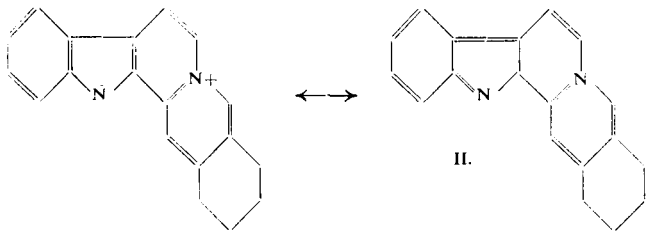
ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Alkaloidal Titanifluorides. M. Janot and M. Chaigneou. (*C. R. Acad. Sci., Paris,* 1949, **229,** 69.) The recent work showing that silicofluorides and zirconifluorides had formulæ different from those originally established by Schaeffer has been extended to include the preparation of titanifluorides. In the preparation of these salts, an aqueous solution of potassium titanihexafluoride K_2TiF_6 was mixed with an aqueous solution of the hydrochloride or sulphate of the alkaloid, when the titanifluoride crystallised out in the cold. Analyses of the salts showed the following formulæ: quinine, $B_2A,5H_2O$; quinidine, $B_2A,2H_2O$; cinchonine, B_2A,H_2O; cinchonidine, $B_2A,2H_2O$; strychnine, $B_2A,3H_2O$; brucine, $B_2A,6H_2O$; morphine, $B_2A.H_2O$; codeine, B_2A; narcotine, B_2A; thebaine, $B_2A,5H_2O$; corynanthine, $B_2A,4H_2O$; corynantheine B_2A,H_2O; where B is the alkaloid in question and A represents H_2TiF_6. The titanifluorides were all perfectly crystalline and could be used for microcrystalline identification of the alkaloids. R. E. S.

Sempervirine, Structure of. R. Bentley and T. S. Stevens. (*Nature,* 1949, **164,** 141.) The structure of sempervirine, the reddish-brown alkaloid of *Gelsemium sempervirens,* previously formulated by Prelog (*Helv. chim. Acta,* 1948, **31,** 588) as having two of its five condensed rings partially reduced, is now considered to be best formulated as an "anhydronium base" (I ⟷ II). Such a structure is considered to be consistent with the deep red colour and great strength of the base ($pK = 10{\cdot}6$).



II.

the pale colour of the salts with acid, the formation of a methosulphate which on reduction with zinc and acetic acid gives a non-quaternary methyloctahydrosempervirine forming a benzyloiodide, and the formation of a benzylobromide which similarly reduced gives benzyloctahydrosempervirine forming the quaternary methiodide. Both nitrogen atoms are therefore involved in these changes. Dipole moment measurements were consistent with the expected high value for the proposed structure. F. H.

ANALYTICAL

Benzene Hexachloride, Spectrophotometric Determination of. B. Davidow and G. Woodward. (*J. Assoc. off. agric. Chem.,* 1949, **32,** (4), 751.) The method described depends on the initial extraction of the insecticide from the material to be examined, the subsequent conversion of the benzene hexachloride with alkali to 1 : 2 : 4-trichlorobenzen , followed by the purification of this compound and its estimation by measurement of

the optical density at 286 mμ. Biological tissues are ground with anhydrous sodium sulphate to ensure dehydration and breaking up of the cells; the dried mixture (containing between 0·5 and 15 mg. of benzene hexachloride) is then extracted with ether. Dry materials, such as animal laboratory feeds, are ground and extracted directly without dehydration. The ether solution is evaporated and the residue refluxed with 20 ml. of a 1·5 N solution of potassium hydroxide in methyl alcohol for 1 hour; after cooling and adding 250 ml. of distilled water the mixture is extracted with 25 ml. of normal hexane and the hexane is washed with 10 quantities, each of 400 ml. of distilled water (without shaking). The hexane solution is dried by filtering through 10 to 12 g. of anhydrous sodium sulphate, which had previously been wetted with hexane, and the volume is adjusted to 25 ml. Most tissues needed no further purification but extracts from liver were purified by chromatography through a magnesium oxide column; extracts from laboratory diets were passed through a column of alumina. Hexane solutions of the unsaponifiable fractions of laboratory diets, spinach, potatoes, apple wax, and biological tissues, were found to have absorption properties in the ultraviolet region which interfered with a quantitative determination of benzene hydrochloride. To overcome this difficulty, absorption curves were prepared of the unsaponifiable fraction of these materials and on the products of alkaline hydrolysis of the isomers of benzene hexachloride. It was found that the spectral absorption curves of the control material were linear through the range of 284 to 290mμ. Resolution of the components contributing to the density at 286 mμ could be accomplished by two methods, the first consisting of a determination of the optical densities at 284 and 286 mμ and application of simultaneous equations for the resolution of a two component colour system. The second method used was the base line method in which the densities at 284, 286 and 290 mμ were determined. Recovery experiments showed a standard deviation of 8 per cent., the error being due to loss of benzene hexachloride and 1 : 2 : 4-trichlorobenzene because of their volatility, and to emulsification of a portion of the 1 : 2 : 4-trichlorobenzene. As little as 500 μg. of benzene hexachloride could be determined in biological tissues, in spray residues on vegetables such as spinach and cabbage, and in animal laboratory diets. R. E. S.

Formaldehyde, Acidimetric Titration of, in the Presence of Ammonium Salts. A. Casini. (*Ann. Chim. appl., Roma.*, 1949, **39,** 600.) Most pharmacopœias determine formaldehyde by oxidising it to formic acid. This method is unsatisfactory, as many side reactions, including the formation of hydrogen, make it inaccurate. The following method is based on the formation of hexamine with the quantitative liberation of acid according to the equation $6\ HCHO + 4\ NH_4Cl = (CH_2)_6H_4 + 6H_2O + 4HCl$. To 2 ml. of formaldehyde solution, add 2 or 3 drops of solution of methyl red (if necessary, neutralise exactly), 5 g. of ammonium chloride dissolved in 15 ml. of water and then 30 ml. of N sodium hydroxide. After thorough mixing, titrate the excess of sodium hydroxide with N hydrochloric acid until a permanent red colour is obtained; 1 ml. of N sodium hydroxide corresponds to 45 mg. of formaldehyde. H. D.

Formaldehyde, Determination of. L. Fiore. (*Ann. Chim. appl., Roma.*, 1949, **39,** 604). The method of the Italian Pharmacopoeia for the determination of formaldehyde depends on oxidation to formic acid by hydrogen peroxide and titration of the acid formed. This method is unsatisfactory, and 10 assays carried out on the same sample of formaldehyde solution gave figures varying from 28·35 per cent. to 34·95 per cent. The U.S.P. gives a similar method with modifications presumably aimed at producing

greater accuracy, but 10 assays on the same sample of formaldehyde used for the Italian method gave figures varying from 29·70 per cent. to 33·00 per cent., so the U.S.P. method is no better than the Italian and both are unsatisfactory. H. D.

Phenazone, Alkalimetric Determination of. F. Monforte and G. Stagno d'Alcontres. (*Ann. Chim. appl., Roma.*, 1949, **39**, 663.) When phenazone is heated with ammoniated mercury, a compound is formed which reacts with potassium iodide, combining with iodine and liberating alkali. This reaction can be used for the determination of phenazone. To a solution of 0·05 g. of phenazone in 400 ml. of water add 0·5 g. of ammoniated mercury and boil for about 15 minutes. Filter and wash the residue on the filter paper. Neutralise the filtrate, which still contains traces of ammonia, with 0·01N hydrochloric acid and, after cooling, add 8 g. of potassium iodide (quite neutral and free from iodate), then add 50 ml. of 0·01N hydrochloric acid and titrate the excess with 0·01N potassium hydroxide in the presence of phenolphthalein; 1 ml. of 0·01N hydrochloric acid is equivalent to 0·00188 g. of phenazone.

ORGANIC CHEMISTRY

Adrenaline, Fluorescent Oxidation Product of. A. Lund. (*Acta pharmacol.*, 1949, **5**, 75.) The fluorescent substance is shown to possess the same oxidation step as adrenochrome; for its formation each molecule of adrenaline is oxidised by 2 atoms of oxygen. It is prepared in 65 per cent. yield by titrating an alkaline solution of adrenochrome with acid to *p*H 6·7. The crystalline precipitate thus obtained is separated by centrifuging, washed with water and dried. The fluorescent substance is yellow to orange-yellow, readily soluble in alcohol and pyridine, slightly soluble in acetone and water and insoluble in chloroform and ether. The aqueous solution is light yellowish green and strongly fluorescent. In crystalline form the fluorescent substance will keep for months, but in solution it is oxidised very rapidly. When heated to just above 100°C., the crystals become black without melting. The velocity constants for the formation of the fluorescent substance from adrenaline in alkaline solution and for the oxidative decomposition of the substance prepared as described were found to be the same and this was taken as good evidence for the identity of the two substances. G. R. K.

Adrenaline-like Compounds, a Synthesis of. G. Fodor and O. Kovács. (*J. Amer. chem. Soc.*, 1949, **71**, 1045.) A new synthesis of hydroxyaryl *N*-alkylamino ethanols is described. Hydroxyaryl methyl ketones and their esters or ethers were oxidised by means of selenium dioxide in good yields to the corresponding aryl glyoxals. On catalytic reduction in the presence of alcoholic methylamine or *iso*propylamine, the glyoxals were converted to the adrenaline-like aryl ethanolamines in good yield. The synthesis, *inter alia*, of 1-(2′-hydroxyyhenol)-2-methylamino-ethanol and of *N-iso*propyl-nor-adrenaline by this method is described.

F. H.

Phenazone, Iodomercuric Compounds of. F. Monforte and G. Stagno d'Alcontres. (*Ann. Chim. appl., Roma*, 1949, **39**, 665.) When phenazone is boiled with ammoniated mercury and potassium iodide added various compounds are formed. The authors purified three, (i) $C_{11}H_{12}ON_2HgIOH$ which occurs in yellow scales, soluble in absolute alcohol, m.pt. 135°C.; it does not combine with further iodine. (ii) $C_{11}H_{11}ON_2HgI$ which occurs in white crystals m.pt. 160° to 161°C.

soluble in alcohol and acetone; it combines with iodine, (iii) $C_{11}H_{12}ON_2$-HgI_2,H_2O which occurs in white needles m.pt. 159° to 162°C.; it combines with iodine. These substances are prepared as follows. Add 8 g. of ammoniated mercury in very fine powder to a solution of 3·76 g. of phenazone in 400 ml. of water, boil for about 15 minutes, filter and cool the filtrate. Then add drop by drop a 3 per cent. solution of potassium iodide until no further precipitate is produced. Set aside to deposit and filter off. The filtrate is opalescent but clarifies on standing and is then evaporated by gentle heat when a heavy yellow oil separates. This is dried at 80°C. in an oven and dissolved in alcohol, from which compound (i) can be crystallized. The original precipitate is extracted with acetone, from which compound (ii) can be crystallised. If the original precipitate is extracted with much cold alcohol and the liquid slowly evaporated in the cold, compound (iii) crystallises.

H. D.

PLANT ANALYSIS

***Strychnos* species from British Guiana, Examination of.** H. King. (*J. chem. Soc.*, 1949, 955.) The finely powdered barks of *Strychnos Erichsonii, S. Melinoniana, S. Mitscherlichii, S. diaboli, S. toxifera, S. guianensis* and *S. hirsuta* were slowly percolated with tartaric acid solution (1 per cent.) until the extracts no longer gave a reaction with Tanret's reagent or until the extracts were very pale. The reaction with Tanret's reagent with a portion of the total percolate gave an indication of the alkaloidal content. The extracts were tested for curare activity, on a neutralised portion of the solution, using the righting reflex of the frog. All the species examined, except one near *S. guianensis*, contained alkaloids. Of four containing alkaloids with curare-action, *S. toxifera* yielded the strongest curare preparations. *S. diaboli* yielded an alkaloid, diaboline, which though itself of little pharmacological activity gave an O-methyl ether, the methiodide of which had a curare potency by the rabbit head-drop test one fifty-fourth of that of *dextro*-tubocurarine chloride.

F. H.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Carotene Solutions, Stabilisation of. M. L. Cooley. (*J. Ass. off. agric. Chem.*, 1949, **32**, 706.) The stabilising effect of mixed tocopherols on carotene solutions has been examined. Identical sets of solutions were stored in daylight (not direct sunlight), at room temperature (20°C.), in the dark at room temperature, and in the refrigerator; the tocopherols used were natural and contained 220 mg. of mixed tocopherols per g. The solutions were stored for 12 weeks, and at regular intervals the colour of each was measured in a spectrophotometer at 440 mμ. At the end of the 12-week period the solutions were analysed chromatographically followed by an assay using the method of Devlin and Mattill (*J. biol. Chem.*, 1942, **146,** 123). It was found that by incorporating 10 to 50 times as much tocopherols as carotene a light petroleum solution of carotene did not deteriorate in 12 weeks; without tocopherols similar solutions were stable only for 2 to 3 days. The use of more than 5 mg. of tocopherols per 100 ml. of light petroleum may produce a measurable colour which would be erroneously recorded as carotene. The destruction of carotene in higher potency solutions is not as rapid as in dilute solutions, possibly due to an antioxidant effect by the

carotene itself. Recovery of carotene from a solution which had been passed through a magnesia adsorption column was not complete although degradation products were removed. R. E. S.

β-Carotene, Stereoisomeric Analysis of. E. M. Bickoff, M. E. Atkins, G. F. Bailey and F. S. Stitt. (*J. Assoc. off. agric. Chem.*, 1949, **32**, 766.) Work has been done on the development of a comparatively rapid, reliable, liquid chromatogram procedure for separating β-carotene extracts into three fractions, each containing primarily only one of the three isomers, all-*trans*, *neo*-B, and *neo*-U, which constitute 95 per cent. of the β-carotene equilibrium mixture. In order to obtain a quantitative chromatographic procedure a study of the adsorbent, developer, adsorbate, size of column and method of packing was made and a method is described for the stereoisomeric analysis of β-carotene extracts by liquid chromatogram procedure into *neo*-B, all-*trans*, and *neo*-U fractions, followed by colorimetric analysis of the fractions, the method being sufficiently simple to make its routine application practical. Calcium hydroxide to definite specification is used as the adsorbent and a sufficient quantity of carotene solution is added to the dry column to give about 40 μg. of carotene. The chromatogram is developed with a solution of 1·5 per cent. of *p*-cresyl methyl ether in light petroleum (b.pt. 88° to 90°C.) and maintained at an absolute pressure of 100 to 200 mm. Hg., the eluate being collected directly in 25-ml. volumetric flasks. The bands are eluted from the column in the following order: *neo*-β-carotene B, all-*trans*-β-carotene, *neo*-β-carotene U. The *neo*-B fraction contains all the eluate leaving the column before the leading boundary of the all-*trans* zone reaches the bottom of the column; further eluate is collected as the all-*trans* fraction until the leading boundary of the *neo*-U zone reaches the column outlet. The elution of the *neo*-U fraction is accelerated by the addition to the column of 15 ml. of 5 per cent. acetone in light petroleum when the collection of the all-*trans* fraction is nearly completed. Each fraction is made up to volume and the optical density determined on an aliquot portion, the concentration of the various isomers being calculated from factors determined on solutions of the pure crystalline *neo*-B, all-*trans*, and *neo*-U isomers. The method should be applicable to the stereoisomeric analysis of β-carotene extracts from any source. Results are given of the application of the method to the analysis of an iodine-isomerised solution of β-carotene. R. E. S.

1:5-Vinyl-2-thiooxazolidone, an Antithyroid Compound. E. B. Astwood, M. A. Greer and M. G. Ettlinger. (*J. biol. Chem.*, 1949, **181**, 121.) Several foods were found to inhibit the uptake of radioactive iodine in a manner similar to that of antithyroid compounds, the most effective of these being rutabaga (yellow turnip or Swedish turnip), which was subsequently utilised for isolation of the active principle. Extraction of rutabaga was carried out as described previously, being followed during the early stages of purification by a simplified rat assay with I^{131}. Examination of the absorption spectra of concentrates showed a strong maximum at 240 mμ, of intensity parallel to antithyroid activity, and since most known sulphur-containing goitrogens are thioamides the ultra-violet absorption spectrum was used to control the final purification of the active principle. Details of the purification by chromatography and by crystallisation are given, this resulting in the isolation of the antithyroid factor as colourless optically active crystals, m.pt. 50°C., $[\alpha]_D^{31°C.}$ − 70·5° (2 per cent. in methyl alcohol). Analyses and a molecular weight determination furnished the molecular formula C_5H_7ONS, the compound being a weak monoacid, pK_a 10·5, without

basic properties, and stable in hot alkali but not in acid. The ultra-violet absorption spectrum in aqueous solution exhibited a single intense maximum at 240 mμ, log ε 4·18, shifted by alkali to 232 mμ, log ε 4·08. The physical properties of the compound suggested the presence of the grouping -NH-CS-O- final evidence in this direction being afforded by infra-red spectra studies which indicated that the substance was either 4- or 5-vinyl-2-thio-oxazolidone. The behaviour of the rutabaga compound on boiling with 4 N hydrochloric acid (the optical activity disappeared and ammonia could not be detected) indicated that the antithyroid factor was actually *l*-5-vinyl-2-thiooxazolidone; this structure has since been confirmed by synthesis. The thiooxazolidone content of seeds of plants of the mustard family was studied spectrophotometrically; it was not detected in any plant except those of the genus *Brassica*, and within that genus it was absent from the seeds of mustard and cauliflower. It was isolated from the edible root of rutabaga and white turnip in amounts which varied from 0·12 to 1·0 g. per kg. but it could not be detected in the edible portions of other *Brassica* vegetables, including cabbage, Chinese cabbage, kale, cauliflower, broccoli, mustard-greens, or horseradish root. The compound appeared to be present in the plant as a water-soluble, ether-insoluble precursor, from which the thiooxazolidone was formed apparently by enzyme action; on boiling the root or seed in water the enzyme was inactivated. When tested in normal human subjects, by the use of radioactive iodine the compound was found to have an antithyroid activity equal to that of thiouracil. R. E. S.

Vitamin B_{12} and Related Growth Factors, Paper Chromatography of. W. A. Winsten and E. Eigen. (*J. biol. Chem.*, 1949, **181,** 109.) A paper chromatographic procedure for separating the six alternative growth factors for *Lactobacillus leichmannii* 313 as previously reported is described, as well as the method of recognising the position of the several factors on a chromatogram by use of *L. leichmannii* 313 as a microbiological indicator. The extension of the method to the examination of other natural materials revealed the existence of at least one other substitute growth factor replacing vitamin B_{12} in the nutrition of the test organism. In the method described, 10 to 30 μl. samples of the solutions under examination (*p*H 5·0) were spotted on Whatman No. 1 paper strips. The chromatograms were developed overnight at room temperature with wet *n*-butyl alcohol; the strips were allowed to dry in air for 1 hour at 30° to 35°C., being laid on agar plates seeded with *L. leichmannii* 313. After leaching for 5 minutes the strips were removed and the plate was incubated overnight at 37°C.; the resulting zones of bacterial growth were light and it was necessary to hold the plate at an angle with a source of light to one side and behind it, when the zones were always sharply defined, well-formed ellipses. Several commercial parenteral liver preparations for use in the treatment of pernicious anæmia were examined by this technique in addition to condensed fish solubles, a commercial stomach concentrate intended for use in pernicious anæmia, corn steep liquor, a commercial trypsin, Wilson's liver powder 1:20, and an enzyme digest of sperm desoxyribonucleic acid. The slowest moving zone at the top of the strip had a double character, suggesting two substances; analysis of crystalline vitamin B_{12} preparations and vitamin B_{12} concentrates indicates that the doublet zone of growth may be due to two forms of vitamin B_{12}. Examination of corn steep liquor showed no vitamin B_{12} doublet but four other growth factors for *L. leichmannii* 313; in addition to at least two forms of vitamin B_{12}, five other substitute growth factors were found to be present in various natural materials. Evidence obtained from analysis of an enzyme digest of desoxyribonucleic acid

indicated that the more rapidly moving growth factors other than vitamin B_{12} were of desoxyriboside character. In a quantitative analysis of vitamin B_{12} and of substitute growth factors in a parenteral liver preparation it was found that after chromatography there was somewhat more apparent vitamin B_{12} activity per ml. at the top of the chromatogram, when compared with the value obtained on the whole preparation without chromatography. The bottom section of the chromatogram, containing as it did the three fast moving alternative growth factors, contributed an additional amount of apparent vitamin B_{12} activity, which was only about 25 per cent. of the activity at the top of the chromatogram; in a total tube assay of an unchromatographed sample, the value obtained (0·67 per ml.) was not the sum of the activities contributed by the top and bottom sets of factors measured separately. R. E. S.

BIOCHEMICAL ANALYSIS

Adrenaline in Blood, Fluorimetric Determination of. L. Lund. (*Acta Pharmacol.*, 1949, **5**, 231.) Adrenaline is oxidised by manganese dioxide to adrenochrome and this is rearranged to adrenolutine on addition of strong alkali and sodium ascorbate; the latter prevents further oxidation of the adrenolutine. Irradiation with ultra-violet light shows a yellowish green fluorescence the intensity of which is measured in Coleman's electronic photofluorimeter, model 12B, using Schlott's glass filter BG 12 as primary filter and Corning's glass filter 3486 as secondary filter. Fresh blood, mixed with heparin, is centrifuged to remove corpuscles; 5 ml. of plasma with 5 ml. of 0·2 N sodium acetate is filtered rapidly through a 7 mm. diameter column of alumina (1 g.), previously washed with 20 to 30 ml. of water to remove fluorescent substances and then with 5 ml. of 0·2 N sodium acetate. The column is washed with 5 ml. of sodium acetate solution and eluted with a mixture of 10 ml. of 0·2 N acetic acid and 10 ml. of water. The eluate is made up to 20 ml. To each of two portions of 9 ml. in centrifuge tubes 0·1 g. of manganese dioxide (washed free of fluorescent substances with acetic acid and water) is added, the tubes are inverted for 1 minute and centrifuged for 30 seconds at 3,000 revolutions per minute; the liquid is filtered through filter paper (washed free of fluorescent substances and dried). Two portions (A and B) each of 8 ml. of the filtrate are taken. To A 0·84 ml. of a 20 per cent. solution of sodium hydroxide is added, and 5 minutes later, 0·16 ml. of 1 per cent. solution of ascorbic acid. To B 1 ml. of a mixture of 1 ml. of 20 per cent. solution of sodium hydroxide and 2·20 ml. of 1 per cent. solution of ascorbic acid, is added. Solutions A and B are exactly alike except that the adrenolutine fluorescence in A has been eliminated by oxidation so that A gives the individual blank value for B. For concentrations above 10 μg. per cent. of adrenaline in plasma the accuracy is ±5 per cent.; for concentrations between 1 and 10 μg. per cent. it is ±10 per cent. G. R. B.

Aneurine, Microbiological Assay of. E. E. Fitzgerald and E. B. Hughes. (*Analyst*, 1949, **74**, 340.) A modification of the method of Sarett and Cheldelin (*J. biol. Chem.*, 1944, **155**, 153) depending on the destruction of the aneurine in an extract of the sample by a modification of the sulphite treatment of Schultz, Atkin and Frey and the use of this sulphite-treated extract to supplement the standard tubes is described, all tubes used in an assay thus contain the same amount of the sample and a comparison can be made between untreated extract and sulphite-treated extract plus standard aneurine, inhibitory or stimulating effects due to substances other

than aneurine being in this way counteracted. The method is described in detail and prevents the unsatisfactory drift in the values obtained at different sample levels, due to the stimulatory or inhibitory effect on *Lactobacillus fermenti* 36 of substances other than aneurine in the sample extract, associated with the process of Sarett and Cheldelin. Using pure aneurine solutions, it was found that the sulphite treatment destroyed about 95 per cent. of the activity, but when the solutions were autoclaved at 15 lb. pressure instead of being steamed for 30 minutes, 99 per cent. of the activity was destroyed in 15 minutes; if this modification of the sulphite treatment was applied to sample extracts supplemented with aneurine, residual activity was absent. *L. fermenti* shows very little growth in the first 20 to 24 hours of incubation in the basal medium to which the hydrolysis products of the aneurine has been added (sulphite treated yeast extract was actually used). After this period, the organism grows rapidly, reaching full growth in a further 40 hours. The growth in the first 20 hours, although small, was difficult to account for until it was found to be due to residual aneurine in the alkali-treated peptone in the medium. When the peptone was prepared by treatment with sulphite, a very low "blank" was obtained in the first 20 hours of incubation. Under normal assay conditions, that is, in the presence of about 0·03 μg. of aneurine per tube, the lag phase is about 6 hours, the logarithmic phase is passed in a further 10 hours, and after this growth continues more slowly. Thus, with an incubation time of 18 hours, growth is independent of aneurine degradation products, whilst the effect of intact aneurine is approaching its maximum. This work indicated that by treating natural products such as peptone and yeast extract with sulphite it should be possible to devise a simpler basal medium with a shorter list of synthetic supplements; this is now being investigated. The method has been applied to a wide range of food materials. The results have been satisfactorily reproducible and have agreed reasonably well with parallel chemical assays. R. E. S.

Aneurine in Yeast and Yeast Products, Assay of. A. Jones and S. Morris. (*Analyst*, 1949, **74**, 333.) A study has been made of the plate method of assay (*Analyst*, 1948, **73**, 334) using *Lactobacillus fermentum*. The final method adopted used the basal medium of Sarett and Cheldelin (*J. biol. Chem.*, 1943, **150**, 1) with the addition of sodium chloride and 1·5 per cent. of agar; the organism used, *Lactobacillus fermentum* P.36, was maintained on the medium of Cheldelin, Bennett and Kornberg (*J. biol. Chem.*, 1946, **166**, 779) and sub-cultured every 3 weeks. For the inoculum *L. fermentum* was grown for 18 hours at 37°C. in 10 ml. of basal medium minus agar, with the addition of 10 μg. of aneurine per ml.; the medium was centrifuged, the supernatant liquor discarded and the organisms washed twice with saline solution, being finally suspended in 20 ml. of sterile saline solution. In the preparation of the plates 5 tubes of medium were used for the standard and for each test sample, the contents of each tube being melted, cooled to 48° to 50°C., and 1 ml. of inoculum added; after thorough mixing the contents were poured into sterile Petri plates, allowed to set, dried for 1½ hours at 37°C. and stored until used, 2 to 3 hours later; 5 holes were cut in each plate, each hole 10 mm. in diameter. The yeast was prepared for assay by taking 1 g. and adding, with 0·05 g. of takadiastase, to 15 ml. of 1 per cent. sodium acetate buffer at *p*H 4·5. The *p*H of the mixture was adjusted to 4·4 to 4·5 with N acetic acid or sodium hydroxide as necessary, 2 drops of benzene were added, and the whole was incubated at 37°C. for 16 to 18 hours. After steaming for 10 minutes, cooling, adjusting to *p*H 6·5, diluting to 25 ml. with water and filtering, the filtrate was diluted 1:2, 1:4,

1:8 and 1:16: 0·1 ml. quantities of each dilution were then measured into the holes in each of the 5 plates. The assay range of 0·25, 0·50, 1·00, 2·00, and 4·00 μg. of aneurine hydrochloride per ml. was used for the preparation of the standard curve; 0·1 ml. of each standard solution was measured into the holes of each of the five plates which were then incubated for 18 hours at 37°C. with the lids raised. The effect of doubling the aneurine concentration was to increase the zone diameters by 2·0 mm., the following equation expressing the relationship: log $D = aZ - b$, where a and b are constants, D is the aneurine concentration in μg. per ml. and Z the zone diameter in mm. corresponding to a yeast concentration of M mg./ml. With the given test conditions, $a = 0{\cdot}1505$ and b is dependent upon the intercept of the standard curve for any given day. From this, the aneurine concentration in the yeast in μg. per g. is given by 1000 D/M. The method gave valid and accurate results, though it was not particularly sensitive. R. E. S.

Ascorbic Acid in Blood, Determination of, by the Dinitrophenylhydrazine Method. W. Daubenmerki. (*Acta pharmacol. toxicol.*, 1949, **5**, 270.) The colorimetric, photo-electric macro method for the determination of ascorbic acid in serum and in whole blood by means of dinitrophenylhydrazine (described by Roe and Kuether, *J. biol. Chem.*, 1943, **147**, 399, and modified by Lowry, Lopez and Bessey, *ibid.*, 1945, **160**, 609) is reliable both quantitatively and qualitatively. By controlled oxidation in presence of suspended charcoal, ascorbic acid is converted to dehydro-ascorbic acid, which forms an osazone with dinitrophenylhydrazine; the intensity of the stable, deep-red colour produced on addition of strong sulphuric acid is measured photo-electrically. It is asserted that in the given circumstances blood contains no substances likely to upset the specificity of the method. In recovery experiments, 0·2 mg. per cent. of ascorbic acid added to serum or citrated blood was recovered with an accuracy of 99 to 100 per cent. with a maximum deviation of ± 4 per cent. Comparison with Farmer and Art's method of titration with dichlorindophenol showed this to be grossly inaccurate. Favourable agreement was found between the dinitrophenylhydrazine method and a photo-electric dichlorindophenol method. Ascorbic acid is unstable in clotted blood. G. R. B.

Aureomycin, Assay of, in Body Fluids. H. D. Brainerd, H. B. Bruyn, Jr., G. Meiklejohn and M. Scaparone. (*Proc. Soc. exp. biol., N.Y.*, 1949, **70**, 318.) Serum or other body fluid is stored at −40°C. prior to testing, and sterilised if necessary by Seitz filtration. Serial dilutions are prepared in a nutrient broth containing 20 per cent. of ascitic fluid, and to 0·2 ml. of each dilution, 0·5 ml. of a broth suspension of a strain of β-haemolytic streptococcus (JB) is added. The mixtures are incubated at 37°C. for 18 hours and the highest dilution which shows no growth visible to the naked eye is noted. Control tests using aureomycin solutions are run simultaneously, and generally indicate that the end-point of the test corresponds to a concentration of 0·031 μg. of aureomycin per ml. After oral administration of 1 g. of aureomycin, the maximum serum concentration, varying from 0·6 to 2·5 μg. per ml., is reached in 4 to 6 hours. When the dose is repeated every 4 to 6 hours, the serum level is gradually increased. Similar or greater serum concentrations are reached 5 minutes after intravenous injection of 50 mg. The concentration decreases rapidly for the first hour and then more slowly. 50 to 200 mg., injected intramuscularly, rarely produces measurable levels in the serum. G. B.

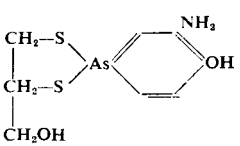
Glucose, Galactose and Rhamnose in Mixtures, Determination of. W. L. Porter and C. S. Fenske, Jr. (*J. Assoc. off. agric. Chem.*, 1949,

32, 714.) In studies of flavonol glycosides various methods, including filter paper chromatography, copper reduction and yeast fermentation were combined to determine the constituent sugars in hydrolysates of buckwheat and other plants. A filter paper chromatogram of the solution to be analysed was prepared and the identity of the sugars present was determined from the position of the spots. An aliquot of the original solution was diluted in the same manner as was done with the fermented samples and using Schoorl's method an aliquot of this solution was measured for total reducing sugars. If the chromatogram indicated that rhamnose was absent, but that either glucose or galactose or a mixture of these two was present, fermentation procedure A was followed; if rhamnose was present in combination with either or both of the two sugars, procedure B was used; if the chromatogram indicated only rhamnose, fermentation was unnecessary. Fermentation procedures A and B are given in detail and employed *Saccharomyces bayanus* (N.R.R.L. No. 966) for fermenting glucose and galactose. The fermentations when completed are followed by copper determinations of the undestroyed carbohydrate. From a combination of the results obtained at each stage the amounts of glucose, galactose and rhamnose can be calculated. Synthetic mixtures of glucose, galactose, and rhamnose could be analysed with recoveries of 98 to 104 per cent.; hydrolysates of a flavonol glycoside gave recoveries of approximately 96 per cent., which were probably low owing to destruction of sugar during the hydrolysis. R. E. S.

CHEMOTHERAPY

Amidone and Isoamidone, Homologues of, and some Related Compounds. E. Walton, P. Ofner and R. H. Thorp. (*J. chem. Soc.*, 1949, 648.) In view of the established value of amidone, four series of related ketones were prepared by condensing diphenylmethyl cyanide with a chlorobase and treating the resulting basic cyanides with Grignard reagents. Corresponding amides, acids, esters and alkanes were also prepared. The compounds were examined for analgesic and respiratory depression activities. The two properties were always associated but not always in the same ratio, maximal activity being attained in the ethyl ketone in all the four series. 3-Dimethylamino-1:1-diphenyl-*n*-butyl cyanide was resolved by means of D-tartaric acid and from the optically active isomers the *dextro-* and *lævo-*amidones were prepared. F. H.

Dimercaprol and Oxophenarsine Hydrochloride, Toxicity and Chemotherapeutic Effect of the Condensation Product. J. L. Sawyers, B. Burrows and T. H. Maren. (*Proc. Soc. exp. Biol., N.Y.*, 1949, **70,** 194.) The equimolecular condensation product of oxophenarsine and dimercaprol is a light pink powder having m.pt. 122° to 124°C., and arsenic content 24·5 to 24·6 per cent., corresponding to a dithioarsenite of the following formula:—



It may be prepared by mixing a solution of 1·88 g. of oxophenarsine hydrochloride in 100 ml. of N hydrochloric acid with 1 g. of dimercaprol in 20 ml. of methyl alcohol, neutralising with sodium hydroxide, and washing the precipitate with water, methyl alcohol and ether. Compared with the original oxophenarsine hydrochloride, the therapeutic effect and toxicity for *T. equiperdum* in mice are both reduced, the former to a greater extent. The therapeutic index (LD_{50}/CD_{50}) for the dithioarsenite is 10, compared with 30 for oxophenarsine hydrochloride. A similar reduction in toxicity is observed in rats. It is suggested that the compound acts by dissociation to a

substance whose arsenic is available to the sulph-hydryl groups in protein, and that combination with dimercaprol alters the distribution of active arsenical between trypanosome and host. The hypothesis that the dithioarsenite acts as a whole cannot be explained by the -SH-arseno receptor theory. G. B.

***p*-Phenanthroline Derivatives as Antimalarials.** B. Douglas and W. O. Kermack. (*J. chem. Soc.*, 1949, 1017.) 4-Chloro- and 4:9-dichloro-*p*-phenanthroline were synthesised from ethyl ethoxymethylenemalonate and the chlorine atoms replaced by basic side chains. 9-Chloro-4-(3-diethylaminopropylamino)-*p*-phenanthroline and 4-(4′diethylamino-1′ methylbutylamino)-*p*-phenanthroline showed antimalarial activity of the same order as mepacrine and quinine against *Plasmodium gallinaceum* in chicks, delaying considerably the appearance of parasites in the blood in doses of 10 mg. per 20 g. body weight. F. H.

PHARMACY

DISPENSING

Penicillin, Stabilisation of Aqueous Solutions of. P. Fleury, G. Schuster, M. Dessus, J. Roux-Delimal and A. Morel. (*Ann. pharm. franc.*, 1949, **8**, 529.) Aqueous solutions containing not less than 20,000 Units per ml. may be preserved at room temperature for several months by the addition of 0·3 to 0·5 ml. of formaldehyde solution per 100 ml. On dilution with water, the potency of the solutions diminishes rapidly. Certain samples of solutions containing, in addition, citrate or phosphate buffers (*p*H 7·2 to 7·5) also deteriorate and the solutions may become slightly more acidic (*p*H not below 6·8). Solutions prepared with distilled water, glucose saline, or water treated with permutit, and containing phosphate buffer, formalin and penicillin (10,000 Units per ml.), all showed some deterioration when assayed microbiologically at intervals. The use of a solution of sodium hyposulphite, stabilised by borate, produced a stable preparation, possibly by preventing oxidation of the formaldehyde. The following method yields a solution stable at room temperature for several months. Dilute 0·2 ml. of neutralised formalin to 100 ml. with distilled water. Dissolve 100,000 Units of penicillin in 2·5 ml. of this solution, heat at 56°C. for 30 minutes and repeat the heating after an interval of 24 hours. Prepare a solution containing crystalline monopotassium phosphate, 0·25 per cent.: crystalline dipotassium phosphate, 1·56 per cent.; sodium hyphosphite (pentahydrate), 1 per cent.; sodium borate, 0·04 per cent.; sodium chloride, 1 per cent., and sterilise by autoclaving. Mix 2·5 ml. with the penicillin solution and store in ampoules. It has been observed that highly refined samples give less stable solutions, and further work is in progress. G. B.

Penicillin G, Stabilisation of Aqueous Solutions of. R. Paul, P. Gaillot and J. Baget. (*Ann. pharm. franc.*, 1949, **8**, 524.) The activity of aqueous solutions of penicillin G is rapidly destroyed by penicillinase and by heat. A sterile aqueous solution containing 200,000 Units in 20 ml. loses 90 per cent. of its activity on keeping at 25°C. for 4 days. The solution is most stable if buffered at *p*H 8·0 to 8·3. Citrate or phosphate buffers are compatible with penicillin G and well tolerated when given by injection, but citrates discolour on heating at 250°C. to destroy pyrogenic matter. The following is a suitable method for introducing the required buffer salts into containers and making a stabilised solution of penicillin G. Prepare a solution containing 0·1 g. mol. per litre of an equimolecular mixture of disodium

phosphate and monosodium phosphate, place 0·5 ml. in each container and heat at 250°C. for 1 hour to sterilise and to destroy pyrogen. Cool and add aseptically 200,000 Units of penicillin G dissolved in 20 ml. of sterile double-distilled water, isotonic sodium chloride or glucose solution. The deterioration of aqueous solutions of penicillin G is not accelerated by traces of alkali, and it is no advantage to use containers which comply with a limit test for alkalinity of glass. G. B.

GALENICAL PHARMACY

Ointment Bases, Absorption of Radioactive Sodium Iodide from. G. N. Cyr, D. M. Skauen, J. E. Christian and C. O. Lee. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38,** 615, 618.) Ointments consisting of 10 per cent. of sodium iodide labelled with iodine^{131} in lard, wool fat and soft paraffin were applied under rigorously standardised conditions to clipped areas of the backs of white rats. After 30 hours, in which time previous experiments had shown that maximum absorption took place, the animals were killed and their thyroids examined for content of radioactive iodine, using a Geiger Muller counter. Conversion of the results to percentages showed that the amount of applied iodine found in the thyroids was 0·045 per cent. from white soft paraffin, 0·041 per cent. from wool fat and 0·037 per cent. from lard. This technique was applied to 37 ointment bases. Whatever the base, there was some degree of absorption or penetration, the greatest amount (0·05 per cent. of iodide applied) being obtained with hydrous wool fat and the least (0·00006 per cent.) with a methylcellulose base. About 50 per cent. of the bases were more efficient than lard and the remainder less efficient. A difference in the type of emulsion, oil-in-water or water-in-oil, did not seem to exert any appreciable effect and with bases of the same type there was considerable variation in the amount of absorption. G. R. K.

Sulphadiazine and Wetting Agents, Diffusion from Ointment Bases. B. Levy and C. L. Huyck. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38,** 611.) The rates of diffusion of powdered and microcrystalline sulphadiazine from various ointment bases with and without wetting agents were compared *in vitro.* Results confirmed that water-soluble drugs should not be incorporated in water-in-oil emulsions since diffusion is greater from oil-in-water type ointments. Microcrystalline sulphadiazine was liberated faster and in greater amounts than ordinary powdered sulphadiazine from hydrophilic ointment U.S.P., and this rate was increased by the addition of the wetting agent, sodium lauryl sulphate. Microcrystalline sulphadiazine in white ointment U.S.P., with sodium lauryl sulphate, diffused slightly immediately after preparation, but did not diffuse after storage for 1 month at 37·5°C. Addition of sodium lauryl sulphate to white ointment containing 5 per cent. of microcrystalline sulphadiazine caused slight diffusion of the drug from the base. From hydrophilic ointment microcrystalline sulphadiazine diffused to a greater extent than ordinary sulphadiazine both at room temperature and at 37·5°C. G. R. K.

PHARMACOGNOSY

Anthraquinone Drugs, Colorimetric and Fluorimetric Studies on the Bornträger Reaction for. B. V. Christensen and I. A. Abdel-latif. (*J. Amer. pharm. ass.*, 1949, **38,** 487.) The authors have re-investigated the published modifications of the Bornträger test and conclude that alcoholic potassium hydroxide is the best hydrolysing agent and that ether is the best

solvent for the liberated anthraquinones. The alcoholic potassium hydroxide extract of senna leaf when viewed under filtered ultra-violet light shows a strong pink fluorescence; during the test this fluorescence passes into the ether layer then into the ammonia layer with the anthraquinones. It is therefore suggested that the fluorescence is due to the anthraquinones and that it may be used as a sensitive test of their presence. The colour varies when other anthraquinone drugs are used in the test. Hydroxyl groups in the 1 and 8 positions of anthraquinone must be present in order that a positive Bornträger reaction as well as the fluorescence may be obtained. J. W. F.

Cholla Gum. F. Brown, E. L. Hirst and J. K. N. Jones. (*J. chem. Soc.*, 1949, 1761.) Cholla gum, an exudate of the cactus, *Opuntia fulgida*, has been shown to consist of L-arabinose (6 parts), D-xylose (2 parts), L-rhamnose (trace), D-galactose (3 parts) and D-galacturonic acid (1 part). Hydrolysis of the methylated derivative of cholla gum yielded 2:3:5-trimethyl L-arabinose (4 parts), 2:3:4-trimethyl D-xylose (2 parts), 2:3-dimethyl L-arabinose (1 part), 2:4-dimethyl D-galactose (3 parts), L-arabinose (1 part), and 2-methyl D-galacturonic acid (1 part). The L-arabinose residues were present in the furanose form. Cholla gum therefore resembled other plant gums in many features of its architecture, the main difference being that its acidity is due to D-galacturonic acid instead of D-glucuronic acid. F. H.

Pyrethrum Flowers, Analysis of. W. Mitchell and F. H. Tresadern. (*J. Soc. chem. Ind., Lond.*, 1949, **68**, 221.) It is shown that the assay of pyrethrum flowers using warm ligroin (Soxhlet) gives high results for pyrethrin II especially when the higher boiling grades are employed. The additional pyrethrins extracted with hot ligroin or chloroform were proved to be non-toxic to house-flies. It was also found that the additional matter obtained by hot extraction was insoluble in kerosene which is the basis of fly spray solutions. The use of cold ligroin (b.pt.: 40°to 60°C. or 20° to 40°C.) both for the assay and for commercial extraction is recommended. It is also suggested that the flowers should be standardised on their content of pyrethrin I only. G. R. A. S.

PHARMACOLOGY AND THERAPEUTICS

Antimalarial Drugs, Response of *Plasmodium berghei* to. L. G. Goodwin. (*Nature*, 1949, **164**, 1133.) A new strain of *Plasmodium berghei* was used for the investigation of a series of 2 : 4 diaminopyrimidines, one of these compounds, 2 : 4-diamino-5-*p*-chlorophenoxy-6-methyl pyrimidine (48-210), being assayed simultaneously against *Plasmodium gallinaceum* in chicks and *P. berghei* in mice at the same dose-levels. Mice were inoculated intraperitoneally with a suspension of infected blood in heparinised saline solution, each mouse receiving *ca.* 5,000,000 parasitised erythrocytes; 6 doses of drug were given by stomach tube, night and morning for the following 3 days. Blood smears were prepared on the fifth and seventh days of the disease, and the percentage of parasitised cells determined for each mouse. Results are given in quinine equivalents and show that, although the quinine equivalents of 48-210 and chloroquine were about equal on the two species of *Plasmodium*, the relative activities of pamaquin and mepacrine were reversed: the low activity of pamaquin against *P. berghei* has been confirmed in further experiments, the variations possibly being due to differences in the rate of absorption and excretion of the drugs in mice and chicks, or by differences in the susceptibility of the parasites. R. E. S.

Artane in the Treatment of Parkinson's Disease. R. S. Schwab and W. R. Tillmann. (*New Engl. J. Med.*, 1949, **241,** 483.) Artane alone, and in combination with other drugs, has been used in 44 cases. Favourable results were obtained in 67 per cent. of cases in which artane was given. After 3 months' treatment only 7 of the patients were relieved sufficiently, as compared with their status on atropine, to remain on artane alone. The initial dose of artane is 1 mg. 4 times a day, and this dose is gradually increased until the patient is taking 3 mg. 5 times a day as a high level, the average being 2 mg. 5 times a day. Overdosage produces side-effects such as giddiness, dryness of the mouth, blurring of vision and headache. It was found less toxic than parpanit in older patients but less effective in reducing tremor; indeed, in some patients the tremor was worse in spite of reduction in rigidity. Nine of the patients required the addition of parpanit, and 7 were best regulated on a combination of atropine drugs and artane.

S. L. W.

Atropine and Tripelennamine (Pyribenzamine) in Treatment of Peptone Shock. J. C. Davis and H. O. Haterus. (*Proc. Soc. exp. Biol., N.Y.*, 1949, **70,** 275.) Intravenous injection of atropine (2 mg./kg.) of tripelennamine (10 mg./kg.) to anæsthetised dogs prevents death from shock subsequent to the rapid intravenous injection, 15 minutes later, of a 20 per cent. solution of Witte's peptone (5 ml./kg.). Neither substance prevents the initial fall in blood pressure, but there is a more rapid recovery of pressure in the animals protected by tripelennamine. A combination of atropine with tripelennamine appears to have no advantage over tripelennamine alone. In the concentration administered, atropine antagonises acetylcholine but not histamine, and so the results confirm that acetylcholine, as well as histamine, is concerned in peptone shock. G. B.

Curare Assays; a modification of the Rat's Phrenic Nerve-Diaphragm Method. G. B. West. (*Analyst*, 1949, **74,** 582.) Employing the usual method for the estimation of curare-like substances of suspending the isolated phrenic nerve-diaphragm in Ringer's solution at 37°C. the author was unable to obtain constant contractions for many hours, when using fluid electrodes and condenser charges from a neon lamp circuit. The temperature of the fluid was therefore reduced to 20°C. With a rate of stimulation of 8 stimuli per minute the tension developed varied from .1 to 10 g. and remained constant for several hours. When a dose of tubocurarine, sufficient to produce an inhibition of 50 per cent. of the muscle contraction in about 5 minutes, was added to the bath at 20°C. and the Tyrode solution changed after 5 minutes' action, the recovery of the preparation to its base-line required about 30 minutes. In an effort to reduce this time, potassium chloride was added to the bath of Tyrode solution. In small doses, up to 45 mg., this resulted in pure potentiation, while doses of more than 50 mg. resulted in initial stimulation followed by depression. It was found, however, that the tubocurarine must be removed from the bath before the potassium (40 to 45 mg. of potassium chloride) could aid recovery. This extra potassium was then washed out before the next dose of curare (100 μg. to a 75 ml. bath) was added. For the assay process, inhibitions of about 50 per cent. of the muscle contractions were produced, doses of the preparation under test being added at 8-minute intervals. With the lower temperature the diaphragm showed little sign of fatigue after 8 hours. A difference in activity of 7 to 10 per cent. could be detected by this means. S. L. W.

Curarising Activity; Assay in the Conscious Mouse and Rat. H. O. J. Collier, E. C. Fieller and R. A. Hall. (*Analyst*, 1949, **74,** 583.)

This is a report on methods used by the authors for the estimation of the activity of *d*-tubocurarine, and, in the rat, for the estimation of the dimethyl ether. The methods resemble in many respects those of Skinner and Young (*J. Pharmacol.*, 1947, **91,** 144), but differ essentially in the use of the intravenous route of administration. If a solution of *d*-tubocurarine of suitable concentration is given intravenously to a number of mice a proportion of them temporarily lose the righting reflex. Analysis of a number of experiments indicates that the log dose-probit line obtained by giving intravenous doses at several levels to groups of mice is both steep and straight, and this response may be made the basis of an assay method. The log dose-probit line obtained in a similar way with *d*-OO-dimethyl tubocurarine in rats is also steep and straight, and this response may also be made the basis of an assay method. S. L. W.

Fat Emulsion, Intravenous Infusion into Human Subjects. G. P. Shafiraff, J. H. Mulholland, E. Roth and H. C. Baron. (*Proc. Soc. exp. Biol., N.Y.*, 1949, **70,** 343.) An emulsion containing fatty oil (10 per cent.), protein hydrolysate (5 per cent.), glucose (5 per cent.) and gelatin (2 per cent.) and having a calorific value of 1300/1. and *p*H about 6·2 was prepared as follows. Refined coconut oil was autoclaved and mixed with sterile non-pyrogenic solutions of protein hydrolysate (amigen, 10 per cent.), glucose (50 per cent.) and intravenous gelatin (Knox P-20, 6 per cent.), and the mixture passed through a special homogeniser until the particle size of the emulsion was less than 1 μ. This emulsion was infused into surgical hospital patients in quantities of up to 16 l., at a rate of 20 to 80 drops per minute. The most severe reaction was a chill, observed in 9 per cent. of the patients and usually accompanied by a high temperature reaction. Allergenic reactions could be controlled by anti-histaminic drugs, and cough and vomiting were usually controlled by reducing the speed of administration. No pulmonary irritation, fatty emboli, serious toxic effects or late sequelae were observed. G. B.

Methadone and its Isomers, Narcotic Power of. J. E. Denton and H. K. Beecher. (*J. Amer. med. Ass.*, 1949, **141,** 1146.) The analgesic power of *dl*-methadone (6-dimethylamino-4:4-diphenylheptanone), *l*-methadone, *dl-iso*methadone (6-dimethyl-amino-5-methyl-4:4-diphenylhexanone-3), and *l*-isomethadone was compared with that of morphine in 429 patients with post-operative pain. The basis of comparison of the analgesic equivalence of morphine and the methadones was an arbitrary standard analgesic dose, the AD 90 per cent., which is defined as the analgesic dose giving moderate to complete relief of pain in 90 per cent. (limits 89 to 93 per cent.) of the trials. *dl*-Methadone and *l-iso*methadone are, mg. for mg., equivalent to morphine (AD 90 per cent. = 7 to 9 mg. /150 lb.) in analgesic power. *dl-iso*Methadone (AD 90 per cent. = 25 to 30 mg./150 lb.) is one-third as potent as morphine in analgesic power. *l*-Methadone (AD 90 per cent. = 4 to 6 mg./150 lb.) has approximately twice the analgesic potency of morphine and of *dl*-methadone. This suggests that the lævo-rotatory isomer has virtually all the analgesic power of the racemate and that the dextro-rotatory isomer is inactive. The reason that *l-iso*methadone has three times the analgesic power of its racemic form is not clear, but one possible explanation is that *d-iso*methadone has an anti-analgesic action. G. R. B.

Quinidine Gluconate; an Intramuscular Preparation of Quinidine. S. Bellet and J. Urbach. (*J. Lab. clin. Med.*, 1949, **34,** 1118.) Quinidine gluconate, $C_{20}H_{24}N_2O_2.C_5H_6(OH)_5COOH$, contains 62·3 per cent. of anhydrous quinidine and 37·7 per cent. of gluconic acid. It occurs as a white,

dextrorotatory powder, soluble 1 in 9 parts of water, giving a neutral or slightly alkaline solution. The solution is stable, and non-irritant. Observations on 15 patients showed that the quinidine effect appears in 15 minutes after a dose of 5 to 7½ gr. Absorption by the intramuscular route is certain, uniform and relatively safe, and its use is suggested for those patients in whom a rapid effect is required or in whom oral administration is not practicable. S. L. W.

Thiomerin, Clinical Studies on. I. W. Winik and R. B. Benedict. (*J. Lab. clin. Med.*, 1949, **34**, 1254.) Thiomerin, the di-sodium salt of N(γ-carboxymethyl-mercaptomercuri-β-methoxy)propyl camphoramic acid, is a new mercurial compound which has been shown by animal experiments to be much less toxic to the heart than the commonly used mercurial diuretics. In 70 trials carried out on 36 patients suffering from congestive heart failure with considerable œdema, the diuresis obtained after the subcutaneous injection of 2 ml. of thiomerin was comparable with, or greater than, that noted after the intramuscular use of equivalent amounts (0·08 g. of mercury) of mercurophylline or mersalyl-theophylline in 29 comparative studies on 21 patients. In some patients the diuresis with thiomerin was more sustained. The diuresis resulted in weight losses ranging from 2 to 17½ lb., the range of weight loss being roughly proportional to the amount of œdema present. The diuretic effect usually appeared within several hours after the injection and lasted for 24 hours or more. No systemic toxic effects were observed, and no kidney damage, as judged by urine analyses, occurred. Local reactions were absent or insignificant. S. L. W.

BACTERIOLOGY AND CLINICAL TESTS

***p*-Aminosalicylic Acid and Salicylic Acid, Antagonism Between, Effects of, on *Mycobacterium tuberculosis*.** G. Iványovics. (*Proc. Soc. exp. Biol., N.Y.*, **70**, 462.) A freshly prepared solution of the sodium salt of *p*-aminosalicylic acid was added to a liquid medium containing 0·05 per cent. of Tween 80 and 0·5 per cent. of bovine albumin fraction. Serial dilutions by a factor of two were made. The tubes were inoculated with 0·0001 mg. (dry weight) of tubercle bacilli grown in Dubos medium and the total volume made up to 5 ml. with water. After 16 days' incubation at 37°C., 0·5 ml. of phosphate buffer, *p*H 7·0, containing 2 per cent. of formaldehyde and 0·1 per cent. of Tween 80 was added to each tube and the amount of tubercle bacilli was determined by turbidimetric readings in a Leitz universal colorimeter. Using a control culture as reference, the growth rate of bacilli in the presence of antiseptics was expressed in percentages of the full growth. Comparisons of the effect of *p*-aminosalicylic acid alone and in combination with sodium salicylate showed that the tuberculostatic action of 1 mol. of *p*-aminosalicylic acid is antagonised by 4 to 12 mols. of sodium salicylate. This effect of salicylate was shown to be highly specific and not shared by related compounds. Pantothenic acid does not inhibit the tuberculostatic effect of salicylate. E. N. I.

Chloramphenicol: Inhibition of Bacterial Esterases. G. N. Smith, C. S. Worrel and A. L. Swanson. (*J. Bact.*, 1949, **58**, 803.) Studies on the inhibitory action of chloramphenicol on phosphatases, nucleases and oxidising enzymes suggested that the antibiotic might act by inhibiting the metabolism of fats and related organic acid esters. Such action might involve either the hydrolysis of the fat or ester, or the utilisation of the acid and alcohol resulting from the hydrolysis. The inhibitory effect of the antibiotic was investigated on *Escherichia coli* esterase, crystalline horse

liver esterase, and the esterase activity of mitochondria. In the main compartment of a Warburg vessel were placed 0·5 ml. of 0·2 M tributyrin emulsified with 2 per cent. of acacia in 0·025 N sodium bicarbonate buffer equilibrated with carbon dioxide to give *p*H 7·40 at 38°C., 2 ml. of the sodium bicarbonate buffer and 0·5 ml. of chloramphenicol solution, the concentration varying from 0·01 μg./ml. to 3 mg./ml. One side arm contained 0·5 ml. of enzyme solution; the other contained 0·5 ml. hydrochloric acid, which was used to determine the amount of carbon dioxide retained by the solutions when testing crude enzyme preparations. With chloramphenicol concentrations in the final solution of 1 to 3 μg./ml. the enzymatic activity of living *E. coli* cells was decreased by up to 25 per cent., whereas at higher concentrations (3 to 50 μg.) there was marked stimulation of activity, the increase being from 50 to 80 per cent. Beyond 50 μg./ml. enzymatic activity was almost completely inhibited. Essentially similar results were obtained with horse liver esterase, but the inhibition at low concentrations and the stimulation at high concentrations were less marked. With mitochondria the drug inhibited only 40 to 50 per cent. of the esterase activity and there was no inhibition at high concentrations, suggesting that some barrier exists preventing reaction between the drug and the esterase within the animal cell. The effect on esterase activity of *E. coli* cells was in close agreement with the effects of the various concentrations on growth and this may provide a clue to the bacteriostatic action of the antibiotic.

H. T. B.

Iodonium Compounds, Bactericidal Efficiency of, against Gram-negative Bacteria. L. Gershenfeld and C. Kruse. (*Amer. J. Pharm.*, 1949, **121**, 343.) Three compounds were tested namely bis(methylphenyl)iodonium chloride (I), bis(3:4-dichlorophenyl)iodonium chloride (II), and bis(2:4-dichlorophenyl)iodonium sulphate (III), the solubility in water being for each compound about 0·1 per cent. The Gram-negative organisms employed were *Shigella alkalescens, Proteus vulgaris, Pseudomonas æruginosa, Klebsiella pneumoniæ, Salmonella enteritidis* and *Neisseria catarrhalis.* Bacteriostatic efficiencies were investigated by the U.S. Food and Drug Administration agar plate procedure and bactericidal efficiencies by the F.D.A. phenol coefficient technique using in each case dilutions of the saturated solutions. The three compounds had approximately the same bacteriostatic activities, being most effective against *Klebsiella pneumoniæ* and least effective against *Ps. æruginosa.* Compound II had the strongest bactericidal action, its effect being demonstrable in 1:20 dilutions of the saturated solution. Compounds I and III only showed bactericidal action when used in the saturated solutions.

H. T. B.

Penicillin and Sulfactin: Drug-fastness Studies with *Staphylococcus aureus*. H. E. Morton and M. J. B. Perez. (*Proc. Soc. exp. Biol., N.Y.*, 1948, **69**, 26.) Sulfactin, an antibiotic produced by a species of *Actinomyces,* resembles penicillin in being active against Gram-positive organisms. Cultures of *Staphylococcus aureus* resistant to sulfactin and others resistant to penicillin were developed *in vitro* by growing the organisms in increasing concentrations of the respective antibiotics. The sulfactin-resistant strain developed a resistance to the drug more than 1,100 times that of the original strain. Under similar conditions, the resistance of the organism to penicillin increased slightly more than 32-fold. In becoming resistant to sulfactin the strain showed no increase in its resistance to penicillin, and in becoming resistant to penicillin there was no increase in resistance to sulfactin.

H. T. B.