.

Hydrocortisone Hemisuccinate by Inhalation in Asthma. J. M. Smith. (*Lancet*, 1958, **2**, 1248.) A controlled trial of hydrocortisone hemisuccinate solution, 5 mg. daily by inhalation for a month, was conducted on 57 children with asthma. Twenty-nine of the children were given hydrocortisone and 28 an apparently identical placebo. It was considered that in order to be of real value the treatment must be shown to be beneficial to at least 50 per cent more children than might benefit from the inhalation of an inert solution. As it was estimated that 15 per cent would be likely to benefit from the placebo, the hydrocortisone had to benefit 65 per cent or more to satisfy the criteria. The treatment failed to benefit 50 per cent more children than did the placebo; over the whole period of the investigation the hydrocortisone benefited 21 per cent of 29 children, and the placebo 14 per cent of 28 children. S. L. W.

Noradrenaline Infusion, Fall of Blood Pressure after, and its Treatment by Pressor Agents. J. H. Burn and M. J. Rand. (*Brit. med. J.*, 1959, **1**, 394.) The experiments were carried out on spinal cats to which, on the two preceding days, resperpine (3 mg./kg.) had been administered intraperitoneally. After reserpine, the store of noradrenaline in the heart and blood vessels disappears and under these conditions an intravenous infusion of noradrenaline caused a rise in blood pressure which was not sustained but which gradually returned to the resting level in spite of the fact that the rate of infusion remained the same. After the infusion the rise in blood pressure normally produced by a rapid injection of noradrenaline did not occur but the response to ephedrine, which is abolished after treatment with reserpine, was restored by the infusion. The authors explain these results by suggesting that during an infusion, noradrenaline is taken up and held in a store from which amines like ephedrine can release it. Apparently because of the increase in the store of noradrenaline, the vessels become insensitive to further injections of this and other directly acting amines. Sympathomimetic amines may be divided into three classes: those which, like noradrenaline, work only by direct action, those which, like tyramine and

ephedrine, appear to act only by liberating noradrenaline from the store and which are, therefore, most active at the termination of a noradrenaline infusion which has been filling the store and finally, those which are intermediate having some direct action as well as some action in discharging the store. In experiments in which both blood pressure and spleen volume were recorded, the actions of six pressor amines were examined. Three of these—methedrine, mephine and vonedrine appeared to act only by liberating noradrenaline from the store, while the other three—aramine, vasoxine and propadrine, were shown to have both a direct action and an action through discharging the store. It is well known that on stopping a drip of noradrenaline, the patient's blood pressure often falls to a low level. Noradrenaline is known to depress ganglionic transmission and the absence of sympathetic tone after an infusion may in part be due to this action. The main conclusion arising from this investigation, however, is that when this occurs, the correct procedure is to inject one of those amines believed to act by discharging the noradrenaline store, rather than to resume the drip. W. C. B.

Polyoestradiol Phosphate, A Long Lasting Oestrogen. O. Fernö, H. Fex, B. Högberg, T. Linderot and S. Veige, (*Acta. chem. scand.*, 1959, **12**, 1675.) This is a high molecular weight polyester of oestradiol-17β and phosphoric acid. The chemical and biological properties are described. The molecular weight was 26,000, indicating a molecule of approximately 80 oestradiol moieties. Enzyme studies showed it to be a powerful inhibitor of acid phosphatase, alkaline phosphatase and hyalurodinase. Pharmacologically the acute intravenous LD50 in mice was 240 mg./kg. and the chronic LD50 by the subcutaneous route 700 mg./kg. The oestrogenic activity in spayed mice showed the log dose to be linearly related to the log duration of oestrogenic activity. Doses of 7μg. and 14 μg. gave a mean duration of 17 and 26·4 days respectively. The compound was inactive orally. G. F. S.

Prednisolone in the Treatment of Chronic Asthma. H. M. Brown. (*Lancet*, 1958, **2**, 1245.) Ninety patients with chronic asthma, in whom bronchodilator drugs had failed, were treated with prednisolone. Complete relief of bronchospasm was obtained in 57 cases, partial relief in 10, slight relief in 7, and no relief in 16. Except when bronchospasm was very severe the dose given was 5 mg. three times daily for a week, followed by reassessment and adjustment of dosage sufficiently to keep the patient free from wheezing. Experience showed that if there was no improvement in the course of two weeks on 15 or 20 mg. of prednisolone daily, in the absence of purulent sputum, no significant benefit would be derived from continuing treatment. The elderly bronchitic with severe bronchospasm rarely benefited. The success of the treatment is dependent on the presence of large numbers of eosinophils in the sputum. In the absence of an eosinophilic sputum a satisfactory response is unlikely and the use of prednisolone is contraindicated. A rapid method of determining eosinophils in the sputum is as follows. Place a small piece of sputum on a slide, spread out with forceps (*not* into a thin film), apply one drop of Leishman strain and one drop of distilled water, put a cover-slip on top and examine under a 2/3 in. objective in daylight in from 10 minutes to several hours. The treatment never does more than suppress the asthmatic state; it does not abolish eosinophils in the sputum though the number is much reduced. S. L. W.

Vitamin B_{12}, Site of Absorption of, in Man. C. C. Booth and D. L. Mollin. (*Lancet*, 1959, **1**, 18.) A study has been made of the distribution of radioactive vitamin B_{12} in the small intestine of patients undergoing abdominal operations and of the absorption of B_{12} after small intestinal operations. Three hours after an oral dose of 1 μg. of ^{56}Co-labelled vitamin B_{12} little or no radioactive material was found in the plasma. There was no radioactivity in the proximal small intestine, all the radioactivity being in the distal portion. Peak plasma activity occurred at 8 to 12 hours after the dose. In patients who had undergone resection or short circuiting of the small intestine, absorption was normal in one who had had 8 feet of jejunum resected while in nine patients whose ileum had been resected absorption of B_{12} was subnormal. Absorption was not improved by the administration of intrinsic factor derived from hog stomach. Examination of patients who had had resection of the ileum from 1 to 6 years previously showed evidence of megaloblastic anaemia which responded to injections of vitamin B_{12}. The results show that absorption of vitamin B_{12} occurs in the ileum, and this is supported by experiments previously reported in animals. It is suggested that patients whose ileum has been resected or short circuited should be kept under close haematological supervision or treated prophylactically with vitamin B_{12}. G. F. S.

APPLIED BACTERIOLOGY

Dihydrostreptomycin and Anaerobiosis—Indirect Evidence for Two Sites of Action of Dihydrostreptomycin. G. M. Williamson. (*J. gen. Microbiol*, 1958, **19**, 584.) The activity of streptomycin and dihydrostreptomycin is known to be decreased under anaerobic conditions. In an attempt to find some reason for the greater resistance to dihydrostreptomycin of a facultive anaerobe when growing anaerobically experiments were carried out under carefully controlled environmental conditions with organisms whose growth rate and general metabolic behaviour was known. The sensitivity to the antibiotic of four strains of *Escherichia coli* and of *Aerobacter aerogenes* when growing under aerobic and under anaerobic conditions in heavily buffered enriched medium was compared. Each comparison covered a series of pH values from 5·5 to 8·0. The results showed that the decreased activity of dihydrostreptomycin appeared to be related to the enzymic make up of the organism in respect to carbohydrate metabolism. It could not be due to a slower rate of growth of the organism under anaerobic conditions nor could it be accounted for by acidic end products of anaerobic growth. In a further series of experiments a comparison was made of the behaviour of *A. aerogenes* under identical conditions, but in a simple medium. The results obtained suggested that dihydrostreptomycin was more effective against synthesising than against energy producing mechanisms.

W. C. B.

Sorbic Acid and Other Preservatives, Fungistatic Effect of. C. Trolle-Lassen. (*Arch. Pharm. Chem.*, 1958, **65**, 679.) The fungistatic activity of six substances against strains of *Aspergillus*, *Mucor* and *Penicillium* spp. was determined at pH 3, 5, 7 and 9, using a serial dilution technique. Benzalkonium chloride and phenylmercuric acetate proved to be the most potent substances tested, while methyl parahydroxybenzoate had a lower activity but was less affected by changes in pH. Sorbic and benzoic acids showed good fungistatic activity at pH 3–5, but were relatively inactive in alkaline solution. Sodium propionate was the least effective substance examined. G. B.

***Staphylococcus aureus*, Dissemination from Woollen Blankets.** K. F. Anderson and R. A. W. Sheppard. (*Lancet*, 1959, **1**, 514.) A series of experiments were carried out to demonstrate the dissemination of *Staph. aureus* from an infected blanket. A new 100 per cent wool blanket, free from pathogenic staphylococci, was infected with a 6-hour broth culture of *Staph. aureus* phage-type 80/81, using a wooden swab-stick lightly drawn across the blanket. The experiments were carried out in an empty room, the air of which was shown to be free of coagulase-positive staphylococci. In Experiment 1 the infected blanket was lightly shaken at waist height over a corresponding marked area on the floor and 16 culture plates were placed at distances of from 1 to 4 feet round the outside of the marked area, the plates were then exposed and 15-minute precipitation samples collected; in Experiment 2, 4 new plates were placed in position at points 8 feet from the marked area, which was then swept with a new broom, precipitation samples being again collected over a 15-minute period; in Experiment 3, 4 plates were placed in position at bed-level at a distance of 4 feet from the marked area, the blanket being shaken as before and 15-minute samples collected. *Staph. aureus* was isolated from 4 plates exposed during Experiment 1, from 2 plates in Experiment 2, and from 3 plates in Experiment 3. Two weeks after the experiments colonies of the test staphylococcus could still be recovered from the infected blanket. While hospital blankets cannot be solely incriminated, these experiments show that they cannot be excluded from measures to reduce the number of pathogenic staphylococci in hospital wards.

S. L. W.

2:4:5-Trichlorophenyl Ester with an Antimycotic Action. L. Hepding, H. M. Henning and U. Jahn. (*Arzneimitt.-Forsch.*, 1958, **8**, 525.) The fungistatic action of carbamic acid esters of chlorinated phenols is described with special reference to the phenylcarbamic acid ester of 2:4:5-trichlorophenol which seemed to be most effective, combining marked antimycotic action with bacteriostatic properties. Although not quite as active as the parent phenol, the substance was better tolerated and less irritant to mucosa and skin. The fungistatic, fungicidal, bacteriostatic and bactericidal activities against several organisms were investigated, and also the virucidal activity against the Newcastle-Allantois virus which was appreciable. The inhibition of the oxygen-uptake of various bacteria and the effect on the growth of fibroblasts from embryonic chick hearts were investigated in order to ascertain the effect on living cells. The acute and chronic toxicities were investigated using mice and rats respectively. By the oral route, acute toxicity was too low to ascertain the LD50 dose under the conditions described, and thus the substance was far better tolerated than the free phenol. The local toxicity was tested on the ear-muscle and conjunctiva of rabbits and chronic irritability was tested on the skin of guinea pigs. The progress of healing of induced or artificial infection on rabbits' ears was followed and gave promising evidence of usefulness clinically. Lastly, the compounding of the substance into pharmaceutical preparations was investigated.

D. B. C.