ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Charcoal, Determination of Adsorptive Power of. R. Charonnat and J. Leclerc. (*Ann. pharm. franc.*, 1949, **7**, 625.) Differences between the adsorptive power of different samples of charcoal are least marked when the concentration of the substance to be adsorbed is either very high or very low. The most favourable character for differentiation is the concentration of solution from which the adsorbent takes up one half of the dissolved substance. For the determination, 100mg. of the charcoal is shaken for 30 minutes with 10 ml. of a solution of iodine, the mixture is centrifuged, the residue is washed with a little water, and the iodine in the solution is determined by titration. From the results of a series of determinations, the concentration of 50 per cent. adsorption is determined by interpolation. Some results obtained are as follows: graphite 0·004N; vegetable charcoal 0·020N; animal charcoal 0·082N; activated charcoal A 0·13N; activated charcoal B 0·235N. G. M.

Glucose, Microdetermination of. M. Herbain. (*Bull. Soc. Chim. biol.*, 1949, **31**, 1104.) A solution of glucose containing 5 to 25μg. in 2ml. is treated with 2ml. of ferric-ferrocyanide reagent and 2ml. of water and is heated for 15 minutes in a boiling water-bath followed by 10 minutes at 20°C. Phospho-ferric reagent is added to a volume of 10ml. and, after mixing, the solution is kept at 20°C. for 1 hour before the colour is measured in a suitable spectrophotometer; a blank determination is taken through the whole procedure. The ferric-ferrocyanide reagent consisted of potassium ferricyanide (0·03g.), potassium ferricyanide (0·165g.) and anhydrous sodium carbonate (2·0g.) dissolved in water to 250ml.; the phospho-ferric reagent consisted of potassium ferricyanide (0·2g.) and phosphoric acid (85 per cent., 23·5g.) dissolved in water to 500ml. Detailed results are given of the development work carried out in the selection of the conditions of the reaction and on the composition of the reagent in order that quantitative results could be obtained. R. E. S.

Oxalate, Colorimetric Determination of. S. Burrows. (*Analyst*, 1950, **75**, 80.) A number of colorimetric methods for the routine determination of calcium oxalate in unknown composts were found to be unsatisfactory and an absorptiometric method based on the fading effect of oxalates on the green complex of tervalent iron and 7-iodo-8-hydroxyquinoline-5-sulphonic acid (ferron) was shown to be suitable. The ground compost is extracted with citric acid solution containing calcium chloride and saturated with calcium oxalate to remove the iron and most of the phosphate and colouring matter. The residue is extracted with hydrochloric acid (0·4N), filtered, and a buffered ferric chloride-ferron solution is added, the light absorption of the solution being measured spectrophotometrically using Ilford filters No. 607; the amount of oxalate is calculated by using a standard curve prepared using solutions containing between 0 and 4mg. of calcium oxalate. The reproducibility obtained (± 4 per cent. in samples containing about 1 per cent. of calcium oxalate) was considered satisfactory in view of the rapidity of the method, although the recovery of added calcium oxalate amounted to only 90

per cent. The test solutions for absorption measurements were stable in the dark for a number of hours, but fading occurred on exposure to sunlight; this fading was related quantitatively to the amount of oxalate added. R. E. S.

Phenylbarbiturates, Detection of. E. Rathenasinkam. (*Analyst*, 1950, **75**, 108.) Three phenyl barbiturates, phenobarbitone, methylphenobarbitone, and 5-methyl-5-phenylbarbituric acid were examined. The barbiturate (about 1·0mg.) was heated with potassium nitrate (200mg.) and sulphuric acid (20 drops) in boiling water for about 20 minutes. After cooling and diluting with water to about 30ml., the mixture was transferred to a separating funnel and extracted first with 30ml. of chloroform, and then with 30 ml. of ethyl ether. Each extract was washed and evaporated separately, the residue being dissolved in acetone (2ml.), and 1 drop of sodium hydroxide solution (50 per cent.) added; after shaking and allowing to stand, the three compounds examined showed colours ranging from blue to purple in the chloroform or ether extracts. R. E. S.

Phenylephrine (Neo-synephrine), in Pharmaceutical Products Colorimetric Determination of. M. E. Auerbach. (*J. Amer. pharm. Ass., Sci. Ed.*, 1950, **39**, 50.) 5 ml. of a solution containing about 25 μg. of phenylephrine in water or alcohol is mixed with 1 ml. of a 5 per cent. solution of borax and 0·5 ml. of diazo reagent. After 10 minutes 1 ml. of 10 per cent. sodium hydroxide is added, the mixture diluted to 10 ml. with water, and the percentage transmittancy measured at 495 mμ. The content of phenylephrine is read from the straight line obtained by plotting percentage transmittancy against concentration for a number of standard solutions similarly treated. The diazo reagent is prepared by adding 0·5 ml. of a 7 per cent. solution of sodium nitrate to an ice-cold solution of 30 mg. of *p*-nitroaniline in 2 ml. of 6N hydrochloric acid, diluting after 2 minutes with 100 ml. of cold water and adding 1 ml. of sulphamic acid; it should be freshly prepared. Penicillin, amethocaine, chlorbutol, procaine, quaternary ammonium germicides and dyes in the proportions normally used in elixirs do not interfere, whereas sulphathiazole and marfanil do. G. R. K.

Procaine, Sulphanilamide and Related Compounds, Determination of. K. R. Srinivasan. (*Analyst*, 1950, **75**, 76.) Satisfactory results are obtained in such determinations by direct titration with bromate and bromide in acid solution, since the reaction stops at the stage of dibromo-substitution. The difficulty in the determination of the end-point is obviated by the use of a pair of polarised platinum electrodes; a sharp end-point is obtained, the slightest excess of bromine in the solution at once depolarising the cathode and causing a large deflection of the galvanometer. An aliquot containing about 5mg. of a sulphonamide is taken, potassium bromide solution and diluted hydrochloric acid are added and the electrodes are dipped into the solution and connected up in circuit; the solution is then titrated with the potassium bromate solution (0·01N) the end-point being indicated by a permanent deflection of the galvanometer from the zero position. Details are given of the preparation of the electrodes and of obtaining the polarising voltage of 10 millivolts. Satisfactory recovery results were obtained for procaine hydrochloride, benzocaine, sulphanilamide, sulphapyridine, sulphaguanidine, sulphathiazole and sulphadiazine. In the determination of procaine penicillin the procaine is freed from the penicillin by extraction with chloroform after liberating the free base with ammonia; the procaine is taken up in

hydrochloric acid from the chloroform solution and the acid solution is then titrated with standard bromate. R. E. S.

Reducing Sugars, Colorimetric Determination with Triphenyltetrazolium Chloride. A. M. Mattson and C. O. Jensen. (*Anal. Chem.*, 1950, **22,** 182.) A method has been worked out for the determination of reducing sugars based on the reduction of triphenyltetrazolium chloride to triphenylformazan which is red and water-soluble. The solution under test in a dry flask is maintained at 25°C., in a water-bath for 10 minutes together with a blank consisting of distilled water. N sodium hydroxide is added to each flask, the solutions are mixed by swirling and returned to the water-bath for 6 minutes; triphenyltetrazolium chloride solution (0·5 per cent., in water) is added to each of the flasks which are then heated for a further 30 minutes in the water-bath. Acidified pyridine (15ml. of hydrochloric acid per 100ml. of pyridine) is added and the light absorption of the clear red solution is taken at 490mμ. on a photoelectric colorimeter; the sugar content can be obtained from a standard curve previously prepared. The graph of optical density and sugar concentration was a straight line, the average slope of the lines being determined for each sugar; values are given for lactose, glucose, fructose and invert sugar. Milk samples, after clarification with lead acetate and ammonium oxalate, were analysed by the above method and by that of Quisumbing and Thomas; the results obtained showed good agreement. The determination of glucose and fructose in mixtures could be accomplished by estimating the total sugars as glucose by the tetrazolium method (S) and by the Quisumbing and Thomas method (S′) and then applying the equation $(S - S') = AF$ where A is a constant (found experimentally to be 6·48) and F is the percentage of fructose. The method was applied to the analysis of honey. R. E. S.

Reducing Sugars, Estimation of by Ferricyanide. M. Plumel. (*Bull. Soc. Chim. biol.*, 1949, **31,** 1163.) A study has been made of the principal factors affecting the oxidation of glucose in an alkaline medium and a method was devised suitable for the determination of glucose in blood. The blood sample (0·1ml.) in a centrifuge tube is diluted with a little water (3·4ml.) and 1ml. of ferric sulphate reagent (2g. of anhydrous ferric sulphate and 25ml. of N sulphuric acid diluted to 100ml. with water) is added followed by N sodium hydroxide (0·5ml.). The supernatant liquid after centrifuging is colourless and slightly acid (*p*H 6·0); 2ml. aliquots are taken for the actual determination, 2ml. of water is added and 2ml. of alkaline potassium ferricyanide solution. The mixture is heated in a boiling water-bath for 8 minutes, cooled, and acid ferric sulphate solution is added followed by centrifuging and the addition of acid sodium fluoride. The precipitate redissolves and the resulting colour is measured spectrophotometrically, the amount of glucose present being determined from a calibration graph prepared previously. R. E. S.

Saponins, Hæmolytic Assay of. W. Awe and H. Häussermann. (*Arch. Pharm., Berl.*, 1950, **283,** 7.) Comparison of the saponin content of vegetable extracts by means of the hæmolytic index is often unsatisfactory on account of the difficulty in determining the end point owing to turbidity in the extracts. This difficulty may be avoided by determining the "Index hæmolyticus initialis" (HI_1), which is defined as the minimum concentration at which the hæmolytic tubes show a reddish-yellow coloured liquid zone above the red blood corpuscles; it can be detected even in slightly turbid liquids.

A comparison was made of the results of this test with determinations of the surface tension, for extracts of senega root and of horse chestnut. Within certain limits the two sets of figures were found to run parallel for each extract. The determination of surface tension is subject to a smaller range of error than that of hæmolytic index, and it would therefore appear that a determination of the former (i.e. measurement of drop size) would be the simplest method of comparison of saponin extracts derived from a single drug. The method would be invalidated by adulteration with surface-active (wetting) agents, but this objection also applies to the hæmolytic method, more especially as such agents, like saponins, are precipitated by cholesterol.

G. M.

INORGANIC CHEMISTRY

Sodium Hydroxide, Carbonate-free, Preparation of. C. W. Davies and J. H. Nancollas. (*Nature*, 1950, **165**, 237.) Rather more than the required amount of the alkali is made up from washed sticks, and a column of the resin Amberlite IRA-400, which may be initially in the form of its chloride, is prepared in the usual way. The capacity of the air-dry resin is 1·4 milli-equivalents/g., and a 50-ml. tube $\frac{2}{3}$ full of the resin would be suitable for preparing 1 l. of 0·1N sodium hydroxide. The alkali is passed through the resin until the effluent is chloride-free (if necessary), and the tube is then transferred to the neck of the stock bottle. When the rest of the alkali has passed through, the resin column is replaced by a soda lime guard tube, and the carbonate-free sodium hydroxide is ready for standardisation. The column can be easily regenerated by the passage of hydrochloric acid, which destroys the carbonate, and the subsequent replacement of the chloride by hydroxide.

S. L. W.

ORGANIC CHEMISTRY

Adrenochrome and Ephedrine, "Biuret reaction" of. P. Bouvet. (*Ann. pharm. franc.*, 1949, **7**, 640.) Both adrenochrome and ephedrine give, apparently, a "biuret reaction" with copper salts in presence of strong alkali. The coloured compounds produced in these two cases were isolated and examined. In the case of adrenochrome, the compound was an organic substance of green colour, containing no copper, and soluble in alkalis to give a violet solution. The compound from ephedrine, separated by extraction with light petroleum from alkaline solution, formed violet crystals of m.pt. 180°C. (with decomposition). It differs from the biuret compounds by its lack of acid properties. A suggested graphic formula is given. G. M.

Glycerophosphates, Stability of. E. Bamann, E. Nowotny and E. Heumüller. (*Arch. Pharm., Berl.*, 1950, **283**, 4.) In testing for free phosphate in glycerophosphates, using nitro-molybdate, a slight positive reaction is permitted in the case of the ferric salt. This is because this salt is much more easily hydrolysed by acid than are those of sodium or calcium. The salts of aluminium and of zinc are also comparatively readily hydrolysed, and there is also a difference between the α and β salts. The β isomer of the cerium salt was found to be hydrolysed twice as quickly as the α isomer.

G. M.

Steroids, Nomenclature of. W. Klyne. (*Nature*, 1950, **165**, 313.) The nomenclature of the cardiac aglycones, toad poisons and steroid sapogenins is unsatisfactory. New names should be introduced for a few fundamental structures and the naturally occurring substances, and their derivatives named as substitution products of these. Thus, most cardiac aglycones are derivatives of the 14-*iso*-*nor*cholenic acid lactone I (lactone structures I to IX are set out in the text), which might be called digitenolide—indicating the source of the compounds and their unsaturated lactone character. Digitoxigenin would then be named 3β : 14β-dihydroxydigitenolide, and strophanthidin 3β : 5β : 14β-trihydroxy-19-aldo-digitenolide. Toad poisons are derivatives of the 14-*iso*-choladienic acid lactone structure V, which might be called bufadienolide. Thus bufalin would be 3β : 14β-dihydroxybufadienolide. The names of the steroid sapogenins might be based on the name steroketal VI, so that sapogenin would be steroketal, and sarsasapogenin would be 3β-hydroxysteroketal. Compounds in which both oxide rings have been opened might be best named as derivatives of coprostane or cholestane, for example, tetrahydrosarsasapogenin =coprostane—3β : 16β : 26-triol. S. L. W.

TOXICOLOGY

Arsenic in the Body, Distribution of. V. Brustier, P. Bourbon and R. Vignes. (*Ann. pharm. franc.*, 1949, **7**, 729.) Samples of blood and hair from patients suffering from arsenical polyneuritis, were examined at intervals, with the following results:—

Case	Blood mg. per cent.	Hair mg. per cent.	Date of Sampling
C	0·108	7·1	18.6.48
	0·04	18·2	5.7.48
M	—	2·77	22.10.48
	0·08	9·5	5.11.48
	0·02	0·38	11.2.49
N	0·13	0·12	11.2.49
	0·08	0·04	19.3.49
	0·08	0·04	26.4.49
L	0·025	0·7	30.11.48
	0·024	0·6	23.4.49

These figures suggest that arsenic only passes from the blood into the hair when the arsenæmia has reached a certain value. In toxicological investigation, the blood level should be a better guide to the probable date of ingestion of the arsenic than the hair, since the latter may have been cut in the interval. The localisation of arsenic in the hair is not solely a function of the time, but depends on the degree of arsenæmia. Consequently an estimate of the probable date of commencement of poisoning, based on the assumption of continuous and regular passage into the hair, and on the determination in different parts of the latter, is very liable to error. G. M.

Bismuth, Toxicological Determination of. R. Castagnou, P. Cazaux and P. David. (*Bull. Trav. Soc. Pharm. Bordeaux*, 1949, **87**, 106.) In solutions obtained by wet combustion, with nitric and sulphuric acids, of organic matter, bismuth may be determined directly by means of hypophosphorous acid, although concentrations of sulphuric acid greater

than 25 per cent. produce a reduction in the sensitivity of the reaction. The determination is carried out nephelometrically, after heating 10 ml. of the solution under examination with 2ml. of hyphosphorous reagent on the water-bath for 30 minutes.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Adrenaline, Oxidation of, by Halogens and Iodic Acid. P. Bouvet. (*Ann. pharm. franc.*, 1949, **7**, 721.) The oxidation of adrenaline by halogens is incomplete, as the formation of acid prevents the continuation of the reaction. In the presence of calcium carbonate, iodine reacts quantitatively, producing adrenochrome. The same result is obtained with bromine in theoretical quantity, but in this case an excess reacts further with production of brominated derivatives. In weak acid solutions, iodic acid also gives adrenochrome, but in presence of mineral acid there is an immediate and quantitative production of iodo-adrenochrome. For the latter reaction it is necessary to have an excess of 6 molecules of iodic acid for 1 of adrenaline.

G. M.

Heparin Sodium, Ultraviolet Absorption of. F. K. Bell and J. C. Krantz, Jr. (*J. Amer. pharm. Ass., Sci Ed.*, 1950, **39**, 95.) The ultraviolet absorption of 15 preparations of heparin sodium of varying potency was measured in a Beckmann ultraviolet spectrophotometer equipped with a hydrogen tube as the source of radiation. The absorption spectra of the low and medium potency preparations showed no consistent relation to the anti-coagulant activity. The high potency preparations gave curves of a recumbent S-shape when optical density was plotted against wavelength, consisting of a region of transmission followed by one of absorption at the higher wavelengths. The lack of any commonly recognised chromophore group in proposed heparin structures suggests that this absorption pattern in the high potency preparations is due to an impurity or to an unidentified part of the heparin molecule.

G. R. K.

Polyhydric Alcohols, Chromatographic Separation of. L. Hough. (*Nature*, 1950, **165**, 400.) The behaviour of a number of polyhydric alcohols in paper partition chromatography has been examined using ammoniacal silver nitrite to develop the spots. The R_F values were determined on Whatman No. 1 filter paper in various solvents using the normal procedure for sugar analysis. Small circular spots of aqueous solutions of the alcohols (approximately 2·5 per cent w/v) were put on the paper strip; much larger amounts of glycerol and ethylene glycol could be used, however, small spots of the syrup giving excellent results. A solution of silver nitrate (5 per cent.) to which ammonia (sp.gr. 0·88) had been added in excess was used for developing the paper chromatogram. Ethylene glycol moves rapidly on the paper chromatogram and is readily separated from glycerol, which in turn, is easily distinguished from the hexitols, which travel relatively slowly. The R_F values of trimethylene glycol, ethylene glycol, glycerol, α-methyl galactoside, α-methyl mannoside, β-methyl maltoside, sorbitol, dulcitol, mannitol, inositol and sucrose are given for *n*-butyl alcohol-water; for *n*-butyl alcohol 4·0, ethyl alcohol 1·1, water 1·9; for *n*-butyl alcohol 4, ethyl alcohol 1, water 5; for benzene 1, *n*-butyl alcohol 5, pyridine 3, water 3; and for *n*-butyl alcohol 5, acetic acid 1, water 2. Ammoniacal silver nitrate

will detect as little as 1μg. of a polyhydric alcohol, 10μg. of ethylene glycol, and 10μg. of sugar glycosides such as α-methyl galactoside, α-methyl mannoside and β-methyl maltoside; sucrose also reduces the reagent under these conditions. R. E. S.

BIOCHEMICAL ANALYSIS

***p*-Aminosalicylic Acid, Determination of.** A. L. Tarnoky and V. A. L. Brews. (*Biochem. J.*, 1949, **45**, 508.) A method for the estimation of "total *p*-aminosalicylate" in capillary blood is described in which *p*-aminosalicylic acid and its *N*-acetyl derivatives are quantitatively converted to *m*-aminophenol; this compound gave deeper colours than *p*-aminosalicylic acid when diazotised and coupled with *N*-1-naphthylethylenediamine. In the details given oxalated or freshly drawn capillary blood (0·2 ml.) is added to water (3·2 ml.) and allowed to stand for 30 minutes, trichloroacetic acid (20 per cent., 0·6 ml.) is added, the mixture is shaken, centrifuged and the supernatant liquid (2·0 ml.), transferred to a centrifuge tube graduated at 4 ml., is heated with sulphuric acid (21·5 ± 0·5 N., 0·5 ml.) in a boiling water bath for 1 hour. The solution is cooled in tap water partially neutralised with sodium hydroxide (8·1N, 1·0 ml.) and again cooled, water being added to adjust the volume to 4·0 ml. Sodium nitrite solution (1 per cent., 0·2 ml.) is added, the solution is shaken and after 5 minutes ammonium sulphamate solution (5 per cent.) is added, followed 20 ± 1 sec. later by naphthylethylenediamine hydrochloride solution (1 per cent. in water, 1·0 ml.) the tube being shaken after each addition. The pink colour is allowed to develop for at least 2·5 hours and is then compared with previous colour standards. In a modification of this method ethyl alcohol (95 per cent., 1·0 ml.) is added 20 ± 1 sec. after coupling with the naphthylethylenediamine solution, the tubes are shaken and the colours compared after 1 hour. The colours given in both cases follow Beer's Law at concentrations from 2·5 to 20 mg./100 ml. using an Ilford 625 filter. Graphs of the absorption spectra of the dyes obtained from equimolar solutions of *p*-aminosalicylate after heating and of *m*-aminophenol were identical; unheated solutions of *p*-aminosalicylate develop colour more rapidly, producing a dye with lower extinction values and an absorption maximum at a shorter wavelength. In estimations carried out on fresh oxalated blood to which a known amount of *p*-aminosalicylate had been added mean recoveries of 96 per cent. (range 89 to 102) on 17 experiments, and 98 per cent. (range 91 to 102) on 8 experiments were obtained. R. E. S.

Citric Acid, Micro-estimation of. P. Cartier and P. Pin. (*Bull. Soc. Chim. biol.*, 1949, **31**, 1176.) A study has been made of the pentabromacetone method of determination of citric acid; the conditions of the oxidation, of the bromination, and of the colour estimation, were examined and varied until the best results were obtained. Preliminary treatment is performed with trichloracetic acid solution (10 per cent. for powdered bones, 20 per cent. for other tissues) so that the filtrate contains between 100 and 1000μg. of citric acid. The extract is taken in a test tube, sulphuric acid and bromine water are added, the mixture is heated in a boiling water-bath for 10 minutes, and then cooled and diluted. Bromide-bromate solution is added followed by potassium permanganate (1·5N) drop by drop; after vigorous shaking the tube is stoppered and allowed to stand for 30 minutes

at room temperature when hydrazine sulphate solution (10N) is added gradually until decolorised. The pentabromacetone is extracted by shaking twice for 3 minutes with light petroleum, washing each extract with water; an aliquot of the light petroleum layer is taken in a tube protected from light and 3ml. of a solution of sodium iodide, 10 per cent. in alcohol (96 per cent.), is added. After shaking for 30 seconds and allowing to stand in the dark for 70 minutes, the light petroleum is removed and the intensity of the yellow colour in the aqueous phase is measured with a suitable spectrophotometer, the amount of citric acid being deduced from a standard curve previously prepared. Using the technique described it is possible to estimate 100 to 1200μg. of citric acid in tissues and biological fluids with an accuracy of the order of ±2 per cent. R. E. S.

Glucose in Blood, Microdetermination of. P. Castaigne. (*Bull. Soc. Chim. biol.*, 1949, **31**, 1184.) A method has been investigated for the determination of small quantities of glucose in blood using the reducing action of glucose on potassium ferricyanide followed by spectrophotometric estimation of the ferrocyanide formed by means of the prussian blue reaction under specified conditions. The sample of blood (0·02ml.) is added to one of two tubes each containing 2ml. of copper sulphate solution (7 per cent.). After shaking and allowing to stand for 5 minutes, 0·4ml. of sodium tungstate solution (1·4 per cent.) is added to each tube with shaking, followed by 0·1ml. of barium chloride solution (1 per cent.). The tubes are shaken for 5 minutes and centrifuged and 1ml. of the clear supernatant liquid in each case is pipetted into a 10ml. graduated tube and 2ml. of Herbain's ferric-ferrocyanide reagent is added with 3ml. of distilled water. After shaking, the tubes are left for 15 minutes in a boiling water bath and the determination is completed as described previously (*Bull. Soc. Chim. Biol.*, 1949, **31**, 1104); the colour is measured in a suitable spectrophotometer, the amount of glucose present being calculated from a standard curve constructed previously. The results obtained on normal subjects generally averaged from 0·7 to 0·9 g./l. The concentrations of glucose were studied for 2 days at varying times using the above method as well as that of Baudouin and Lewin as modified by Fleury and Marque; good agreement between the two methods was shown.

R. E. S.

Iodine in Blood, Micro-estimation of. F. Lachiver and J. Leloup. (*Bull. Soc. Chim. biol.*, 1949, **31**, 1128.) A method is described for the determination of iodine present in organic material, in which oxidation is first performed with chromic and sulphuric acids. The iodic acid formed is reduced by phosphorous acid to elemental iodine which is steam distilled in a special apparatus, being absorbed in an alkaline solution on which the determination is made. Where proteins are present, they are precipitated by heating with acetic acid or by the addition of zinc sulphate solution and after 15 minutes in a thermostat at 35°C., ceric sulphate is added. The optical density of the solution is then measured at time intervals of 10, 20, 30 and 40 minutes. After plotting a curve with known amounts of iodine the data can be used to determine the iodine in an unknown sample using an algebraically deduced expression. Detailed results are given showing the variation in colour obtained with temperature and time, with *p*H, on the effect of the addition of sodium chloride, and on the effect of differing concentrations of ceric sulphate. Using this method the recoveries of iodine from potassium iodide, di-iodotyrosine and thyroxin in different sera, varied from

93 to 100 per cent. The method enables quantities of iodine between 0·005 and 0·2μg. to be determined with an accuracy of 3 to 7 per cent. R. E. S.

Mercury, Bismuth, Antimony and Arsenic, in Biological Material, Detection of. A. O. Gettler and S. Kaye. (*J. Lab. clin. Med.*, 1950, **35**, 146.) To a 20 g. sample of finely macerated tissue, stomach contents, or urine, in a 50-ml. Erlenmeyer flask is added 4 ml. of concentrated hydrochloric acid and 10 ml. of water. A spiral is prepared by winding a length of 20 gauge copper wire tightly and closely over a piece of glass rod 10 times. The copper spiral, after washing with alcohol and with ether, is introduced into the material contained in the flask. The contents of the flask are gently boiled for 1 hour and the original volume maintained constant by the addition of 10 per cent. hydrochloric acid (by volume) from time to time. The spiral is then removed and washed with water. A large amount of mercury or bismuth may require a longer heating period. A silvery coating on the spiral may indicate mercury. A dark discoloration may indicate antimony, arsenic, bismuth, selenium, sulphur or tellurium, or any combination of these substances. To the deposit on the copper spiral a series of tests are then applied. First a contact agent is employed to detect and estimate the mercury ; then appropriate solvents are used completely to remove a particular metal or group of metals from the spiral. To the solutions thus obtained appropriate reagents are added to identify each metal. The procedure is so arranged that an estimation may be made simultaneously. Several of the tests employed were found to be sensitive and specific for quantities as small as 0·01mg. S. L. W.

Penicillinase Activity, Determination of. W. S. Wise and G. H. Twigg. (*Analyst*, 1950, **75**, 106.) The rate of production of the extra acidic grouping in penicillin by penicillinase is followed by electrometric *p*H titration using sodium hydroxide. A penicillin solution containing 200 to 300 units /ml. is placed in a beaker in a thermostat at 25° ±0·2°C., and the electrodes of a *p*H meter are inserted. The contents of the beaker are stirred with a stream of carbon dioxide-free air, the solution is adjusted to *p*H 7·8, a known volume of the penicillinase solution is added and, to prevent a lowering of *p*H, sodium hydroxide (0·01N) is added continuously from a burette at such a rate that the *p*H is constant at 7·8. The slope of a graph of burette readings plotted against time, in ml. of 0·01N sodium hydroxide/minute, is a measure of penicillinase activity. Results, showed graphically, indicated that the rate of reaction was constant with respect to time, i.e., independent of the penicillin concentration, but dependent on the enzyme concentration over a wide range. The rate of reaction was dependent on the purity of the penicillin used, very impure samples giving lower rates; commercial penicillins of potency greater than about 1200 units/mg. were generally satisfactory. The purity of the penicillinase used did not affect the determination unless it was so highly buffered that the change in *p*H on formation of the acid group from penicillin was too small to be measured easily; the penicillinase preparation was then dialysed before examination. R. E. S.

Phenadoxone in Urine, Determination of. J. E. Page and H. King. (*Analyst*, 1950, **175**, 71.) A colorimetric method has been devised for determining phenadoxone (DL-6-morpholino-4:4-diphenylheptan-3-one) in urine. The method depends on the reaction of equimolecular quantities of phenadoxone and bromophenol blue to form a toluene-soluble compound. The free base of the drug is extracted into toluene and the toluene extract is shaken with an aqueous solution of the dye buffered at *p*H 4·0. An amount

of dye, equivalent to the weight of drug in the toluene layer, is carried over into the toluene as a yellow compound, which is decomposed by shaking the toluene with aqueous alkali; the sodium salt of the dye enters the aqueous layer and is then determined colorimetrically. Phenadoxone was examined polarographically; in 0·1N potassium chloride it gave a polarographic step with a characteristic peak at $-1{\cdot}75$v., the step height being approximately proportional to the concentration over the range 0·0075 to 0·075 per cent.; the step height was however sensitive to small amounts of surface-active material and appeared at a relatively high potential, so that the method was unsuitable for biological fluids. Interference with the determination of phenadoxone in urine is caused by basic substances that react with bromophenol blue to form a toluene-soluble compound e.g. alkaloids, such as codeine, and synthetic analgesics, such a pethidine and amidone; bacterial growth also leads to the production of basic substances and a small amount of an antiseptic (e.g., mercuric chloride or toluene) must therefore be added to the urine. R. E. S.

Potassium in Biological Materials, Estimation of. N. I. Joukovsky and A. Lowenthal. (*Bull. Soc. Chim. biol.*, 1949, **31,** 1190.) The method adopted consists of ashing the organic material at 350°C., the precipitation of the potassium by silver cobaltinitrite reagent and the colorimetric estimation of the cobalt. A quantity of biological material containing 100 to 400 μg. of potassium in a silica dish is evaporated on a water-bath with 4 volumes of nitric acid followed by heating at 350°C., until a white ash is obtained. The residue is dissolved in 3ml. of water, 2ml. of this solution is mixed with 2ml. of silver cobaltinitrite reagent in a graduated centrifuge tube and after thoroughly mixing, is allowed to stand for half an hour. The reagent is prepared by powdering 22·6g. of cobalt acetate with 16·9g. of silver nitrate, adding to the mixture 30ml. of acetic acid (8ml. of water and 22 ml. glacial acetic acid), followed by sodium nitrite solution (44g. in 80ml. of water); after standing for 14 to 16 hours the mixture is filtered and a current of air is passed through the filtrate for 4 hours. 4ml. of acetone (50 per cent.) is added to the tube which is centrifuged for 10 minutes; the supernatant liquid is decanted, the precipitate is washed twice with 2ml. of acetone (50 per cent.) and dried on a boiling water-bath. After dissolving in 1ml. of nitric acid (7 per cent.), 14 ml. of alcoholic ammonium thiocyanate is added and the colour is measured after 10 minutes in a photoelectric colorimeter using a standard curve prepared previously and making allowance for the reduced volume taken. R. E. S.

Vitamin A in Whale Liver Oils, Determination of. O. R. Braekkan. (*Anal. Chem.*, 1949, **21,** 1530.) Ultra-violet absorption studies were carried out on purified kitol. It gave an absorption curve in the ultraviolet with absorption maximum at approximately 285 mμ with a corresponding $E_{1\text{ cm.}}^{1\text{ per cent.}}$ value of 465 in absolute ethyl alcohol and 469 in isopropyl alcohol; in cyclohexane the absorption maximum was found at 287·5 mμ with $E_{1\text{ cm.}}^{1\text{ per cent.}}$ equal to 461, indicating an approximate purity of 60 per cent. (assuming pure kitol to have $E_{1\text{ cm.}}^{1\text{ per cent.}}$, 290 mμ = 707). Only in strong concentrations could a colour be obtained with activated glycerol dichlorhydrin, giving an $E_{1\text{ cm.}}^{1\text{ per cent.}}$ (555 mμ) value approximately 3. The absence of a consistent curve between 400 and 700 mμ suggested that the colour was caused by small amounts of impurities, rather than by kitol itself. Examination of mixture of vitamin A, kitol and cholesterol

by the glycerol dichlorhydrin reaction (*Ind. Engng. Chem. Anal. Ed.*, 1946, **18**, 570) indicated that neither kitol nor cholesterol interfered. The values obtained in the determination of vitamin A in whale liver oils by using activated glycerol dichlorhydrin were 10 to 30 per cent. lower than those obtained by the spectrophotometric method based on $E^{1\ per\ cent.}_{1\ cm.}$ (325 mμ). The absorption maxima are given for a number of samples of whale oil; the displacement of the peak wavelength ranged from 10 to 17 mμ below 325 mμ. The results obtained indicated that there is considerable irrelevant absorption in addition to that caused by the presence of kitol, but there did not seem to be any interference with the colour reaction: the conclusion is drawn that determinations by the use of activated glycerol dichlorhydrin give the true values of the vitamin A content of whale liver oils.

R. E. S.

CHEMOTHERAPY

Œstrogenic Action and Chemical Constitution in Azomethine Derivatives. H. H. Keasling and F. W. Schueler. (*J. Amer. pharm. Ass., Sci. Ed.*, 1950, **39**, 87.) The assumption that compounds with a large, rigid, relatively inert, fat-soluble molecular structure containing two active hydrogen-bond-forming groups at an optimum distance of 14·5Å apart possess œstrogenic activity was tested by preparing 16 4 : 4′-substituted benzylideneanilines and examining for œstrogenic activity. The compounds comprised all the substitution combinations of the four groups H, CH_3, OCH_3 and OH, and were prepared by reaction between the appropriate benzaldehyde and aniline in ethyl alcohol. Œstrogenic activity, tested by the vaginal smear test, was shown by 4 : 4′-dihydroxy-benzylideneaniline when injected subcutaneously in a total dose of 12·5 mg. and when applied to the vagina in a total dose of 25 μg. A total subcutaneous dose of 25 mg. of each of the other 15 compounds produced no effect. The distance between the hydroxyl groups in the active compound was 14·5Å, in agreement with the general assumption. Other compounds showing the same characteristic distance were the 2 hydroxy-methyl compounds and the dimethyl compound ; these were inactive because the methyl group does not form hydrogen bonds. The methoxy-hydroxy and dimethoxy compounds, which might show activity following hydrolysis in the body to the dihydroxy compound, were apparently precluded from doing so by the more rapid detoxification of the azomethine link. The big difference between the effective subcutaneous and intravaginal doses of the dihydroxy compound is also attributed to this detoxification process. G. R. K.

Neostigmine-like Compounds, Curarising and Anti-curarising Action of. H. F. Chase, B. K. Bhattacharya and E. M. Glassco. (*J. Pharmacol.*, 1950, **97**, 409.) A series of carbonyl congeners of neostigmine were studied by the authors by means of the rabbit head-drop assay for curare. Most of the compounds studied were shown to have two characteristic and opposing actions, a curare-like and an anti-curare action. Two of the compounds, however, were shown to be purely curare-like ; these were the diethylcarbamate of (2-hydroxy-3-cyclohexylbenzyl) trimethyl-ammonium bromide (NU 906) and the diethylcarbamate of (2-hydroxy-3-cyclohexylbenzyl) methyl-piperidinium bromide (NU 911). The curare-like action of these compounds differs from the true curare action in that it is summated with, not antagonised by, neostigmine. This synergistic action raises the possibility that the curare-

like action of neostigmine here is exaggerated by the presence of molecules of a compound of similar structure and that the anti-curare and the curare-like actions may be due to different chemical properties. Even though relatively large amounts of NU 906 and NU 911 were necessary to produce curare-like effects it is possible that compounds of similar structure might be more potent paralysants and yet be as free of objectionable cholinergic side actions. S. L. W.

PHARMACY

GALENICAL PHARMACY

Suppository Excipients, Physiological Evaluation of. R. Charonnat, L. Chevillard and H. Giono. (*Ann. pharm. franc.*, 1949, **7**, 627.) Suppositories, containing 5·5mg. of methyl nicotinate, are administered to guinea pigs and the rise of temperature is measured in the ear of the animal by means of a thermocouple. Measurements are made every 2 or 3 minutes, the results being recorded on a graph. The length of the base of the curve represents the duration of the action; the height corresponds to the intensity, and the area of the curve to the total amount of effect. Mean results, obtained with 5 to 6 animals, showed that glyco-gelatin caused a maximum rise in temperature of 5·65°C. lasting 30 minutes and gave a curve with an area of 65·3 sq. cm. Corresponding figures for cocoa butter were 5·1°C., 20 minutes and 35·7 sq. cm., and for polymerised ethylene oxide 3·4°C., 20 minutes and 26·3 sq. cm. G. M.

NOTES AND FORMULÆ

Methenamine Mandelate (Mandelamine). (*New and Nonofficial Remedies ; J. Amer. med. Ass.*, 1950, **142**, 487.) Methenamine mandelate is obtained by reaction between equimolecular amounts of hexamine and mandelic acid. It occurs as a white, almost odourless, crystalline powder with a sour taste, m.pt. 127° to 130°C., very soluble in water and soluble in alcohol (1 in 10), chloroform (1 in 20) and ether (1 in 30) ; a 1 per cent. aqueous solution has *p*H 4·2 to 4·4. A 0·1 per cent. solution exhibits an ultraviolet absorption maximum at 2576 to 2577Å. It complies with limit tests for halides, sulphate and heavy metals ; the loss in weight when dried over sulphuric acid for 18 hours is not more than 1·5 per cent. Methenamine mandelate contains 50 to 54 per cent. of mandelic acid when titrated with sodium hydroxide using phenolphthalein as indicator, and 46 to 50 per cent. of hexamine calculated with reference to the dry substance. The content of hexamine is determined by refluxing for 15 minutes with diluted hydrochloric acid, adding the resulting solution to a modified Nessler's reagent cooled in ice and allowing to stand for 1 minute, when acetic acid and iodine are added and the excess iodine titrated with sodium thiosulphate. Methenamine mandelate combines the actions of hexamine and mandelic acid and is used as a urinary antiseptic. It is given by mouth in a dose of 0·75 to 1 g. 3 times daily. G. R. K.

PHARMACOGNOSY

***Hyoscyamus niger*, Cultivation of.** R. Laruelle. (*J. Pharm. Belg.*, 1949, **4**, 281.) Experiments with both the annual and biennial varieties were carried out at the experimental station at Lessines. Considerable difficulty in germinating the seeds was encountered; about 30 per cent.

germinated after 3 weeks, 10 per cent. after 6 weeks, 10 per cent. after 2 months and the remainder did not germinate at all. Lvov and Jakovleva state that preliminary freezing favours germination and it is hoped to apply this technique next winter. Freshly collected seeds, sown in August, did not germinate, thus indicating that the power to germinate is not acquired till some time after collection. Annual plants are not a good source of the leafy drug, but produced good yields of seed; probably 10 kg./100 sq.m. could be obtained for commercial purposes. J. W. F.

Quassia and Quassin, Characterisation of. P. Duquénois and O. Colbe. (*Ann pharm. franc.*, 1949, **7**, 660.) The method of the German Pharmacopœia for the characterisation of quassia wood is useless, since all lignified parts give up a proportion of lignin to alcohol, causing a positive reaction. The reaction of the French Codex for quassin is also unsatisfactory, and should be replaced by the following: to 1 mg. of quassin add a crystal of phloroglucinol and 1 micro-drop of concentrated hydrochloric acid; a rose colour appears immediately. The limit of sensitivity appears to vary with different samples of quassin. G. M.

PHARMACOLOGY AND THERAPEUTICS

Analgesics, Pharmacodynamic Effects of. F. P. Luduena and E. Ananenko. (*Arch. int. Pharmacodyn.*, 1950, **81**, 259.) The pharmacodynamic effects of some new synthetic analgesic drugs have been studied on dogs, using morphine for comparison. In the unanæsthetised dog the progressive intravenous or intramuscular administration of *l*-methadone, *l-iso*-methadone, 1-methyl-4-(3-hydroxyphenyl)-4-piperidyl ethyl ketone (WIN 1539), *l*-3-dimethylamino-1 : 1-diphenylbutyl ethyl sulphone (WIN 1161) and morphine first produced sedation, respiratory depression, analgesia, cardiac slowing, miosis and less frequently salivation and defæcation. With high total doses there was hyperexcitability and even convulsions except with morphine. In most of the dogs at this stage tachypnœa, tachycardia and mydriasis were present. With *d*-methadone the same symptoms were observed, except that the respiratory rate was increased. With doses of 1 to 3mg/kg. intravenously the analgesia produced by *l*-methadone, *l-iso*methadone, WIN 1161, or less frequently WIN 1539, was sufficient to permit surgical operations without any other medication. Larger doses were required for *d*-methadone. This degree of analgesia was not obtained with morphine with doses up to 100 mg./kg. In dogs anæsthetised with soluble thiopentone the first dose of 1 to 1·5 mg./kg. of *l*-methadone, *l-iso*methadone or WIN 1539, by intravenous injection, produced a moderate fall of blood pressure, subsequent doses producing a brief fall followed by a rise. In contrast, *d*-methadone in the same doses produced only a fall of pressure both initially and subsequently. S. L. W.

Dibromoprocaine Hydrochloride ; a Radioactive Spinal Anæsthetic. F. Howarth. (*Brit. J. Pharmacol.*, 1949, **4**, 333.) This substance was prepared, by a method described by the author, for the purpose of studying the fate of a spinal anæsthetic with special reference to its ultimate distribution among the tissues of the body and the routes by which it leaves the spinal theca. It was shown that the concentration in the spinal subarachnoid space rapidly declines associated with a rapid rise in urine concentration, the blood

level remaining persistently low. A study of the tissue distribution showed that only the spinal roots show any capacity to concentrate the drug above the level existing in the cerebrospinal fluid at the site of injection. Of the tissues examined only kidney (and urine), and liver (and bile) appear able to concentrate the anæsthetic above the circulating blood concentration. Large variations in time and dose did not produce large changes in the tissue/blood ratios. It was shown that the anæsthetic enters the spinal cord during spinal anæsthesia, though it is improbable that it is able to produce a functional cord transection. Of the various routes of departure of the anæsthetic from the spinal theca, the venous system appeared to be the most important. S. L. W.

Digitalis, Some Extraction Studies on. R. E. King and O. Gisvold. (*J. Amer. pharm. Ass., Sci Ed.*, 1950, **39**, 109.) Aqueous extracts of fresh leaves of *Digitalis purpurea* were prepared by disintegrating the broken leaves in water, heating to 70°C. to coagulate the chlorophyll and proteins and inactivate the enzymes, and filtering. A 2 per cent. extract thus prepared was inactive when assayed by the frog method, but conversion to an alcoholic tincture by evaporation to low bulk, diluting with alcohol and filtering indicated that the original aqueous extract gave 87 per cent. extraction of the activity as compared with a control tincture prepared by the U.S.P. method ; 5 and 10 per cent. aqueous extracts similarly treated represented 78 and 57 per cent. extraction respectively. The active material from fresh leaves may be stored by saturating the aqueous extracts with anhydrous sodium sulphate, filtering and drying the precipitate. When required, the active material can be extracted from the precipitate with butyl alcohol, methyl ethyl ketone or tetrahydrofuran. The active principles may be obtained directly from the aqueous extract by adding tetrahydrofuran, saturating with anhydrous sodium sulphate and preparing a tincture from the aqueous layer which separates. Fresh digitalis leaves appear to retain their potency when stored frozen for 4 months. Attempts to remove the pigments from butyl alcohol extracts by washing with alkalis failed because the active principles were destroyed. The active principles were separated into two fractions from a purified extract by using the selective solvent action of acetone and benzene. One fraction gave a positive Keller-Kiliani test for digitoxin and purpurea glycoside A and the other a positive test for gitoxin, purpurea glycoside B and gitalin. G. R. K.

Digitoxins, Pharmacology of. D. I. Macht. (*Arch. int. Pharmacodyn.*, 1950, **81**, 345.) In view of reports which have appeared in the literature describing difficulties experienced with digitoxin U.S.P. in the management of congestive heart failure, the author conducted an experimental investigation on Digitaline Nativelle and digitoxin U.S.P. Comparisons were made by three types of experiment : (1) phytopharmacological tests on root growth of *Lupinus albus* seedlings, (2) pharmacological measurements after irradiation with X-Rays, (3) colorimetric assays. All three sets of experiments showed a consistent and marked quantitative difference in the pharmacological properties of the two digitoxins, Digitaline Nativelle revealing a greater activity than digitoxin U.S.P. The reason for this difference remains unexplained but it can be stated that it is not due to the presence of digitonin. It was noted that combinations of digitoxins with blood serum give synergistic effects ; here also Digitaline Nativelle was found to be more potent than digitoxin U.S.P. S. L. W.

Dihydrostreptomycin in Procaine-Pectin Solution. F. Zini. (*Acta med. scand.*, 1950, **146**, 209.) The intramuscular injection in man of 0·5 g. of dihydrostreptomycin or streptomycin in a 2 per cent. pectin solution, with 1 per cent. of procaine (buffered at *p*H 7) produces a concentration of the antibiotic

in the serum corresponding, in 50 per cent. of cases, to 17 to 96 S.U. ml. at the 24th hour. When the antibiotic is diluted in pectin solution the sharp fall in the serum concentration in the 2nd and 3rd hour described by many investigators does not occur. The concentration of antibiotic in the serum at the 24th hour was much greater than that in the whole blood. In the urine, inhibition values corresponding to 6 to 8 S.U. were observed 32 hours after a first intramuscular injection of 0·25 g. of the antibiotic in pectin-procaine solution, and the inhibition values in urine specimens examined in hourly fractions of the 24 hours were consistently higher than those found in serum or blood. S. L. W.

Heparin, Intramuscular Administration of. G. Bauer. (*Acta med. scand.*, 1950, **136**, 188.) A study of the clinical course in 16 cases of acute thrombosis indicates that heparin administered intramuscularly, in a dosage of 150mg. 3 times a day does not have the same immediate and satisfactory influence on the thrombotic process as the same dosage given by intermittent intravenous injection. This is due mainly to the fact that after intravenous injection the coagulation time is raised to a much higher level for 1 to 2 hours than after intragluteal injection. Even if intramuscular injection of heparin is easier than intravenous this is offset by the fact that it is more painful and frequently gives rise to large local hæmatomata, and also that it requires larger total amounts of heparin for each patient. Moreover, assessment of the stage of healing of the thrombotic process is more difficult than in cases treated by intravenous injection. Thus, after intramusuclar injections, in several cases patients found it difficult to state clearly whether they felt pain when their deep veins were palpated. Because of this heparin treatment was stopped and the patients allowed to get up without satisfactory evidence that the pathological process was terminated. This may account for the much greater number of recurrences observed in comparison with those observed in cases treated intravenously ; it was also responsible for one fatal case of pulmonary embolism due to insufficient dosage. Intermittent intravenous injections, 4 times daily, constitute the best method of administration of heparin, though the intramuscular route may be employed as an alternative where intravenous injections are impracticable. S. L. W.

Neoarsphenamine, Toxicity Determination by a Method based on Survival Time. M. G. Allmark and D. Lavallee. (*J. Amer. pharm. Ass. Sci. Ed.*, 1950, **39**, 81.) Neoarsphenamine in 15 per cent. aqueous solution was injected into the saphenous veins in the hind legs of rats of either sex and the time of death recorded ; 3 doses of standard and 3 of sample were used in each test, and each dose was given to 5 rats weighing 125 to 150 g. The doses were chosen so that all the rats would die within 10 hours. The method was quicker and more economical than the usual quantal response method. but in 3 of the 15 samples tested it gave the toxicity as equal to that of the standard preparation whereas the quantal response method showed it to be much greater. G. R. K.

Œstrone, Effect of Crystal Size on the Activity of. A. Simond, K. M. Lindquist, F. H. Tendrick and L. W. Rowe. (*J. Amer. pharm. Ass., Sci. Ed.*, 1950, **39**, 52.) Using adult female, ovariectomised rats and the principles of the Allen-Doisy test, the duration of œstrus produced by single massive subcutaneous injections of aqueous suspensions of œstrone crystals of varying size was determined for individual animals in small groups and then

(*Continued on page* 608)