ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Capsaicin in Oleoresin of Capsicum, Colorimetric Determination of. H. North. (*Anal. Chem.*, 1949, **21**, 934. Vanillin is used instead of pure capsaicin as a standard in the determination which utilises the Folin and Denis phosphotungstic-phosphomolybdic acid reagent. The sample of oleoresin (approximately 1 g.) is transferred to a separating funnel using purified kerosene, sodium chloride dissolved in acetone-water is added and the mixture shaken gently. The lower layer is removed and the extraction of the oleoresin solution is continued to completion using the same solvent. The separated bulked extracts are clarified and filtered and an aliquot portion is evaporated to an oily sediment, cooled, and the residue is dissolved in 0·5 N sodium hydroxide solution. Sodium bicarbonate is added and the mixture is extracted with light petroleum which is then shaken with 0·5 N sodium hydroxide. After washing the light petroleum with water the extracts are bulked, made up to volume, and an aliquot taken for the colorimetric determination. This is performed in the normal manner with slight modifications. With samples poor in capsaicin, a slight difference in colour between the standard solution and the test solution may be seen, because traces of colour carried through from the oleoresin have an influence on the total colour; this does not, however, interfere with the usefulness of the method. For the determination of capsaicin in spice, 5 to 10 gm. dry material are extracted with acetone or ether in a Soxhlet apparatus; the extract is then tested as before. R. E. S.

Grote's Reagent for Sulphur Compounds. J. J. M. van Sonsbeek. (*Pharm. Weekbl.*, 1949, **84**, 433.) The preparation of Grote's reagent may be simplified by omitting the bromine, as follows: 2 g. of hydroxylamine hydrochloride is mixed with 7.2 ml. of 4N sodium hydroxide and 25 ml. of water, then with 2 g. of sodium nitroprusside. After gas evolution ceases, the solution is made up to 100 ml. and filtered. This formula, though somewhat less stable owing to the higher alkalinity, does not become turbid to the same extent as the original formula. The reaction for the -C=S grouping (a blue colour) proceeds best at *p*H 7, and is apparently due to $Na_3[Fe(CN)_5H_2O]$ formed by the replacement of the NO in the nitroprusside by H_2O. In the reaction for the -C-S-H and -C-S-S-C- groupings, a purple-red colour is produced in alkaline solution (*p*H about 10). For reliable results in this test it is necessary to add cyanide, which also reduces the -C-S-S-C- group to -C-S-H, so that it is not possible to distinguish between the latter two groupings. The reagent is therefore inferior to nitroprusside. With thiopental, neither Grote's reagent nor nitroprusside gave the expected reaction for -C-S-H. On the other hand, at a *p*H of 4 to 8 Grote's reagent gave a fine red colour, which would appear to be a useful identification reaction. G. M.

Hexachloro*cyclo*hexane (Benzene Hexachloride Infra-red Spectroscopic Analysis of Mixture of Stereoisomerides. L. W. Morrison. (*J. Soc. chem. Ind., Lond.*, 1949, **68**, 192.) More than 100 samples of mixtures of the stereoisomers can be analysed per week, the results

being accurate to within 0·5 to 2·5 per cent. of the correct result, by means of infra-red spectroscopy. The α, β and δ isomers are determined from a spectrogram of a solution of the sample in methyl acetate, and the γ isomer from that of a solution in nitromethane between 800 cm.$^{-1}$ and 900 cm.$^{-1}$. Absorption measurements are made at 745 cm.$^{-1}$ (β), 762 cm.$^{-1}$ (α) and 772 cm.$^{-1}$, (δ) on the methyl acetate trace and at 845 cm.$^{-1}$ (γ) on the nitromethane trace. A number of minor constituents occur in varying proportions in different samples, and consist of heptachloro*cyclo*hexanes, octachloro*cyclo*hexanes, *p*-dichlorobenzene, and ε-hexachloro*cyclo*hexane; the absorption bands of certain of these are listed. G. R. K.

Mercuric Chloride Tablets, Determination of. N. Silvestri. (*Boll. chim.-farm.*, 1949, **88**, 205.) The following is a quick and easy way of testing mercuric chloride tablets. Put one 2 g. or two 1 g. tablets, previously powdered, in a graduated 100 ml. flask and add 40 to 45 ml. of cold water. Without mixing add 50 ml. of 0·2 N sodium hydroxide, shake and make up to 100 ml. After a few minutes shake again and filter through a dense double filter. Reject the first 30 to 35 ml. which are turbid, measure 50 ml. of the clear filtrate and titrate the excess of sodium hydroxide with 0·2 N hydrochloric acid in the presence of methyl orange. The method is accurate to 0·1 per cent. It can also be used for pure mercuric chloride using 1 g. with 1 g. of sodium chloride. Heavy metals which would react with sodium hydroxide must be absent. H. D.

Potassium Iodide in Tincture of Iodine, Determination of. P. Mesnard. (*Bull. Soc. Pharm. Bordeaux*, 1949, **87**, 40.) By the following method, both iodine and potassium iodide may be determined in the same sample of tincture of iodine, using a single standard solution: 2.5 g. of the tincture is titrated with 0·1 N arsenious acid in presence of sodium bicarbonate and starch. The mixture is then treated with 5 ml. of a 5 per cent. solution of potassium iodate and 2 ml. of 20 per cent. sulphuric acid. The acidity is neutralised by the addition of 20 ml. of 20 per cent. solution of sodium bicarbonate, and it is again titrated. Five sixths of the last titration corresponds to the total iodine, and deduction of the first titration gives the amount of iodine corresponding to the iodide in the tincture. G. M.

Starch and Cellulose, Determination of, with Anthrone. F. J. Viles Jr., and L. Silverman. (*Anal. Chem.*, 1949, **21**, 950.) A solution of anthrone (0·05 to 0·20 per cent.) in concentrated sulphuric acid was added to an aqueous solution or suspension of the carbohydrate to be determined and mixed immediately; under controlled conditions the amount of green colour produced was found to be proportional to the carbohydrate content. Heat was produced on mixing the acid and water and was necessary to the reaction. The colour produced in this reaction was measured spectrophotometrically and variations in the age of the anthrone reagent, in the water content, in the anthrone concentration, in the age of the colour, and in the nature of the carbohydrate were studied. Although the spectrum transmittance curve shape was altered by these factors, the maximum of the absorption band remained at 625 mμ. Investigations into the effect of heat upon the reaction showed that consistent readings were obtained after 10 minutes of air cooling. Maximum colour development occurred with rapid cooling, and in hot water immersion tests the colour rapidly deteriorated. The colour produced in the reaction was found to be stable for from 5 minutes to 3 hours, after which slight fading occurred. Maximum sensitivity occurred with 0·16 per

cent. anthrone solution, although 0·1 per cent. solution was found to be the most satisfactory for the reaction; this solution, however, deteriorated on storage. Since the reagent was unstable the use of a single standard curve was not practicable and in order to obtain accurate results one or more known standards are required for each group of analyses. Details of the final method chosen are given. R. E. S.

Thiocyanate, Titration of. E. W. Hammock, D. Beavon and E. H. Swift. (*Anal. Chem.*, 1949, **21**, 970.) Values obtained using the iodine monochloride method for the determination of thiocyanate in acid solutions were found to be erratic and an investigation was undertaken into the causes of these variations. It was found that direct titration of soluble thiocyanates with iodate in hydrochloric acid solution to the iodine monochloride end-point, resulted in less than the stoichiometric volume of iodate being used owing to partial decomposition of the thiocyanate before oxidation. Experiments showed that by previous addition to the acid of approximately four-tenths the equivalent amount of iodine monochloride required for the oxidation of the thiocyanate the error is considerably decreased, provided that the initial acid is not too concentrated and that the mixture is not allowed to stand. Slowly reacting products of the acid decomposition of the thiocyanate, rather than atmospheric oxygen, were largely responsible for the error, as indicated by the dependence of the error on the time of standing especially in the more concentrated acid, and by the fact that titrated solutions showed a return of end-point on standing; in several cases solutions that had stood for several days and were then retitrated gave substantially correct titrations. A procedure was adopted therefore in which iodine monochloride was added to the hydrochloric acid before addition of the thiocyanate; a series of titrations were then made with iodate, permanganate and ceric sulphate solutions. It was found that quantitative determinations could be made by using this procedure and titrating with iodate; the titration was not quantitative however under similar conditions with permanganate or ceric sulphate, as negative errors resulted. R. E. S.

Thyroxine, Polarographic Determination of. E. T. Borrows, B. A. Hems and J. E. Page. (*J. chem. Soc.*, 1949, Supp. I. S 204.) Methods hitherto proposed for the determination of thyroxine are discussed. The polarographic behaviour of 23 aromatic iodo-compounds has been examined and the conditions for the hydrolysis of iodinated casein has been studied, in consequence of which it has been found possible to detect 0·1 per cent. of thyroxine in 1·0 g. of iodinated protein polarographically. The polarographic method has the advantage that it can differentiate between thyroxine and 3 : 5-di-iodotyrosine and does not depend on the non-extraction of di-iodotyrosine by *n*-butyl alcohol. It is shown that chemical methods for the determination of thyroxine based on Leland and Foster's butyl alcohol extraction procedure (*J. biol. Chem.*, 1932, **95**, 165) give high results. The procedure recommended for determination of thyroxine in iodinated casein containing large amounts of di-iodotyrosine is as follows. Hydrolyse 1·0 g. of iodinated casein (containing about 5 mg. of thyroxine) by heating for 6 hours with 100 ml. of barium hydroxide solution (40 per cent.) added in 10 ml. portions. Cool, adjust the *p*H to 3·5 with dilute hydrochloric acid and extract with 50-ml. followed by 2 25-ml. quantities of *n*-butyl alcohol. Wash the butyl alcohol extract with 2 50-ml. quantities of a solution containing 1·6 per cent. of sodium hydroxide and 5 per cent. of sodium bicarbonate and evaporate to dryness under reduced

pressure. Dissolve the residue in 10 ml. of N sodium carbonate, transfer to a 20-ml. graduated flask, add 2·0 ml. of tetramethylammonium bromide solution (10 per cent.) and 4·0 ml of *iso*propyl alcohol and make up to 20 ml. with distilled water. Polarograph a 3·0 ml. oxygen-free portion of the final solution over the potential range of −0·6 to −2·0 volts. Measure the height in microamps of the first thyroxine step and of the combined thyroxine and di-iodotyrosine steps. Calibration curves of the diffusion currents obtained with thyroxine and di-iodotyrosine are shown in the paper. F. H.

FIXED OILS, FATS AND WAXES

Bacury (*Platonia insignis*) Seed Fat, Component Glycerides of. T. P. Hilditch and S. P. Pathak. (*J. chem. Soc.*, 1949, Supp. 1, S.87.) The seed fat of *Platonia insignis*, Mart. (family Gutteriferæ), is a yellowish solid becoming completely liquid at 51° to 52·5°C. After removal of free fatty acid (about 5 per cent.) the neutral fat was resolved into three groups by systematic crystallisation from ether and the component acids in each group were determined by ester fractionation. It was found that the chief component acids were palmitic (55 per cent.) and oleic (32 per cent.) with smaller proportions of stearic (6 per cent.) and hexadecenoic (3 per cent.) with probably traces of myristic, arachidic and linoleic acids. In spite of a total molar content of 35 per cent. of unsaturated acids, the fat contained over 20 per cent. of trisaturated glycerides (largely tripalmitin) thus causing it to comprise with the seed fats of *Laurus nobilis* and *Myristical malabarica* the only known exceptions to the "rule of even (or widest) distribution" of acyl groups amongst the glycerol molecules of a fat. A possible explanation is suggested for the departure in bacury fat from the generalisation followed in most seed fats that trisaturated glycerides are not encountered in appreciable amounts unless their content of oleic (or other unsaturated) acid is insufficient to provide one acyl group in each triglyceride molecule.

F. H.

Ergot, Oil of, Unsaponifiable matter of. E. Ruppol. (*J. Pharm. Belg.*, 1949, **4**, 55.) Although a large number of constituents have been reported in the unsaponifiable matter of ergot, many of these have not been characterised with sufficient certainty. The author treated 6g. of the unsaponifiable matter, from which most of the sterols had been removed, by chromatography on alumina. The products identified were cerevisterol, cerebrin, the "sterol C" of Vandermeulen (identified as dehydroergosterol), and another substance, which was apparently stigmasterol but was not identified with certainty. Questions which require further investigation are: complete analysis of cerebrin and its decomposition products; examination of the volatile oil; identity of the higher alcohols; constancy of composition of the sterol fraction. G. M.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Digitalinum Verum, Isolation of, from *D. purpurea* and *D. lanata*. K. Mohr and T. Reichstein. (*Pharm. Acta Helvet.*, 1949, **24**, 246.) Digitalinum verum has till now been isolated only from the seeds of *D. purpurea*, of which it forms the main glycoside, and it has not been obtained crystalline. As no good method of preparation appears to be on record, the authors used the following process. The finely powdered seeds were defatted with light petroleum, and then extracted with hot alcohol (50 per cent.) until all bitter tasting substance

was removed. The extract was purified with lead hydroxide in the usual way, freed from alcohol *in vacuo*, and purified by shaking with ether and also with chloroform (chloroform treatment is desirable, but sometimes impracticable on account of emulsion formation). Digitonin was then removed by treatment with cholesterol, and after removal of the excess of cholesterol, the glycoside mixture was acetylated to form the hexa-acetate of digitalinum verum. This may be obtained crystalline from a mixture of benzene and ether. From 500 g. of seeds, 1·5 g. of the acetate was obtained. The acetate, like that of gitoxigenin, easily loses acetic acid when chromatographed on alumina. Careful saponification with potassium bicarbonate in aqueous methyl alcohol gives the free digitalinum verum in a crystalline state. With *D. lanata*, which contains more digitonin than *D. purpurea*, the digitonin should first be removed before the shaking out, by extraction with choloroform-alcohol. The yield in this case was 0·564 g. of pure hexa-acetate from 900 g. of seeds. G. M.

Maize Starch, Waxy, Amylose Component of. E. J. Bourne and S. Peat. (*J. chem. Soc.*, 1949, 5.) A sample of waxy maize starch was defatted by treatment with aqueous methyl alcohol and dioxan and then submitted to the action of hot aqueous acid, when it was converted completely into glucose. Hydrolysis using β-amylose (from soya bean) however, ceased at a conversion limit (to maltose) of 49 per cent. The blue-value of the starch was 0·10 compared with average values of 1·10 and 0·22 found respectively for amylose and amylopectin isolated from potato starch by the thymol method. The lower staining power of waxy maize starch relative to potato amylopectin is emphasised by the light absorption curves of their iodine complexes. Attempts to fractionate waxy maize starch using the amylose-precipitants thymol or cyclohexanol produced a small fraction (3 to 5 per cent.) which had blue-values of 0·17 and 0·14, and a limiting conversion into maltose of 51 per cent. and 49 per cent. respectively, thus yielding some indication that these fractions were enriched in amylose although the light absorption curves for these fractions were of amylopectin character. Fractionation using the aluminium hydroxide precipitation method wielded among others a fraction with a blue-value of 0·40 and a limit of β-amylose conversion of 59 per cent. and obviously similar in composition to potato starch. The properties of fractions isolated by this and other procedures are consistent with the view that this starch does contain the largely unbranched component amylose to the extent, however, of less than 2 per cent. The recent work of Pascu and Hillier (*Text. Research J.*, 1946, **16**, 243) is discussed although the authors do not depart from the view that amylose is a pre-formed component of the starches, including that of maize search. R. E. S.

Potato Starch, Fractionation of, by means of Aluminium Hydroxide. E. J. Bourne, G. H. Donnison, S. Peal and W. J. Whelan. (*J. chem. Soc.*, 1949, 1.) Aqueous dispersions of potato starch (3 per cent.) were treated with varying amounts of hydrated aluminium sulphate (ranging from 0·3 g./g. of starch to 4·0 g./g. of starch) followed by a slight excess of ammonia. In each case a portion of the starch was not adsorbed and could be recovered from the supernatant liquid by precipitation with alcohol. This fraction, representing 1·2 to 10·3 per cent. of the starch, invariably had a higher blue value (varying from 1·11 to 1·36 in 6 experiments), than that of thymol-amylose. Exhaustive extraction of the aluminium hydroxide with boiling water gave fractions some of which consisted mainly of amylose, while others contained a large proportion of amylopectin. When the precipitate

remaining after exhaustive aqueous extraction had been redissolved in dilute alkali, neutralised, and dialysed to remove inorganic material, an amylopectin fraction was recovered by precipitation with alcohol which stained red-purple with iodine, and had blue value 0·18 to 0·22, comparable with that of an average amylopectin fraction separated from potato starch by the thymol method. The aluminium hydroxide method was tried for the separation of amylose fractions of high blue value as it was no convenient as the thymol method for the routine isolation of the amylopectin of starch. A number of factors, namely the temperature of precipitation and of "ageing" of the hydroxide, the time of "ageing" of the hydroxide, and the mode of preparation of the starch paste were examined with regard to their influence on the purity of the amylose fraction. A group of separations conducted on portions of the same starch paste, showed that when the aluminium hydroxide was both precipitated and "aged" at 30°C. the amylose fraction had a higher blue-value (1·03) than that obtained when the temperatures of precipitation and "ageing" were 30°C. and 14°C. respectively, or when both operations were carried out at 14°C. The time of "ageing" played an important part in the fractionation, as shown by separations in which the starch-aluminium hydroxide suspension was kept at 15°C. and aliquot portions were removed at intervals for the isolation of the amylose fraction; the blue-value of the product was 1·01 when no "ageing" occurred, although it rose to a maximum of 1·27 after "ageing" of the hydroxide suspension for 3 days, and diminished slightly to 1·22 after 7 days. "Ageing" at a higher temperature (30°C.) for 3 days considerably raised the blue value of the product. When a 3 per cent. paste was stirred at 100°C. and portions removed at intervals for aluminium treatment, the amylose fraction with maximum blue-value was isolated from the paste which had been boiled for 1 hour. Details are given of a method which was worked out for the isolation of amylose using aluminium nitrate; samples of amylose isolated by this technique were consistently of high blue value (1·35 to 1·40). The yield was somewhat variable (6 to 13 per cent.) and was always lower than the usual yield (*ca.* 20 per cent.) obtained with organic precipitants, presumably due to the difficulty of separating completely the amylose solution from the aluminium hydroxide gel. The light absorption curves of the polysaccharide-iodine complexes of a number of "amylose" fractions obtained are given: they confirm that adsorption on aluminium hydroxide effects a true fractionation of the starch. R. E. S.

ORGANIC CHEMISTRY

Dramamine, Chemistry of. J. W. Cusic. (*Science*, 1949, **109**, 574.) Attempts have been made, unsuccessfully, to obtain chemical compounds of antihistamines with methylxanthines. The methylxanthines do not form stable salts because of their low ionisation constants. 8-chlorotheophyllin does form stable salts and is used to prepare dramamine (β-dimethylamino-ethyl benzohydryl ether 8-chlorotheophyllinate). A slight excess of the base is dissolved with the 8-chlorotheophyllinate in hot methyl ethyl ketone or ethyl alcohol. On cooling, an almost quantitative yield of salt is obtained with m.pt. 101° to 103°C.; empirical formula $C_{24}H_{30}O_3N_5Cl$. Found: Cl., 7·45, 7·46 and 7·51 per cent.; Basic N, 2·98, 2·98 per cent.; 8-chlorotheophylline 45·65, 45·62 per cent.; $C_{24}H_{30}O_3N_5Cl$ requires Cl, 7·55 per cent.; Basic N, 2·98 per cent.; 8-chlorotheophyllin, 45·67 per cent. A. D. O.

Fluorescent Compound from Adrenaline, Structure of. I. Ehrlèn. (*Farm. Revy.*, 1949, **48**, 485.) The formation of a fluorescent compound from adrenaline

via adrenochrome, has been used by the author for the fluorimetric determination of adrenaline. A polarographic study of the transformation of adrenochrome into the fluorescent compound indicated that the spontaneous transformation of adrenochrome in neutral or alkaline solutions was a intramolecular rearrangement with formation of an *o*-dihydroxy compound. The secondary cathodic wave, ascribed by Wiesner to reaction with an added reducing substance, also occurs in the absence of the latter, and is probably due to the conversion of an enol into a ketone. The fluorescent compound is probably 1-methyl-3-oxo-5 : 6-dihydroxy-2 : 3-dihydroindol. Actually indoxyl itself shows a resemblance to the fluorescent compound. Although the latter is insoluble in ether, it can be extracted from an aqueous solution at *p*H 4 by shaking with ether containing some alcohol. The spectrum of such a solution showed 4 maxima at respectively 232·0, 258·0, 294·0 and 415·0 mμ. G. M.

Thyroxine and Related Substances, Synthesis of. E. T. Borrows, J. C. Clayton and B. A. Hems. (*J. chem. Soc.*, 1949, Supp. 1, S 185.) DL-Tyrosine has been synthesised in 55 per cent. overall yield by condensation at 160°C. of *p*-hydroxybenzaldehyde with hydantoin using morpholine as catalyst, reduction of the *p*-hydroxybenzylidene-hydantoin quantitatively in alcohol with Raney nickel at 130°C. under 60 atmospheres pressure of hydrogen, and hydrolysis of the resultant *p*-hydroxybenzyl hydantoin on refluxing with 2 N sodium hydroxide solution. DL-3 : 5-Di-iodotyrosine was obtained in 86 per cent. yield on iodinating tyrosine in hot dilute hydrochloric acid with a solution of iodine monochloride in concentrated hydrochloric acid. The attempted iodination of thyronine and related diphenyl ethers are described and discussed, and contrary to a previous report it has been found possible to tetrazotise 2 : 6-diaminodiphenyl ethers and to replace the diazo-groups by iodine atoms. This reaction has been used to develop an alternative synthesis of thyroxine. Condensation of methyl 4-chloro-3 : 5-dinitrobenzoate with quinol monomethyl ether in the presence of potassium hydroxide at 150°C., gave in good yield the dinitromethoxyphenoxy benzoate which was reduced to the diamine catalytically using palladised charcoal. Tetrazotisation was achieved when a solution of the diamine in glacial acetic acid was run into a cold stirred solution of nitrosyl-sulphuric acid in concentrated sulphuric acid and on decomposition by aqueous potassium iodide solution yielded 3 : 5-di-iodo-4-(4′-methoxyphenoxy)benzoic acid This was converted to the acid chloride with thionyl chloride and thence to the hydrazide by heating with hydrazine hydrate in methyl alcohol. The toluene-*p*-sulphonyl derivative was formed with toluene-*p*-sulphonyl chloride in dry pyridine and on heating for 30 seconds in ethylene glycol with sodium carbonate gave the crude aldehyde which on condensation with hippuric acid gave 2-phenyl-4-[3′ : 5′-di-iodo-4′-(4″-methoxyphenoxy)benzylidene] oxazol-5-one identical with the oxazolone obtained by Harington and Barger in their synthesis of thyroxine. F. H.

Thyroxine, Synthesis from 2:6-Dinitrophenyl Ethers. E. T. Borrows, J. C. Clayton and B. A. Hems. (*J. chem. Soc.*, 1949, Supp. 1, S 199.) Thyroxine has been synthesised in 14 per cent overall yield from *p*-hydroxybenzaldehyde in the following manner. 5-(4′-Hydroxybenzyl)-hydantoin, obtained on condensation of hydantoin with *p*-hydroxybenzaldehyde in the presence of morpholine and catalytic reduction of the product using Raney nickel, gave 5-(3′ : 5′-dinitro-4′-hydroxybenzyl)hydantoin in 83 per cent. yield when added in portions to concentrated nitric acid at 25° to 30°C., this temperature being maintained for 2 hours. This was converted

to its toluene *p*-sulphonyl ester in dilute alkaline solution and on then heating with quinol monomethyl ether in pyridine gave 5-(3′:5′-dinitro-4′-*p*-methoxyphenoxybenzyl)hydantoin which was reduced catalytically at elevated temperature in an autoclave to the diamine. In acetic acid of phosphoric acid the freshly prepared diamine added to a cooled stirred solution of nitrosylsulphuric acid in concentrated sulphuric acid gave a solution of the tetrazonium compound which on drop by drop addition to a solution of potassium tri-iodide gave 5-(3′:5′-di-iodo-4′-*p*-methoxyphenoxybenzyl)-hydantoin. This was best demethylated by refluxing gently with hydriodic acid (57 per cent.) and glacial acetic acid and on hydrolysis by heating in stainless steel with 2N-sodium hydroxide solution for 15 hours 3:5-di-iodothyronine was obtained. Iodination to thyroxine was accomplished in concentrated ammonia using drop by drop addition of a solution of iodine in potassium iodide. F. H.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Adrenaline, Separation of nor-Adrenaline from Natural. B. F. Tullar. (*Science*, 1949, **109**, 536.) Paper chromatography studies have shown that crystalline epinephrine U.S.P. derived from adrenal glands contains appreciable quantities of *l*-arterenol (*nor*-adrenaline) and direct chemical evidence has therefore been sought in support of the physiological observations that the adrenal medulla may elaborate more than one hormone having adrenaline-like properties. Examination of commercial samples of natural adrenaline and also of a sample from the bulk stock of U.S.P. Reference Standard epinephrine has led to the isolation from them of *l-nor*-adrenaline. *l-nor*-adrenaline, *l*-bitartrate (m.pt. 163° to 164°C, $[\alpha]_{25^\circ C.}^{D}$ -40·2°) and *l-nor*-adrenaline *d*-bitartrate (m.pt. 101° to 102°C. $[\alpha]_{D}^{25^\circ C.}$ -12°) and *l-nor*-adrenaline hydrochloride (m.pt. 147·5° to 148·5°C., $[\alpha]_{D}^{25^\circ C.}$ -39·8°) were prepared from the samples of natural adrenaline and identified with the corresponding salts prepared from synthetic *l-nor*-adrenaline. It is confirmed that natural adrenaline contains appreciable quantities of *l-nor*-adrenaline and the isolation of the latter in chemically pure form from biological material furnishes the final step of proof in establishing its hormonal nature. F. H.

nor-Adrenaline in Adrenal Medullary Tumours. P. Holton. (*Nature*, 1949, **163**, 217.) An acid extract of a portion of human suprarenal tumour, found histologically to be a typical phæochromocytoma consisting of chromaffin tissue, has been shown to contain both adrenaline and *nor*-adrenaline. Using the rat's uterus and the frog's perfused heart, which have been shown to be insensitive to *nor*-adrenaline, each ml. of the extract was found to be equivalent to 0·37 mg. of adrenaline. When tested on the rabbit's duodenum and the spinal cat's blood pressure, which are approximately equally sensitive to adrenaline and *nor*-adrenaline, the extract was found to be equivalent to 1 mg. of adrenaline. It was therefore concluded that each g. of tumour contained 3·7 mg. of *l*-adrenaline and 6·3 mg. of *nor*-adrenaline. The presence of both amines was confirmed by tests with polyphenolase, a mixture of the extract and enzyme on incubation giving a brown colour typical of *nor*-adrenaline during 10 minutes changing after 20 minutes to a rose-pink colour typical of adrenaline. By paper chromatography, using phenol as solvent and identifying the spots by spraying with potassium ferricyanide, adrenaline and *nor*-adrenaline were separated from the extract. Two

other adrenal medullary tumours examined, contained 5·3 mg. of *l-nor*-adrenaline and 0·32 mg. of l-adrenaline, and 5·5 mg. of *l-nor*-adrenaline and 0·37 mg. of *l*-adrenaline respectively per g. of tumour. It is suggested that since *nor*-adrenaline has slightly greater pressor activity than adrenaline, the attacks of high blood pressure caused by the tumours were probably mainly due to *nor*-adrenaline. F. H.

***l*-nor-Adrenaline in the Suprarenal Medulla.** U. S. von Euler and U. Hamberg. (*Nature*, 1949, **163**, 642.) The demonstration that *nor*-adrenaline constitutes the specific neurohormone (sympathin N) of adrenergic nerve fibres has directed attention to the occurrence of this substance in biological material. Suprarenal glands from cattle were extracted with trichloracetic acid (5 per cent.) and the acid removed with ether. The extracts were examined for the presence of *nor*-adrenaline, chemically, biologically and by paper chromatography. It has been shown that the rates of formation of adrenochrome and of *nor*-adrenochrome on oxidation of the parent amines with iodine depends on the *p*H. At *p*H 4 and an oxidation time of 1½ minutes the formation of adrenochrome is complete whereas only about 7 per cent. of the *nor*-adrenaline is then oxidised. At *p*H 6 an oxidation time of 3 minutes gives the sum of adrenochrome and *nor*-adrenochrome. Although the colorimetric method does not differentiate between *dl-* and *l-nor*-adrenaline, the results obtained agreed well with those obtained from comparison of the effect of extracts on the blood pressure of the cat with those of mixtures of adrenaline and *nor*-adrenaline, samples of extracts of whole glands containing the equivalent of 0·48 to 0·70 μg. of *nor*-adrenaline hydrochloride and 1·80 to 2·16 μg. of adrenaline hydrochloride per mg. of fresh tissue and a sample of adrenal medulla containing the equivalent of 2·68 μg. of *nor*-adrenaline hydrochloride and 11·3 μg. of adrenaline hydrochloride per mg. of fresh tissue. Paper chromatography using *n*-butyl alcohol saturated with N/1 hydrochloric acid as solvent gave a close correspondence between the migration of a suprarenal gland extract purified by adsorption to alumina and a mixture of *nor*-adrenaline and adrenaline. The results showed that the normal suprarenal gland from cattle contains *l-nor*-adrenaline in appreciable amounts as previously shown for adrenergic nerves and support Blaschko's concept (*J. Physiol.* 1942, **101**, 337) of adrenaline formation in the chromaffin cells. F. H.

Aureomycin and Blood Coagulation. D. I. Macht and R. Farkas. (*Science*, 1949, **110**, 305.) Experiments were made on rabbits and cats, and clinical tests were made on patients who had not received any previous medication. In all the experiments the drug was administered by stomach and the clotting time measured before administration and at various intervals afterwards. In both rabbits and cats the clotting time after administration of aureomycin showed a progressive diminution. This finding was confirmed in 14 human subjects following administration of one or two capsules of aureomycin of 250 mg. each, the usual clinical dosage. Tests made on both human subjects and lower animals revealed no difference in prothrombin time, thus indicating that the diminution in clotting time is due to other factors involved in blood coagulation. The authors suggest that in order to avoid the possibility of thrombo-embolic accidents during antibiotic therapy prophylactic measures by the use of anticoagulant drugs may be instituted. S. L. W.

Chloramphenicol (Chloromycetin), Enzymatic Hydrolysis of. G. N. Smith, C. S. Worrel, and B. L. Lilligren. (*Science*, 1949, **110**, 297.) In an

initial series of experiments with *E. coli*, *P. vulgaris* and *B. subtilis* it was found that chloramphenicol could be hydrolysed when introduced into actively growing broth cultures of these organisms. Subsequent experiments showed *P. vulgaris* and *B. subtilis* to be the best sources of the enzyme. Filtrates from cultures 2 to 4 weeks old actively destroyed chloramphenicol and a fairly active preparation of the enzyme could be obtained by concentration of the filtrate. The action of this enzyme, which has been tentatively designated enzyme A, is to hydrolyse the amide linkage of chloramphenicol and thus liberate the corresponding basic amine and dichloroacetic acid. The optimum conditions for the enzymatic hydrolysis have been found to be *p*H 7·5 and a temperature of 37·5 to 40·0°C. The rate of enzymatic hydrolysis increases with substrate concentration up to 2 mg./ml. One unit of enzyme activity has been arbitrarily chosen as that amount of the enzyme which will hydrolyse 1μg. of chloramphenicol in 1 hour at *p*H 7·5 and 37·5°C. The enzyme is probably very similar to other proteolytic enzymes that have been isolated from bacterial cells. It is neither a true papain nor a trypsin though it is probably more closely allied to the former group of enzymes than to the latter. S. L. W.

BIOCHEMICAL ANALYSIS

Bacitracin, Assay of. G. D. Darker, H. B. Brown, H. Free, B. Biro and J. T. Goorley. (*J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 156.) Two methods for assaying bacitracin, an antibiotic produced by an organism of the *Bacillus subtilis* group, are described. The turbidimetric assay is rapid, sensitive for samples containing from 0·2 to 0·8 units per ml., and shows an accuracy of ± 20 per cent. Strictly aseptic technique is not necessary. *Staphylococcus aureus* grown on Bacto-yeast broth is incubated with various dilutions of the sample and with a standard solution for 4½ hours at 38°C., when growth is stopped by steaming. The turbidity is determined in a Soleman Universal Spectrophotometer at 600 mμ wavelength. The amount of antibiotic activity, as measured by the turbidity, is given by the difference between the growth obtained in the bacitracin sample and that obtained with the organism alone. This is compared with the inhibition produced by the standard bacitracin solution. The second method, the cylinder-plate method, is suitable for solutions containing from 1·5 to 8 units of bacitracin per ml., and has an accuracy of ± 15 per cent. The procedure is the same as for penicillin, except that the inoculated plates with the cylinders holding the test-solutions should be kept at 4°C. for 6 to 10 hours before incubation, to allow for the slower diffusion rate of bacitracin. Methods for establishing standard curves and charts for the cylinder-plate assay are described.

L. H. P.

Penicillin. Report of the Analysts' Sub-Committee of the Ministry of Health Conference on the Differential Assay of Penicillin. Part I. The Determination of Benzyl Penicillin by Precipitation with N-Ethylpiperidine. (*Analyst*, 1949, **74**, 79.) The method was that of Sheehan, Mader and Cram (*J. Amer. Chem. Soc.*, 1946, **68**, 2407) and the Food and Drug Administration of the United States, depending on the extraction of an acidified aqueous solution of the penicillin salt with amyl acetate and subsequent precipitation of the N-ethylpiperidinium salt from a mixture of amyl acetate and acetone. Details are given of the reagents and the tests for limits of impurities, together with the exact procedure to be adopted. Two standards were used, one the "M.U. Standard" or Manufacturers'

Ultimate Standard, and the "A.S.C. Standard" or Analysts' Sub-Committee Standard. After collaborative determinations it was found that, assuming the purity of the M.U. Standard to be 100 per cent. 98·6 per cent. recovery was obtained in the process; accordingly as the (weighted) mean assay result for the A.S.C. Standard was 96·1 per cent., after correction on a 98·6 per cent. recovery basis it was assigned a sodium benzyl penicillin content of 97·5 per cent. Results produced in microchromatographic examinations showed that the precipitation procedure gave a precipitate, which except with the already pure M.U. Standard, was not composed of the N-ethyl-piperidinium salt of pure benzyl penicillin; in the range 90 to 100 per cent. of benzyl penicillin, there was some agreement between the calculated benzyl penicillin content of the samples examined and the amount determined by the proposed method, indicating a possible compensation of errors. The Sub-Committee recommended that the procedure described be used as a tentative method in the examination of samples of penicillin consisting substantially of benzyl penicillin. In view of the appreciable error to which the method is subject and the number of variable factors, it was considered desirable that a standard penicillin should be assayed alongside the test sample and a correction applied equal to the difference between the benzyl penicillin content found for this standard and its actual value. The Sub-Committee, with the approval of the Ministry of Health, has standardised a quantity of sodium benzyl penicillin designated the "Benzyl Penicillin Gravimetric Standard" to which a value of 95·8 per cent. sodium benzyl penicillin has been assigned. R. E. S.

Streptomycin, Colorimetric Determination of, in Urine. M. J. Masquelier. (*Bull. Soc. Pharm. Bordeaux,* 1949. **87,** 53.) By treatment with acetylacetone, the methylglucosamine of streptomycin is converted into pyrrole derivatives which give a red colour with Ehrlich reagent. 1 ml. of solution, containing 100 to 500 μg. of streptomycin, is heated for 10 minutes on the water-bath with 1 ml. of a 2 per cent. aqueous solution of acetylacetone and 2 drops of sodium hydroxide. After cooling, there are added 2 ml. of alcohol (95 per cent.) and 2 ml. of a solution of 0.8 g. of *p*-dimethylaminobenzaldehyde in 30 ml. of alcohol (95 per cent.) and 30 ml. of hydrochloric acid. In order to allow for indole derivatives, etc., in the urine, another portion of the solution is treated similarly, but omitting the acetylacetone. The colour is compared with that obained with a standard solution of streptomycin. It is sometimes of advantage to treat the urine first with one tenth of its volume of basic lead acetate solution, removing excess of lead with sodium sulphate. The streptomycin passes into the filtrate and gives a purer colour. The concentration of streptomycin in the urine, after the administration of 1.5 g. in 24 hours, is generally about 100 to 500 μg. per ml. G. M.

Trichloroethylene in Blood, Estimation of. F. H. Brain and P. J. Helliwell. (*Biochem. J.,* 1949, **45,** 75.) New conditions are described for the quantitative production of the coloured compound from trichloroethylene and pyridine in the presence of alkali. The sample of blood is steam-distilled and collected in anisole, the anisole solution of trichloroethylene being added to dry, colourless, redistilled pyridine. The mixture is stirred mechanically for 10 minutes while being heated in a boiling water-bath: tetra-ethylammonium hydroxide is then added with stirring, and after cooling the two layers separated. The upper pyridine layer is removed by a pipette and delivered into an absorptiometer cell containing sodium hydroxide solu-

tion. The colour is measured immediately using a Spekker photoelectric absorptiometer with an Ilford spectrum blue-green filter No. 603. The colour began to fade in subdued light after 20 minutes; the absorptiometer was calibrated with standard solutions of trichloroethylene in anisole. Details are given of an efficient apparatus for the absorption of the trichloroethylene steam-distilled from blood. The addition of tetraethylammonium hydroxide was necessary owing to the sensitivity of the coloured material to carbon dioxide. The amount of alkali in the pyridine-anisole layer was so much reduced by the use of 10M sodium hydroxide that, in the process of transferring the coloured layer to the absorptiometer cell, sufficient carbon dioxide was absorbed to neutralise it, and the blue-red colour which is formed only in alkaline solution, changed to a pale yellow. Care was taken to ensure that there could be no variation in the amount of water taken up from the alkali solution by the pyridine-anisole layer by using always exactly 10M sodium hydroxide which had been checked by titration. A simple procedure for colour development was also investigated in which the pyridine-anisole solution and alkali were not stirred during heating. This gave reproducible absorptiometer readings proportional to the trichloroethylene concentration up to about 0·05 mg./5 ml. of anisole, but above this the absorption varied considerably from one estimation to another and was always too large to fit the proportionality of the lower concentrations. Using the method devised the concentration of trichloroethylene on approximately 1 ml. blood samples could be estimated to the nearest μg. at concentration levels between 1 and 12 mg. per 100 ml. R. E. S.

PHARMACY

GALENICAL PHARMACY

Emulsifying Agents. α-Monostearin and Sodium Stearate. H. H. G. Jellinek and H. A. Anson. (*J. Soc. Chem. Ind.*, 1949, **68**, 108.) A tensionmeter was devised to measure interfacial tensions by a capillary height method and with the apparatus described a number of systems were studied at 70° C. The areas (in Ångstrom units) occupied by each molecule at the interface were evaluated from the interfacial data obtained for the systems: (a) water—white oil containing α-monostearin against water; (b) water—white oil against water containing sodium stearate; and (c) water—white oil containing α-monostearin against water containing sodium stearate. The separation of phases at 70°C. of emulsions of water—white oil and water containing either α-monostearin in the oil or sodium stearate in water or both emulsifying agents, was studied under controlled conditions, the phase volume ratio being kept at 1 : 1. α-Monostearin was found to give water/oil emulsions but the addition of sodium stearate led to the formation of oil/water emulsions, the amount of sodium stearate necessary to cause the inversion being only slightly dependent on the concentrations of α-monostearin. A special apparatus is described for preparing the emulsions, and details are given of the methods used for the determination of emulsion type, together with micro-photographs and solid phase diagrams. Sodium stearate and α-monostearin together gave more stable emulsions than equivalent amounts of either emulsifying agent. R. E. S.

Injections, Bactericidal Action of. K. Alin and N. Diding. (*Farm. Revy.*, 1949, **48**, 545.) The autobactericidal action of a number of solutions

for injection was tested by inoculating such solutions with pure cultures. The results are summarised in the table below, which shows the results of sterility tests made 30 days after the inoculation.

Injection	Organism		
	Escherichia coli	*Staphylococcus aureus*	*Bacillus subtilis*
Ascorbic acid	—	—	+
Adrenaline	—	—	—
Atropine	—	—	—
Carbachol	—	—	—
Dihydromorphinone	—	—	—
Ephedrine	—	—	+
Methadone	—	—	—
Morphine with scopolamine	—	—	+
Nicethamide	—	—	—
Oxicone (Eucodal)	—	—	+
Papaverine	—	—	+
Pentazol	—	—	—
Pethidine	—	—	—
Phenemal (phenobarbitone)	—	—	+
Phenopromine (Amphetamine)	—	—	—
Picrotoxin	—	—	+
Prostigmine	—	—	—
g-Strophanthin	—	—	—
Sulphur	—	—	—
Tetrapon	—	—	+

These results show the necessity of the addition of bacteriostatic substances to solutions dispensed in multiple dose containers. G. M.

Ringer's Solution, Isotonicity of. J. Michaels and K. Münzel. (*Pharm. Acta Helvet.* 1949, **24,** 199.) A comparison of experimentally observed depressions of freezing points, for Ringer's solution prep—according to various Pharmacopœias, showed that only the Swedish formula gave a solution having the correct value of 0·55 to 0·57°C., all of the others being hypotonic. For Ringer-Locke solution nearly all the formulæ gave the correct depression. There appears however to be no justification for a concentration of calcium and potassium different from that of Ringer's solution, and the following formula is recommended: sodium chloride, 8·5 g.; anhydrous potassium chloride, 0·3 g.; calcium chloride 0·3 g.; glucose, 1·0 g.; sodium bicarbonate, 0·5 g.; water, to 100 ml. The Ringer-lactate solution of the British Pharmacopœia, 1948, is hypotonic; it may be made isotonic by reducing the concentration of sodium chloride to 0·73 per cent. G. M.

Theophylline Ethylenediamine, Solution of, for Injection. J. Büchi and F. Hippenmeyer. (*Pharm. Acta. Helvet.*, 1949, **24,** 326.) Commercial theophylline ethylenediamine shows a considerable variation in the proportion of the two components. In order to determine the optimum proportions for a stable solution of relatively low *p*H value, a number of solutions were made up with different proportions of the two components. As a result the following two formulæ were recommended. Strong solution: theophylline (1 H_2O), 20·60 g.; ethylenediamine hydrate, 5·630 g.; water, to 100ml. Dilute solution: theophylline (1 H_2O), 2·06 g.; ethylenediamine hydrate, 0·510 g.; water, to 100 ml. These solutions contain respectively 126 and 139 per cent. of the amount of ethylenediamine corresponding to the formula $1\,2\,C_7H_8O_2N_4 \cdot 1C_2H_8N_2.2H_2O$; the *p*H values, after sterilisation, being 9·45 and 9·10. They show no tendency to crystallisation, and no appreciable change after 6 months' storage. The reaction is less alkaline than that of most commercial preparations. G. M.

PHARMACOLOGY AND THERAPEUTICS

Aureomycin in the Treatment of Late Cutaneous Syphilis. P. A. O'Leary, R. R. Kierland and W. E. Herrell. (*Proc. Mayo. Clin.*, 1949, **24,** 302.) Aureomycin administered orally produced healing in 2 patients with late nodulo-ulcerative lesions of syphilis. The dose was 250 mg. every 6 hours for 2 or 3 days, increased to 500 mg. to 1 g. 6-hourly. One patient received a total of 56·5 g. and the other a total of 60 g. Nausea, vomiting and diarrhœa were the only complications encountered. Encouraged by these results the authors have commenced the treatment of patients with various types of neurosyphilis with aureomycin administered orally and have found that the serologic changes in the cerebrospinal fluid are similar to those noted after penicillin. S. L. W.

***para*-Aminosalicylic Acid Therapy, Complications of.** J. M. Swanson. (*Lancet*, 1949, **257,** 175.) 5 out of 6 cases of rheumatoid arthritis treated with *para*-aminosalicylic acid developed hypoprothrombinæmia. 15 g. of the drug had been given daily in 5 doses at 3-hourly intervals. A. D. O.

Digitalis, Effect of the Rate of Injection on the Lethal Dose in Guinea-pigs. I. S. Miya and H. G. O. Holck. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38,** 64.) Eighty four male guinea-pigs arranged in seven groups, were anæsthetised with urethane and injected with tincture of digitalis U.S.P. diluted with normal saline to contain 1·60, 2·26, 3·20, 4·53, 6·40, 9·05 and 12·80 per cent. of the tincture respectively. The average lethal dose was determined by the U.S.P. method for the assay of digitalis except that the injections were made into the jugular instead of the femoral vein. In general the average lethal dose increased as the concentration of the tincture decreased except that the highest concentration gave a slightly higher lethal dose than the next lowest. The end-point was difficult to determine because the respiration became irregular some time before the heart beats ceased, and the beats became sporadic before finally stopping. Comparison of the results with those of other workers suggest that the guinea-pig is less suitable for the assay of digitalis than the cat. G. R. K.

Dramamine for the Prevention of Airsickness. B. A. Strickland, Jr. and G. L. Hahn. (*Science*, 1949, **109,** 359.) 108 young men were treated with dramamine (β-dimethylaminoethyl benzohydryl ether 8-chlorotheophyllinate) and then taken on specially controlled flights in an aeroplane. 100 mg. of the drug was given 25 to 45 minutes before taking off. 31 men were airsick and, of a similar number of controls, 60 were sick. A. D. O.

Dramamine in the Prevention and Treatment of Motion Sickness. L. N. Gay and P. E. Carliner. (*Science*, 1949, **109,** 359.) Clinical trials of dramamine (β-dimethylamine ether benzohydryl ether 8-chlorotheophyllinate) were carried out on a troopship. In each case the dose was 100 mg. every 5 hours and before retiring. Seasickness was prevented in all but 2 of 134 men who received the drug at the time of departure, whereas 34 men comprising a control group were sick. In the latter group complete relief of symptoms were obtained 1 hour after commencing treatment. Out of 195 other cases of severe seasickness 187 were completely relieved of symptoms 30 minutes after treatment was started. Relapses occurred when a placebo was substituted for the drug and relief was obtained again when treatment recommenced. In another series of 359 cases, 372 recovered within 1 hour of commencing treatment. Rectal administration was equally as effective as oral treatment. There were no reactions to the drug. A. D. O.

Iron, Intravenous, and Anæmia of Pregnancy. A. D. T. Govan and J. M. Scott. (*Lancet*, 1949, **256**, 14.) A stable saccharated oxide of iron was given by intravenous injection to 25 pregnant women suffering from iron-deficiency anæmia. All showed a rapid response to treatment, the hæmoglobin increasing in almost all cases by 8 per cent. in the first week of treatment. During the first week injections were given daily, starting with a dose equivalent to 30 mg. of iron on the first day, 60 mg. on the second day, and 100 mg. thereafter, the injections being reduced at the end of a week to 100 mg. on alternate days. It apparently requires about 40 mg. of iron to increase the hæmoglobin by 1 per cent. in an anæmic pregnant woman. The solution appears to irritate the vessel wall, and rapid injection causes an immediate vasospasm, though only one case showed a severe reaction, probably due to vagal stimulation. A comparison of the hæmoglobin readings of the patients treated by intravenous injection and of 62 cases treated with iron by mouth showed a very striking difference, not only as regards time but also because in the patients treated intravenously succeeding increments of hæmoglobin increased instead of diminishing as in the patients treated with iron by mouth. S. L. W.

Morphine Derivatives: Chemical Constitution and Analgesic Action. F. W. Schueler, E. G. Gross and H. Holland. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 74.) In a review of the pharmacodynamic action of morphine and eighteen of its derivatives, the authors have concluded that the analgesic action may be due to the presence of both sympathomimetic and parasympathomimetic portions in the molecule connected by the same amino-nitrogen atom. Previous workers found that for a maximum muscarine-like action the average optimum limiting distance between the methyl group of the amino-nitrogen and the ether oxygen or carboxyl oxygen are respectively 5·3 Å and 7·0 Å; using Fisher-Hirschfelder-Taylor models the corresponding distances in the nineteen molecules under discussion varied between 5·0 and 9·0 Å. For sympathomimetic action the distance between the methyl groups of the amino-nitrogen and the aromatic carbon atom joining the ring to the alkyl carbon chain appears to be of paramount importance it varied between 4·5 and 6·5 Å in these molecules. It is suggested that the analgesic action of morphine and its analogues is due to the presence of both these autonomic nervous system stimulating portions and acts through a peripheral mechanism. The parasympathomimetic group may be active in stimulating the output of inhibitory action on cholinesterase and the sympathomimetic group may adrenaline by the suprarenal glands either by direct action or through the enhance the peripheral perineural vasoconstrictor response of the adrenaline either by its direct action or by preserving adrenaline. G. R. K.

Nisin, Some Recent Applications of. A. Hirsch and A. T. R. Mattick. (*Lancet*, 1949, **257**, 190.) Nisin is an antibiotic produced by *Streptococcus lactis*. It is mainly effective against Gram-positive organisms including the tubercle bacillus and has the properties of a polypeptide or protein of low molecular weight. Nisin is soluble in dilute acids and the solution is stable to heat, but it tends to precipitate at neutrality and is slowly labile under alkaline conditions. With *Strep. agalactiæ* and *Strep. pyrogenes* nisin is rapidly bactericidal. The intravenous LD50 in rabbits is between 20 and 23 mg./kg. The intramuscular LD50 is about 200 mg./kg. and the subcutaneous dose was greater than 1,000 mg./kg. Rabbits which had received a fatal intravenous dose died quickly and quietly, but guinea-pigs suffered very

violent spasms resembling "peptone shock." Necropsies showed that nisin was present in all the internal organs with the highest concentration in the lungs. Lesions at the site of subcutaneous injection contained almost all the nisin which had been injected and this probably explains the low toxicity by this route. The "peptone shock" symptoms of fatal doses of nisin are usually avoided when the injection was given slowly with a perfuser. No apparent effect on leucocytes and red blood cells could be observed. After initial saturation injections, satisfactory blood-nisin levels (against mycobacteria, 100 units/ml.) were obtained by 12 hourly injections. Blood levels of 1 to 10 units/ml. of serum in rabbits were obtained by oral doses. The *in vitro* activity of nisin against mycobacteria in the Dubos and Davis medium, was equivalent to that of streptomycin. Combinations of nisin and streptomycin or nisin and licheniformin were neither synergistic nor markedly additive, but a combination of all three was. A similar behaviour was shown by nisin, licheniformin, and sulphathiazole, and a more than a mere additive action occurred with nisin and sulphathiazole together. Sulphathiazole resistant cultures were sensitive to nisin, as were streptomycin cultures. Nisin resistant cultures remained susceptible to streptomycin. *In vivo* experiments with subcutaneous doses of crude nisin resulted in a reduction in the spread of infection. After intravenous treatment, histological examination revealed early tuberculosis but this could not be detected macroscopically as in the control animals. A.D.O.

Salicylates. Toxicity during Pregnancy. A. V. Jackson. (*J. Path. Bact.*, 1948, **60**, 587.) Pharmacological text-books commonly contain references to the danger of using large doses of salicylates during pregnancy but as there is little clinical support for this view the matter was investigated in rabbits and rats the placental permeability of which resembles that of man. It was found that salicylate readily passed the placental barrier in rabbits, the concentration in the fœtal serum being about two-thirds that in the mother. Even when doses sufficient to kill one out of four of the mothers were given most of the fœtuses survived. In rats, whenever the mother survived so also did the fœtuses. It is concluded that there is no special liability to abortion or fœtal death in salicylate poisoning until doses approaching maternal lethal levels are reached. No specific histological changes occur in salicylate poisoning and the harmful effects are thought to be referable to interference with intracellular enzyme activity. H. T. B.

Senna, Biological Assay of. L. C. Miller and E. B. Alexander. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 417.) The assay procedure described involves administering the preparation under test at three dosage levels selected with a view to producing laxative effects in approximately 25, 50 and 75 of three similar groups of mice. Concurrently, a suspension of a standard senna powder was given at three appropriate dosage levels. The number of mice receiving each dose of unknown and standard and showing laxative effects during the 5½ hours after dosing were recorded and from these data the potency and confidence limits were calculated by conventional methods. The method is applicable to senna in its various pharmaceutical forms; the material used in this investigation consisted of proprietary preparations containing about 50 per cent. of sugar and senna extractives equivalent to 50 to 100 mg. of fresh drug per ml. Satisfactory dosage-response relationships were found in experiments on human beings and the potency ratios found on human subjects agreed well with those of mice. S. L. W.

Sodium Carboxymethylcellulose, Evaluation of Hydrophilic Properties of Bulk Laxatives containing. R. H. Blythe, J. J. Gulesich and H. L. Tuthill. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 59.) In a study to find a laxative agent superior to those now in use, the hydrophilic properties of standard bulk laxatives were compared with two new synthetic agents by new and modified methods *in vitro*. The significant properties evaluated were (a) the volume of water absorbed in various media (water and artificial gastric and intestinal juices), (b) the viscosity and texture of the gel formed, and (c) the ability of the gel to retain water. To avoid causing gastric discomfort the agent should swell in the alkaline intestinal tract but not in the acid gastric juice. To simulate *in vivo* conditions polyethylene glycol was used an an osmotic agent. Karaya, psyllium and the synthetic agents methylcellulose and sodium carboxymethylcellulose were compared. It was found that methylcellulose went slowly into solution when agitated in artificial gastric juice while sodium carboxymethylcellulose remained essentially insoluble. The synthetic substances proved superior to the natural gums in hydrophilic capacity and the formation of viscous, homogeneous solutions which permit uniform distribution and eliminate any tendency to blockage. It was concluded that sodium carboxymethylcellulose was the most promising substance of those tested because of these properties and its insolubility in artificial gastric juice. G. R. K.

BACTERIOLOGY AND CLINICAL TESTS

***p*-Aminobenzoic Acid and Related Compounds, Fungistatic Properties of.** G. W. K. Cavill and J. M. Vincent. (*J. Soc. chem. Ind., Lond.*, 1949, **68**, 189.) This paper deals with the relative fungistatic actions of the methyl to *n*-amyl *p*-aminobenzoates and of *o*-, *m*-, and *p*-aminobenzoic acids and their methyl esters The fungistatic activities were determined from the inhibition of the growth rates of *Aspergillus niger, Byssochlamys fulva* and *Penicillium roqueforti*. It was noted that the inhibition of *P. roqueforti* was unusually increased if the nitrogen was supplied as nitrate and not as ammonium. The results were treated in two ways. On the basis of the reciprocal of the concentration required for 50 per cent. inhibition (I_{50}), there was a general increase in activity in ascending the series methyl to *n*-amyl *p*-aminobenzoate, and also the *m*-isomer was markedly less effective than the corresponding *o*- or *p*-isomer. On the basis of an adsorption approach, using an adsorption equation relating inhibition to the Langmuir isotherm, the method proved of little use with *P. roqueforti*, but with *B. fulva* it seemed to permit a useful analysis of the activity of the esters, particularly in terms of increasing biological adsorbability. The esters tested against *A. niger* did not give the same clear cut picture. With both *B. fulva* and *A. niger* the low I_{50} values of the *m*-isomer could be explained on the basis of the adsorption approach. G. R. K.

***p*-Aminosalicylic Acid and Resistance to Streptomycin.** O. E. Graessle and J. J. Pietrowski. (*J. Bact.*, 1949, **57**, 459.) The effect of *p*-aminosalicylic acid on the development of resistance to streptomycin by *Mycobacterium tuberculosis* was investigated *in vitro*. The strain of *M. tuberculosis* used had an initial sensitivity of 0·8 unit/ml. By repeated exposure to the antibiotic the organism was rendered resistant to 20,000

[*Continued on p.* 63