ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Alkaloids, Hydrofluorides of. M. M. Janot and M. Chaigneau. (*Ann. pharm. franc.*, 1950, **8**, 812.) The hydrofluorides of a number of alkaloids are described. They may be prepared by dissolving the alkaloid in hydrofluoric acid, and evaporating the solution in the cold over phosphorus pentoxide. All the compounds, except that of narcotine, contain water of crystallisation, and all are soluble in water. G.M.

***Nicotiana* Alkaloids, Nornicotine and Anabasine, New Tests for.** L. Feinstein and E. T. McCabe. (*Science*, 1950, **112**, 534.) A new colour test for nornicotine and anabasine, and another new test for nornicotine are reported. Nicotine fails to give similar colour reactions in both tests. A solution of quinhydrone (0·5 g. in 100 ml. of ethanol) react with nornicotine and anabasine at *p*H 7 (phosphate buffer) to give a cherry red solution. A similar quantity of nicotine gives no colour. The second test (for nornicotine) consists of the violet colour produced as follows—(the quantities illustrate the sensitivity of the test): nornicotine (363 μg.) in 5 ml. of acetone, plus 15 ml. of di-isopropyl ketone, 2 ml. of 2 per cent. *p*-hydroxybenzoic acid in di-isopropyl ketone and 2 ml. of 0·3 per cent. 1:3-diketohydrindene in di-isopropyl ketone. The violet colour produced gives a reading of 384 at 540 mμ on the Klett-Summerson colorimeter within 60 minutes after the start of the reaction. The same quantity of nicotine or anabasine does not give this violet colour under the same conditions.

A. H. B.

Strychnos Alkaloid, A New. F. A. L. Anet, G. K. Hughes and E. Ritchie. (*Nature*, 1950, **166**, 476.) Leaves from *Strychnos psilosperma* were found to contain 1 per cent. of total alkaloid from which a new alkaloid named strychnospermine was isolated. It formed colourless needles, m.pt. 209°C., had $[\alpha]_D$ + 58°C. (C = 2·07 per cent. in chloroform) and analysis of it, its bromo-derivative (m.pt. 245°C.), its hydrochloride (m.pt. 330° to 332°C. with decomposition) and its picrate (m.pt. 254°C. with decomposition) indicated the formula $C_{21}H_{28}O_3N_2$. One methoxyl, one methimino group and a tertiary N atom were shown to be present. Strychnospermine could not be hydrogenated in acetic acid solution in presence of palladium charcoal. Hydrolysis with acid or alkali yielded deacetylstrychnospermine (m.pt. 222°C.), which was smoothly reconverted to the parent alkaloid by acetic anhydride. The deacetyl derivative with nitrous acid gave a pale yellow crystalline nitroso compound (m.pt. c.195°C. decomp.). The remaining oxygen atom is inert. By the application of Woodward's theory (*Nature*, 1948, **162**, 155) of the biogenesis of the alkaloids of the strychnine group, two possible structural formulæ for strychnospermine were deduced which are consistent with the above properties. A. H. B.

ANALYTICAL

Khellin and Visnagin, Determination of. W. C. Ellenbogen, E. S. Rump, P. A. Geary and M. Bourke. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40.** 287.) Five methods for the determination of khellin and visnagin are discussed. (1) The alkaline hydrolysis colorimetric method was not satisfactory because of many variables such as the exact method of adding and mixing the reagents, etc., which led to non-reproducibility of results. The two substances gave the same colour although the intensity differed. (2) The sulphuric acid hydrolysis colorimetric method (slightly modified) led to greater reproducibility of the results, but khellin and visnagin gave the same colour. Furthermore, the possibility of error from excipient materials in the assay of tablets is great. (3) The ultra-violet absorption method was convenient and gave reproducible results. Using a solution in cyclohexane it makes possible analysis of the individual components in mixtures of khellin and visnagin except for those occurring in crude extracts. (4) The polarographic method gave results equivalent to those from the ultra-violet determination. (5) The infra-red absorption method may be used to obtain ratios of one component to another but at present is not satisfactory for absolute quantitative measurements. New infra-red methods being developed, using a double-beam instrument, indicate good accuracy and convenience.

A. H. B.

Magnesium, Volumetric Determination of. E. Bovalini and E. Mannucci. (*Ann. Chim.*, 1951, **41,** 237.) The method of L. R. Williams (*Ind. Eng. Chem. Anal. Ed.*, 1946, **18,** 542) for the volumetric estimation of magnesium in the presence of other metals is simple and rapid. It consists in treating the specimen (e.g., a mineral magnesium carbonate) with sulphuric acid (this removes silica and most of the calcium), taking up in water, neutralising with a suitable indicator (methyl red) to precipitate the oxides of iron and aluminium, and then adding a measured volume of standard sodium hydroxide to precipitate magnesium hydroxide, filtering and titrating the excess of sodium hydroxide with standard sulphuric acid using methyl red as indicator. In the presence of calcium, however, results tend to be high. This is due to the precipitation of calcium carbonate by carbonate present in the sodium hydroxide solution and by absorption of carbon dioxide from the air. This can be avoided by the use of solution free from carbonate and carrying on the operations in an atmosphere free from carbon dioxide. This, however, is troublesome and difficult, and good results may be obtained by adding two volumes of 95 per cent. ethanol to each volume of the liquid with which the residue from the treatment with sulphuric acid is taken up, and washing the residue with 70 per cent. ethanol. H. D.

Morphine in Poppy Plants, Determination of. W. Poethke and E. Arnold. (*Pharm. Zentralh.* 1951, **90,** 145.) The authors have previously described two processes for the determination of morphine in parts of the poppy plant (*Pharm. Zentralh.* 1949, **88,** 1). It is now shown that neither the gravimetric method, using chlorodinitrobenzene, nor the colorimetric one with nitrous acid, is affected by the presence of narcotoline, which may be present in the capsules in an amount equal to 10 per cent. of the morphine. For the assay of the leaves and all other parts of the plant, only the latter method is suitable on account of the small proportion of alkaloids present. G. M.

Morphine, Microscopic Identification of. G. Denigès. (*Bull. Soc. Pharm. Bordeaux* 1951, **89,** 3.) A quantity of morphine, not exceeding 1 mg., is placed on an object glass and dissolved in 1 drop of N sodium hydroxide. The addition of 1 drop of hydrochloric acid (1 + 2) leads to the formation of a characteristic crystalline appearance of the hydrochloride of the alkaloid. Crystals of the nitrate may also be obtained by using, in place of hydrochloric acid, nitric acid (1 + 3). G. M.

Quinine and Strychnine, Infra-red Determination of. W. H. Washburn and E. O. Krueger. (*J. Amer. pharm. Ass., Sci. Ed.,* 1951, **40.** 291.) An infra-red method for the simultaneous determination of quinine and strychnine in Elixir of Iron Quinine and Strychnine Phosphates N.F. is reported. Because of the 1:20 relationship of strychnine to quinine, both components cannot be determined accurately on a single dilution. The optical density at 6·06 μ is measured when the concentration of strychnine is about 4 mg./ml. and then the solution diluted to bring the concentration of quinine down to the range of 25 to 30 mg./ml. and the optical density measured at 6·2 μ. The concentration of each component is computed by a two-component graphical calculation. A method has also been developed for the determination of quinine hydrochloride by fluorimetric means and strychnine sulphate by an infra-red method. The procedures are simple to perform and their reproducibility is in the range of ±2·5 per cent. These procedures give greater accuracy in the determination of small amounts of strychnine than methods previously described. A. H. B.

ESSENTIAL OILS

Cajuput Oil, Adulteration of. (*Konink. Inst. Tropen., Mededeeling* 95, 1951, 8.) A sample of cajuput oil, with a deep green colour, showed the following figures, compared with the official requirements of the Dutch Pharmacopœia.

	Sample	Official requirements
*d*15°C/15°C	0·9104	0·919 to 0·930
n 20°C	1·4648	1·466 to 1·471
Solubility in 80 per cent. ethanol...	in 2 to 10 vols.	in 1 vol.
α 1 dm.	+ 3·8°	—
cineol, per cent.	63·7	—
distillate between 170°C to 190°C, per cent. ...	66	66

The green colour was apparently due to an artificial colour, and not to copper. The rotation is normally lævo, not dextro as with this sample, and the odour was somewhat abnormal. It is possible that this sample was actually a eucalyptus oil, coloured green. G. M.

Citronella Oil, Ceylon. (*Konink. Inst. Tropen., Mededeeling* 95, 1951, 7.) The citronella oil of Ceylon comes from *Andropogon nardus* L., which is a different variety from that of Java. Comparative figures for the two oils are as follows:

	Java oil	Ceylon oil
density, 20°C	0·881 to 0·889	0·894 to 0·910
n 20°C	1·467 to 1·473	1·479 to 1·485
α 1 dm.	up to −4°, rarely +	−7° to −15°
citronellal, per cent.	33 to 37	maximum 10p
total geraniol, per cent.	minimum 85	minimum 57

There is some difference in the odour of the two oils. G. M.

INORGANIC CHEMISTRY

Bentonites, Cation-saturated. M. Barr and E. P. Guth. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 9.) Sodium bentonite may be prepared as follows. Shake 20 g. of Volclay bentonite with 175 ml. of 1·0 M sodium acetate for 30 minutes, centrifuge and discard the supernatant liquid. Repeat the process 4 times, but shaking for 10 minutes each time. Wash by shaking for 10 minutes with 50 per cent. ethanol, centrifuge and shake 5 times with 80 per cent. ethanol, centrifuge, shake twice with 95 per cent. ethanol, centrifuge, add 95 per cent. ethanol, evaporate and dry at 110°C. Potassium, calcium, magnesium and hydrogen bentonites may be prepared similarly. but water can be used for washing the calcium and magnesium bentonites which do not hydrate to any appreciable degree. The products may be assayed for exchangeable cations by shaking 2·5 g. with 50 ml. of a barium chloride-triethanolamine replacement solution, centrifuging and repeating with a further 50 ml., centrifuging shaking with 25 ml. of 2 a 2·5 per cent. w/v solution of barium chloride, centrifuging and repeating the process with three 40-ml. quantities of water. Aliquot quantities of the combined supernatant liquids are used for the determination of the cations. The results indicate that the bentonites were nearly saturated with the cation used in their preparation. G. B.

ORGANIC CHEMISTRY

***N*-(β-Aminoethyl)-morpholine as a Reagent for Esters.** R. W. Best and L. V. Mullen, Jr. (*J. Amer. chem. Soc.* 1951, **73**, 1967.) The use of *N*-(β-aminoethyl)-morpholine as a reagent for the characterisation of esters has been examined experimentally for some 39 esters of both aliphatic and aromatic acids. *N*-(β-aminoethyl)-morpholides are readily formed by direct reaction under reflux (2 to 3 hours) of this reagent with an ester; for the most part, they are stable crystalline solids of characteristic melting-point. The presence in the molecule of the morpholine group affords a ready means of securing a second derivative by quaternisation of the tertiary nitrogen, in the few cases where the primary product is an oil.

J. B. S.

Benzylpenicillin, New Salts of. L. N. Westfelt. (*Acta chem. scand.*, 1951, **5**, 327.) A series of organic amines has been investigated in an attempt to find bases which might be useful for the isolation of penicillin. The penicillin salts were all prepared by the addition of a slight excess of the base, dissolved in acetone, to a solution of pure free benzylpenicillin in amyl acetate-acetone; some of the salts separated in crystalline form, others as oils. The salts of the following bases were prepared: *N*-monomethylethylenediamine. *N*-monoethylethylenediamine, 2-methylimidazoline, 2-thylimid-

azoline, 2-propylimidazoline, 2-amylimidazoline, 2-benzylimidazoline, 2,4(5)-dimethylimidazoline, 2-ethyl-4(5)-methylimidazoline, 2-propyl-4(5)-methylimidazoline. R. E. S.

Chloramphenical, Synthesis of Analogues of. F. Huebner and C. R. Scholz. (*J. Amer. chem. Soc.* 1951, **73**, 2089.) Modifications of the 2-dichloro-acetamidopropanediol side chain of chloramphenicol have been examined with a view to producing new biologically active analogues. *N*-Dichloroacetyl-*p*-nitrophenylserine (I) is synthesised starting from the ethyl ester of DL-2-phenylserine, which is *N*-dichloroacetylated, *O*-acetylated and nitrated in turn to yield ethyl *O*-acetyl-*N*-dichloroacetyl-*p*-nitrophenylserine; alkaline hydrolysis of the latter yielded the required compound (I), together with a small quantity of a by-product, identified as ethyl α-dichloroacetamido-*p*-nitrocinnamate. The preparation of a homologue of chloramphenical, 1-(*p*-nitrophenyl)-2-dichloracetamido-2-methyl-1 : 3-propanediol (II) is described. α-Benzamidopropiophenone is condensed with formaldehyde and the product reduced, hydrolysed and acetylated to yield 2-acetylamino-1 : 3-diacetoxypropylbenzene, separable into 2 distinct racemates by their differential solubility in ether. Nitration, hydrolysis and *N*-dichloro acetylation of each yielded two crystalline racemates of II. The configurations of these two racemates were not ascertained. 1 : 3-Di-(*p*-nitrophenyl)-2-dichloroacetamido-1 : 3-propanediol (III) was obtained from 1 : 3-diphenyl-2-phenylhydrazono-1 : 3-propanedione by catalytic reduction and the reaction sequence, *N*-dichloroacetylation, *O*-acetylation, nitration and, finally, selective hydrolysis of the *O*-acetyl groups. The stereochemical configuration of III is unknown. A miscellaneous group of substances more distantly related to chloramphenicol is also described, which contain *p*-nitrophenyl groupings and a carbohydrate type, polyhydroxy-amino side chain. None of these compounds nor the compounds I, II and III described above showed antiobiotic activity. J. B. S.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Adrenaline Solutions, Racemisation of. J. Kisbye and S. A. Schou. (*Dansk. Tidsskr. Farm.*, 1951, **25**, 185.) The racemisation of adrenaline hydrochloride is a monomolecular process. The following table shows the values obtained for the reaction constant at different *p*H values at 100°C. and at 110°C., and the corresponding temperature coefficient. The time

*p*H	k_{100}°C.	k_{110}°C.	Temp. coefficient
1·8	0·28	0·84	3·0
2·1	0·17	0·46	2·7
2·4	0·098	0·25	2·6
2·6	0·072	0·19	2·6

required at 100°C. for 50 per cent. racemisation, i.e. 25 per cent. loss of biological activity, is half an hour at *p*H 1·36, 4½ hours at *p*H 2·13; 9 hours at *p*H 2·43; 18 hours at *p*H 3·00, and 30 hours at *p*H 4·28. The velocity constant of racemisation increases rapidly when the *p*H drops below 2. G. M.

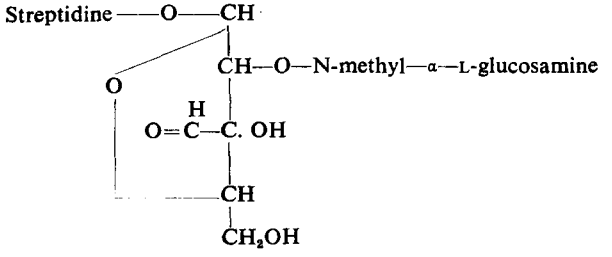
Benzylpenicillin, *d*- and *l*-*N*-methyl-1:2-diphenyl-2-hydroxyethylamine Salts of. V. Y. Young. (*J. Amer. pharm. Ass. Sci. Ed.*, 1951, **40**, 261.) The

l-*N*-methyl-1 : 2-diphenyl-2-hydroxyethylamine salt of benzylpenicillin was obtained by reacting aqueous solutions of potassium benzylpenicillinate and racemic *N*-methyl-1 : 2-diphenyl-2-hydroxyethylamine hydrochloride in molar ratio of 1 : 2. The above penicillin salt separated as a crystalline precipitate. The *d*-*N*-methyl-1 : 2-diphenyl-2-hydroxyethylamine salt of benzylpenicillin was obtained by reacting butyl acetate solutions of *d*-*N*-methyl-1 : 2-diphenyl-2-hydroxyethylamine (obtained from the filtrate from the above penicillin salt) and free benzylpenicillinic acid. These penicillin salts are white, needle-like crystalline compounds whose micro-biological activity approaches the theoretical potency of 1058 I.U./mg. The preparation of these benzylpenicillin salts affords a method for the resolution of racemic *N*-methyl-1 : 2-diphenyl-2-hydroxyethylamine. A. H. B.

Cortisone, *in vitro* Production by Mammalian Cells. H. Seneca, E. Ellenbogen, E. Henderson, A. Collins and J. Rockenbach. (*Science*, 1950, **112**, 524.) Because of the favourable clinical results obtained through the combined use of desoxycorticosterone acetate and ascorbic acid in the treatment of rheumatoid arthritis, it was considered probable that oxidising and reducing agents, especially in conjunction with enzyme systems of the adrenal cortical cells, could synthesise cortisone from desoxycorticosterone or its precursors. Desoxycorticosterone was therefore incubated with various types of tissue in suitably enriched media containing added ascorbic acid, thiamine hydrochloride, insulin, glutathione, riboflavine and pyridoxine. These substances were added singly and in combination. The steroids were extracted from the media and tested chemically by a paper chromatographic method. The following results were obtained. Extracts of adrenals, liver, kidneys, testis, and human placenta were negative for cortisone. Incubation of adrenal gland in media containing desoxycorticosterone, insulin, ascorbic acid, thiamine, pyridoxine, riboflavine and nicotinic acid gave the most constant and potent paper chromatography test. Desoxycorticosterone plus adrenal gland and insulin gave the second highest positive; vitamin B complex was next, followed by ascorbic acid, although sometimes simple incubation of adrenals with desoxycorticosterone yielded positive results. Consistently negative results were obtained with glutathione or with glutathione, insulin, ascorbic acid and vitamin B complex. Liver, testis, kidney, and ovary to a lesser extent, gave some positive results when incubated with desoxycorticosterone, insulin, ascorbic acid, thiamine, riboflavine, pyridoxine and nicotinic acid. Other tissues tested gave negative results. The adrenals of cat and man gave the highest positives. A. H. B.

Hydroxystreptomycin, a new Antibiotic from *Streptomyces griseocarneus*. F. H. Stodola, O. L. Shotwell, A. M. Borud, R. G. Benedict and A. C. Riley, Jr. (*J. Amer. chem. Soc.*, 1951, **73**, 2290.) An account is given of the degradation of hydroxystreptomycin (I) (*Science*, 1950, **112**, 77) a new member of the streptomycin series produced by *Streptomyces griseocarneus*, which is obtained from a Japanese soil. Cleavage of (I) with methanolic hydrochloric acid gave streptidine and a disaccharide hexa-acetate. Streptomycin under similar conditions yields streptidine and a disaccharide penta-acetate. Isolation of a penta-acetyl-*N*-methyl-α-L-glucosamine indicated that the additional hydroxyl group was located in the streptose moiety. This was confirmed by alkaline degradation of (I) to the pyrone CO;C(M):C(CH_2OH):O.CH:CH isomeric with kojic acid. The

corresponding product from streptomycin is meltol $\dot{C}O;C(OH):C(CH_3);O;\dot{C}H:\dot{C}H$. The following formula is therefore indicated for hydroxystreptomycin:



The use and effectiveness of paper chromatography in the detection and separation of the various streptomycins is described with illustrations.

J. B. S.

Noradrenaline; Formation *in vivo* from Dihydroxyphenylserine. C. G. Schmiterlöw. (*Brit. J. Pharmacol.*, 1951, **6**, 127.) Following the administration of 3:4-dihydroxyphenylserine (noradrenaline carboxylic acid) to rabbits by intravenous injection in a dose of 5 mg./kg. varying amounts of noradrenaline were found in the urine. The injection was made when diuresis was established, the urine was collected, concentrated and freed from depressor substances, and the extract was tested on the blood pressure and nictitating membrane of the cat under chloralose. It was shown that the active material in the urine was noradrenaline alone and that no adrenaline was present. Normal rabbit urine so extracted contained no pressor substances in the doses used. The transformation still occurred in animals after adrenalectomy. The injection of the corresponding adrenaline carboxylic acid was not followed by the appearance of any pressor substance in the urine. The possibility that noradrenaline carboxylic acid is a natural precursor to noradrenaline has been supported by experiments *in vitro*. The present finding that this decarboxylation also occurs *in vivo* throws some light on the biosynthesis of noradrenaline. S. L. W.

Oral Penicillin; Enhancement of Effect by Use of Oil Containing Aluminium Resinate. Y. C. Hsu and G. A. H. Buttle. (*Brit. J. Pharmacol.*, 1951, **6**, 89.) Aluminium resinate, a water-repelling substance, given orally to mice with penicillin in oil (20 mg. of resinate in 0·2 ml. of arachis oil per 20 g. mouse with 500 units of calcium or sodium penicillin) is effective in increasing the blood-penicillin levels. This effect is also shown in rats, guinea-pigs and rabbits, but not in cats. Aluminium resinate increases the effect of oral penicillin in protecting mice against a hæmolytic streptococcal infection and, in this respect, oral penicillin with aluminium resinate proved rather more effective than an equivalent amount of procaine penicillin injected intramuscularly. This action of aluminium resinate appears to be due to increased absorption and delayed excretion of the penicillin. Several derivatives of abietic acid (the essential component of aluminium resinate) and various copals, all water-repelling agents, were also tested, but only a few of them showed activity in enhancing blood-penicillin levels in mice after oral penicillin. The acute toxicity of aluminium resinate to mice is low, the LD50 by mouth being 8·75 g./kg. S. L. W.

Salicylates, Effect of, on Serum. F. Tayeau, E. Neuzil and R. Nivet. (*Bull. Soc. Pharm. Bordeaux.* 1951, **89,** 16.) Certain cyclic compounds are known to be able to release serum cholesterol so that it can be extracted with ether; sodium salicylate does not have this action *in vitro.* On the other hand, serum taken from patients treated with salicylates show this effect, although the extraction of the cholesterol by ether is slow and takes some days. By separating the globulin and albumin fractions from such sera, it was shown that the cholesterol directly extracted by ether was that corresponding to the albumin fraction, i.e., that on which the salicylate is fixed. G. M.

Streptomycin-Fatty Acid Complexes, Biological Activity of. F. Gros, M. Machebœuf, M. Blejanski, F. Grumbach and F. Boyer. (*C.R. acad. Sci., Paris,* 1951, **232,** 764.) Streptomycin-oleic acid complex is precipitated when aqueous solutions of streptomycin sulphate and sodium oleate are mixed. The complex, named oleostreptomycin, is of constant composition, consisting of 4 mol. of oleic acid and 1 mol. of streptomycin; it melts at 160° to 170°C. with decomposition. Its properties have been studied in solution in ethylene glycol. *In vitro* it is approximately 20 per cent. more active than dihydrostreptomycin sulphate against *Staph. aureus, E. coli and Diploccocus pneumoniæ*; against *M. tuberculosis* the two substances are of equivalent activity. *In vivo* studies in mice indicate acute and chronic toxicities of a low order compared with dihydrostreptomycin; in the rabbit oleostreptomycin is much more slowly eliminated from the body than is dihydrostreptomycin. Groups of mice infected with *M. tuberculosis* and treated with dihydrostreptomycin and oleostreptomycin respectively received a marked degree of protection, there being zero mortality after 21 days; by comparison, control groups showed 30 per cent. mortality and the survivors exhibited well-developed lesions. Oleostreptomycin and dihydrostreptomycin are equally effective against *Diplococcus pneumoniæ.* The preparation of similar complexes of streptomycin with undecylenic, isolinoleic and hydnocarpic acids is also reported. J. B. S.

BIOCHEMICAL ANALYSIS

Benzene, in Blood, Determination of. D. M. Buis and H. Jansen. (*Pharm. Weekbl.,* 1951, **186,** 357.) A suitable quantity of the blood (3 to 10 ml.), diluted with an equal volume of water and acidified with 2 ml. of 4N sulphuric acid, is distilled from a glass apparatus into 20 ml. of carbon tetrachloride. To prevent foaming 10 ml. of carbon tetrachloride and 1 g. of sodium chloride are added to the blood before distillation. When most of the liquid has distilled over, fresh carbon tetrachloride is added to the flask through a tap funnel and the distillation is repeated. This procedure is repeated twice. The water layer is removed from the distillate, and the benzene in the lower layer is nitrated by treating for 15 minutes with 10 ml. of a mixture of 2 volumes of fuming nitric acid and 1 volume of concentrated sulphuric acid. The carbon tetrachloride is removed on the water-bath, and the residual acid mixture is dissolved in 20 ml. of water, made alkaline with about 70 ml. of 4N sodium hydroxide, and shaken with 15 ml. of methylethyl ketone. The violet colour obtained reaches a maximum after 15 minutes, and is determined photometrically at 550 to 600 mμ. A standard curve is prepared from known quantities (4 to 10 ml.) of a solution of benzene in carbon tetrachloride (1 ml. = 1 μg.). G. M.

Calcium in Serum, Colorimetric Determination of. A. C. Kibrick, D. Palmer and S. Skupp. (*Proc. Soc. exp. Biol., N.Y.*, 1951, **76,** 115.) Transfer 0·5 ml. of serum to 5 ml. centrifuge tubes and add 0·5 ml. of water and 0·25 ml. of 4 per cent. ammonium oxalate; mix well and stand at room temperature overnight. Centrifuge for 10 minutes at 1500 to 2000 r.p.m. and drain off all supernatant fluid, except about 0·02 ml. Wash the precipitate 3 times by adding 0·8 ml. of dilute ammonia down the sides of the tubes and stirring with a fine glass rod; one rod is used for each tube and rinsed with the succeeding wash solution. During centrifuging, the rods are placed in separate test tubes. Add 1 ml. of N sulphuric acid and return the rods to the centrifuge tubes. Include a blank tube containing 1 ml. of sulphuric acid and a standard tube containing 1 ml. of working standard (concentrated calcium oxalate solution 25 ml. diluted to 200 ml. with N sulphuric acid) with each series of determinations. Dissolve the precipitate by placing the tubes in a boiling water-bath; allow the tubes to cool. Add 4 ml. of ceric sulphate-cerous sulphate reagent, stir, and remove the rods; mix by inversion and allow to stand for 15 minutes (use parafilm, not rubber stoppers). Transfer to colorimeter tubes and measure the colour in an Evelyn colorimeter with filter 42 at the 4 ml. aperture. Obtain the concentration of calcium from the difference in density between the blank and unknown solutions on a standard curve prepared from solutions of known amounts of calcium oxalate. The values obtained for the standard should be close to 10 mg./100 ml. Data are presented to show that the results agree within 0·3 mg./100 ml., with those determined by titration with permanganate.

S. L. W.

Glucose in Blood, New Colorimetric Determination of. M. Peronnet and J. Hugonnet. (*C.R.Acad. Sci. Paris.* 1951, **232,** 2150.) For the determination of glucose in blood or cerebrospinal fluid, a quantity of the liquid, deproteinised by the addition of 4 volumes of ethanol (95 per cent.), is treated successively with a 5 per cent. sodium hydroxide sufficient to produce an alkalinity of N/16, and excess of 1 per cent. alcoholic solution of *o*. dinitrobenzene. The ethanol content of the solution is adjusted to about 70 per cent., and it is heated for 2 minutes on the water-bath. The violet colour obtained is proportional to the quantity of glucose present, and is stable for more than 1 hour. The sensitivity is 1 in 100,000. The absorption may be measured at 650 mμ. In normal concentrations, no colour is given by uric acid, creatinine, the chief amino-acids, glutathione or by fluorides or oxalates.

G. M.

Mercury in Urine, Determination of. D. M. Buis and H. Jansen. (*Pharm. Weekbl.*, 1951, **86,** 359.) Mercury may be determined in urine, without destruction of the organic matter, by the following method. 100 ml. of the urine is allowed to stand overnight with 50 mg. of copper powder and 5 ml. of 4N sulphuric acid. The copper is filtered off and washed with warm water till free from chlorine (this is difficult as the copper holds chlorine ions). The copper is dissolved in the smallest possible quantity of 4N nitric acid, and the solution is just boiled. After cooling, nitrite is removed by the addition of a slight excess of permanganate, which is destroyed by oxalic acid. The acid solution is then shaken for 1 minute with 2 ml. of dithizone solution (6 mg. in 100 ml. of carbon tetrachloride), and the orange mercury dithizone solution is run off, the aqueous phase being washed with carbon tetrachloride. The shaking out with 2 ml. of dithizone solution is repeated

until the carbon tetrachloride layer is twice violet. The quantity of dithizone solution taken is noted (A ml.). The combined tetrachloride solutions are shaken a few times with 1 per cent. nitrite-free nitric acid to remove traces of chlorine, and an excess (c ml.) of silver solution (1 ml. = 10 μg. of Ag) is added. After shaking for a few minutes the dithizone layer is run off and the aqueous layer is washed with carbon tetrachloride. The silver in the aqueous layer is then titrated with dithizone solution (B ml.). The dithizone solution is standardised against the silver by titrating 5 ml. of the latter, the dithizone being added in small portions, shaken and run off until the solution remains green (C ml. being used). The quantity of dithizone solution combined with the mercury is then $A + B - c.\frac{C}{5}$. An alternative method is colorimetric: the nitric acid solution is treated with dithizone solution until it shows a weak violet colour. A blank solution, made from 50 mg. of copper powder and the same volume of dithizone solution, is titrated with a mercury solution (1 ml. = 2 μg. of Hg) until the same colour is obtained. G. M.

Sodium Salicylate in Blood, Determination of. F. Tayeau and R. Nivet. (*Bull. Soc. Pharm. Bordeaux.* 1951, **89,** 10.) The improved method of the authors for the determination of salicylates in blood is as follows. 2 ml. of plasma, or 1 ml. diluted with 1 ml. of saline solution, is treated with 0·5 ml. of 6 N hydrochloric acid, added very slowly. After 5 minutes, 30 ml. of carbon tetrachloride is added, the mixture is shaken and transferred to a centrifuge tube. After centrifuging, 20 ml. of the lower layer is placed in a stoppered flask with 10 ml. of water and 0·25 ml. of a 1 per cent. solution of ferric nitrate in 0·07 N nitric acid. After shaking for 5 minutes, the colour of the aqueous layer is determined at 540 mμ. When sodium salicylate is added to serum *in vitro,* it is fixed on the albumen fraction and may be recovered from the latter. This also occurs *in vivo.* G. M.

CHEMOTHERAPY

Curare, Dicarboxylic acid bis(β-tertiaryaminoalkyl)amides as Substitutes. A. P. Phillips. (*Science,* 1950, **112,** 536.) These bis-amides, such as bis-dimethylaminoethyl oxamide, bis-dimethylaminoethyl succinamide, etc., were obtained in excellent yield by a brief refluxing of a slight excess of the unsymmetrical disubstituted ethylene diamines with the dimethyl or diethyl esters of the dicarboxylic acids. The corresponding bis-quaternary ammonium salts were also prepared. Both the bis-amides and the derived quaternary ammonium salts were relatively devoid of curarelike activity. However, both types of compound showed anticholinesterase-like activity in prolonging the duration of the block of neuromuscular transmission produced in the cat by the powerful curarelike acting bis (β-dimethylaminoethyl) esters of dicarboxylic acids. A. H. B.

Local Anæsthetics. I. Some Aryl Alkamine Ethers. H. B. Wright and M. B. Moore. (*J. Amer. chem. Soc.* 1951, **73,** 2281.) A series of alkamine aryl ethers based on the molecule of the active local anæsthetic, β-(α-methylbenzylamino)-ethyl *o*-anisyl ether, $o\text{-}CH_3O.C_6H_4OCH_2.CH_2.NH\ CH(CH_3)C_6H_5$ (I), has been prepared. The synthesis of γ-diethylaminopropyl 4-methoxynaphthyl ether is typical, 4-methoxyl-1-naphthol being dissolved in ethanolic potassium hydroxide and heated with γ-diethylaminopropyl chloride under a reflux condenser. None of the compounds, which were

examined for local anæsthetic effect, was more promising than (I), which is twice as active as procaine. J. B. S.

Sulphones: Derivatives of 4 : 4-Diaminodiphenol sulphone and Related Substances. H. Bauer. (*J. Amer. chem. Soc.*, 1951, **73**, 2113.) Seven types of substituted diphenyl sulphones have been synthesised for evaluation in the chemotherapy of experimental tuberculosis. The following compounds are described: 4-amino-4′-sulphaminodiphenyl sulphone, a diaminopyrophosphoric acid derivative $[O_2NC_6H_4SO_2.C_6H_3NHP(:NH_4ONa]_2O$, 4:4′-dinicotinylaminodiphenyl sulphone, 4-amino-4′-(6″-nicotinyl-) aminodiphenyl sulphone, *N*-*p*-sulphanilylphenylglycine amide, 4-(4′nitrophenylsulphonyl)-phenylacetamidine, 4-amino-4′-aminoethylaminodiphenyl sulphone, 4:4′-diaethylaminophenyl sulphone, 3-methoxy-4:4′-diaminodiphenyl sulphone, 3-methoxy-4-hydroxy-4′-aminodiphenyl sulphone. Chemotherapeutic evaluation revealed that derivatives containing one free amino group are more active than compounds substituted in both amino groups. Acylation of the type described above reduces the activity more than alkylation. The *in vivo* activity of *N*-*p*-sulphanilylphenylglycine amide is about half that of 4:4′-diaminodiphenyl sulphone. The introduction of methoxyl substituents in position 3 gives a fourfold reduction of toxicity. J. B. S.

PHARMACOLOGY AND THERAPEUTICS

Anthraquinone Drugs, Toxicity of. W. Hallermann. (*Med. Monatschr.*, 1951, **5**, 328.) Toxic effects resulting from the administration of vegetable drugs containing anthraquinone derivatives are rarely reported. Aloes is considered to be the most toxic. The author reports a fatal case following the administration of a preparation containing about 1 g. each of rhubarb, senna pod, and aloes, with liquorice, oil of absinthe, etc. The symptoms and post-mortem indicated yellow atrophy of the liver and nephrosis. Thus it appears that even the official dosage is sufficient to have a toxic effect, in sensitive patients, and especially where kidneys and liver are not normal. G. M.

Arterenol, Adrenaline and Isopropylarterenol, Comparative Pharmacology of. E. Chen, R. Portman, D. Russell and C. R. Ensor. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 273.) In these experiments *l*-adrenaline, *l*-arterenol bitartrate, and *dl*-isopropylaterenol hydrochloride were used. A comparison was made of the cardiovascular, bronchodilatory, spasmogenic, hyperglycæmic, and adrenal-stimulating effects at equivalent dose levels. The following results were obtained. Adrenaline acts like arterenol to produce the vasopressor effect, and like isopropylarterenol to produce the vasodepressor effect, and is twice as potent as arterenol in producing vasoconstriction of the vascular bed of skeletal muscles. Isopropylarterenol is 3 times more effective than adrenaline in the prevention of histamine-induced bronchospasm in guinea-pigs, while arterenol is 1/60th as effective as adrenaline. Arterenol is less active than adrenaline in causing contraction of isolated guinea-pig's seminal vesicle. Isopropylarterenol is devoid of a spasmogenic action. Arterenol is more active than isopropylarterenol but less active than adrenaline in its hyperglycæmic activity. They are equally influential on the ascorbic acid metabolism of the adrenals. A. H. B

Chloramphenicol Resistance. M. Meads, C. M. Harris, N. M. Haslam and W. A. Cline. (*J. clin. Invest.*, 1950, **29**, 1474.) The

sensitivities of organisms isolated from the urine of patients during treatment with chloramphenicol were determined by a serial dilution method and studies of the development of resistance were made on a highly susceptible strain of *Klebsiella pneumoniæ*, type B, isolated from the spinal fluid of a patient with meningitis. Resistance developed during treatment in 2- to 8-fold increments which occurred at unpredictable intervals during the first week. If the number of urinary bacteria decreased by 99·999 per cent., continued treatment was successful even if the residual bacteria became drug-fast. In 5 out of 7 patients strains showing 8- to 64-fold increase in resistance persisted during treatment or recurred after treatment ceased. Approximately 25 per cent. of the bacterial species recovered from the urine produced resistant variants, but usually in small numbers which could be dealt with by the body defences. In the *in vitro* investigation repeated tests showed that the untreated strain of *K. pneumoniæ* produced about 0·0001 per cent. of cells showing small degrees of resistance. Substrains from these resistant organisms contained cells having greater degrees of resistance. After 11 successive transfers of a resistant strain through a chloramphenicol-free medium, the resistance remained at the previous level and there was no evidence of the production of any less resistant mutants. The acquired resistance was specific to chloramphenicol. The wide fluctuations in the number of resistant variants appearing in different cultures shows that the development of resistance is by mutation and not by physiological adaptation.

H. T. B.

Colchicine, Effect on Tumour Growth. L. Bloch-Frankenthal and A. Back. (*Proc. Soc. exp. Biol. N.Y.*, 1951, **76**, 105.) This paper describes the influence of colchicine on the growth in rats of a benzopyrene-induced sarcoma and on the pyrophosphatase contained in it. The tumour was transferred from rat to rat by subcutaneous implantation in the dorsal region. Each growth experiment lasted 6 to 8 weeks and at the end of each experiment the tumour was excised, measured and weighed. The colchicine was given as a 0·05 per cent. solution in distilled water, from 1 to 3 ml. being injected subcutaneously in the vicinity of the tumour, usually from 5 to 6 days after inoculation, the period of colchicine treatment lasting from 7 to 20 days. Suspensions of tumour tissue excised from the rats were tested for their activity on sodium pyrophosphate. In most cases, the colchicine treatment caused a considerable decrease in the growth of the tumours; regression was complete in some cases. No case of hæmorrhage was observed in any of the treated tumours. The colchicine treatment had an inhibiting effect on the pyrophosphatase activity in most of the cases treated. S. L. W.

Digitalis, Preservation of Infusion of. O. Izzo. (*Boll. chim. farm.*, 1951, **90**. 133.) Infusion of digitalis does not keep and the preservatives so far suggested, alcohol, chloroform and thymol have disadvantages. The author suggests that the infusion should be prepared by using a solution of 0·05 g. of the methyl ester of *p*-hydroxybenzoic acid and 0·02 g. of the propyl ester of the same acid, dissolved by boiling in 100 ml. of distilled water. Infusions made with plain water and with this solution were tested physiologically each day for 5 days and then on the tenth day. The ordinary infusion had lost half its strength on the second day, nearly two-thirds on the third day and over two-thirds on the fourth day. The preserved infusion lost no strength for 5 days and had only lost 20 per cent. on the tenth day. The infusions were tested by Hanzlik's method on pigeons. H. D.