ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ANALYTICAL

Aspirin, Phenacetin and Caffeine, Analysis by Partition Chromatography. T. Higouchi and K. P. Patel. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 171.) A solvent combination of water and a nonpolar solvent was used, since caffeine has a definite affinity for water and considerably less affinity for nonpolar solvents such as ethers, whereas the contrary is true for phenacetin. Water was used as the stationary phase and the elution curve showed that it was possible to obtain a clean separation of the two components. Silica gel was used for the column and a chloroform solution containing phenacetin and caffeine was added. Elution with a 75:25 di*iso*propylether-chloroform mixture removed the phenacetin, the caffeine component which remained on the column being then stripped out by elution with chloroform. Recovery data for both constituents were satisfactory. If aspirin was present in the samples it was removed first by extraction of the chloroform solution with a mildly alkaline solution, the aspirin being determined separately. Typical analyses of commercial preparations are reported. R. E. S.

Atropine, Photometric Determination of. A. Romeike. (*Pharm. Zentralh.*, 1952, **91**, 80.) The vegetable material is dried at 60° to 70° C., and powdered. A weighed portion of the powder is moistened with ammonia solution (5 per cent.), transferred to a Soxhlet thimble, and extracted for several hours with chloroform. The extract is concentrated to about 15 ml. and shaken out twice with 5 ml. quantities of hydrochloric acid (0·4 per cent.). If the amount of alkaloid expected is very small, the amount of acid should be reduced to one-half. The alkaloid is precipitated with 3 ml. of 1·7 per cent. solution of sodium silicomolybdate, allowed to stand for 24 hours, filtered on a sintered glass filter, and washed with small quantities of 0·5 per cent. hydrochloric acid containing 1 per cent. of sodium chloride. The residue is dissolved in 10 ml. of reduction solution (glycine, 0·5; sodium sulphite, 1·5; water 15; 5 per cent., ammonia, 83) and made up to a definite volume. The absorption is then determined using filter S72. The standardisation graph is straight between 61 and 40 per cent. absorption, and the minimum concentration of atropine at which a satisfactory determination is possible is 7·7 mg./100 ml. Seeds should be defatted with petroleum ether, after drying and powdering. G. M.

Heavy Metals in Pharmaceutical Chemicals. F. N. Stewart and C. W. Strode, Jr. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 242.) Heavy metals are determined as sulphides by colour measurements under standardised conditions with compensation for sample colour and adjustment of the *p*H to 3 to 4 before addition of hydrogen sulphide, acetone being used to decrease the solubility of metal sulphides, while increasing that of some of the organic compounds to be tested. 50 ml. of the test solution is placed in each of 2 graduated cylinders, 25 ml. of acetone is added and the *p*H adjusted to 3·5. To one cylinder is added 20 ml. of a saturated solution of hydrogen sulphide, and the volume in each cylinder is adjusted to 100 ml. by the addition of water. After 10 minutes, the

light absorption of the solutions is compared, using a photoelectric colorimeter and a 420 mμ broad band filter. The content of heavy metals, calculated as lead, is found from a curve prepared by similarly treating quantities of U.S.P. standard lead solution and plotting the light absorption against lead content. For weak acids, the test solution is prepared by dissolving a sample in 50 ml. of acetone and adding sufficient water to produce 100 ml. Water-soluble neutral compounds are dissolved in 40 ml. of water, 8 ml. of acetic acid (6 per cent.) and 50 ml. of acetone added, and made up to 100 ml. with water. Salts of weak acids are treated with a few ml. of water and an equivalent of hydrochloric acid, and acetone and water added. A wet-ashing process is recommended for organic compounds which are deeply coloured or which react with hydrogen sulphide. Recovery of added lead in the experiments was accurate to within 2 p.p.m. in tests on a variety of pharmaceutical chemicals. Experimental results with and without compensation for colour of sample are compared. G. B.

Histamine, Gravimetric Determination of. M.Schmall, E. G. Wollish and J. Galender. (*J. Amer. pharm. Ass. Sci. Ed.*, 1952, **41**, 138.) The following method is recommended. Dissolve about 100 mg. of histamine dihydrochloride or 150 mg. of histamine phosphate in 10 ml. of water and add slowly, while stirring, 10 ml. of a 3·5 per cent. solution of nitranilic acid in ethanol (95 per cent.). Allow to stand for 15 minutes, add 10 ml. of ethanol (95 per cent.), leave in a refrigerator for 3 hours, filter, wash the precipitate with cold ethanol followed by ether, dry at 120° C. for 2 hours and weigh. Each g. of precipitate is equivalent to 0·5395 g. of the dihydrochloride or 0·9009 g. of the phosphate. Ointments may be warmed with chloroform-light petroleum mixture and extracted twice with water, the extracts being separated by centrifuge and assayed. The precision is about ± 1·0 per cent. on the parent substances and ± 2·0 per cent. on pharmaceutical preparations. During the assay of histamine phosphate the proportion of ethanol should not exceed 60 per cent., or histamine phosphate may be precipitated. Glycine and histidine give precipitates with nitranilic acid and consequently their absence should be confirmed by the ninhydrin reaction. G. B.

Papaverine and Phenobarbitone in Compound Preparations, Potentiometric Titration of. P. Roland. (*J. Pharm. Belg.*, 1952, **7**, 86.) Titrations were performed in ethanol (75 per cent.) to avoid the formation of precipitates, the ethanol concentration being adjusted to 75 per cent. at the end of the titration so as to obtain a sharper end-point for phenobarbitone. Nitrogen was used to stir the solutions and prevent absorption of carbon dioxide by alkaline solutions. For the assay of pills, a sample containing 0·1 to 0·2 g. of papaverine hydrochloride and 0·15 to 0·25 g. of phenobarbitone was triturated with calcium carbonate and water to a paste, dried with anhydrous sodium sulphate, powdered and extracted with chloroform. The residue after drying the chloroform extract was dissolved in ethanol and titrated with 0·1N hydrochloric acid (one end-point due to papaverine). The solution was back-titrated with 0·1N sodium hydroxide (first end-point due to papaverine and second to phenobarbitone). The papaverine content was calculated from the mean of the two titrations. If the chloroform extract was coloured owing to the presence of liquorice as an excipient, a correction was applied to the phenobarbitone titration. Satisfactory results were obtained on the pure substances and tablets, without the preliminary extraction treatment. The method was extended to a determination of mixtures of phenobarbitone and methylphenobarbitone, based on the reaction of these substances with 2 and 1 molecules of silver nitrate respectively. G. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Insulin, Maximum Molecular Weight of. J. M. Creeth. (*Nature, Lond.*, 1952, **170**, 210). The sedimentation and diffusion constants of the untreated crystalline protein have been redetermined. In phosphate buffer, *p*H 7·4, the mean of 7 determinations in the Spinco ultracentrifuge gave $S_{20,w} = 3{\cdot}12 \pm 0{\cdot}02$ (S.D.) Svedberg units. This value was independent of concentrations in the range 0·4 to 0·8 per cent. of temperature in the range 17° to 26° C., and of ionic strength in the range 0·10 to 0·30. A table is given of diffusion coefficients, determined with the Gouy diffusiometer at *p*H 7·4 and ionic strength 0·10 together with notes on the method of calculation used; calculation of D*m* for the most concentrated solution gave 8·16. This work provides qualitative confirmation for the dissociation phenomena reported from osmotic measurements by Gutfreund (*Biochem. J.*, 1948, **42**, 156 and 544), for concentrations lower than about 0·3 per cent., but gives a different value for the molecular weight in the stability range. Thus, using the value $s = 3{\cdot}12$, $D = 8{\cdot}2$, application of the Svedberg equation gives $M = 34{\cdot}800$ or 36,700 according to the value of $\bar{v}$ used. The value of 35,000 to 36,000 refers to the maximum molecular weight of insulin as it exists in solution (investigations at *p*H 7·0 and 7·2 gave similar results) agreeing with the solid molecular weight (from X-ray determination) of 36,000. R. E. S.

Radioactive Penicillin, Preparation of. E. L. Smith and D. J. D. Hockenhull. (*J. app. Chem.*, 1952, **2**, 287.) Details of the preparation biosynthetically of crystalline radioactive penicillin with a specific activity of over 1 c./g. are given. The medium was a modification of that recommended by Jarvis and Johnson (*J. Amer. chem. Soc.*, 1947, **69**, 3010), the sulphur content being reduced to 0·1 mg. of sulphur per ml., and 0·1 per cent. of phenylethylamine hydrochloride being included as a benzylpenicillin precursor. One preparation gave 2·2 mg. of crystalline penicillin at 0·74 μc. per unit (1·23 c./g.); it was purified by 2 steps of solvent extraction, followed by crystallisation as the *N*-ethylpiperidine or *cyclo*hexylamine salt. R. E. S.

BIOCHEMICAL ANALYSIS

Acetone Bodies, Estimation of. C. Thin and A. Robertson. (*Biochem J.*, 1952, **51**, 218.) The method depends on the development of an orange to red coloration when an alkaline solution of salicylic aldehyde is left in the presence of acetone, the depth of colour formed being directly proportional to the amount of acetone present; the test can be used to estimate free acetone, the acetone formed by hydrolysis of acetoacetic acid, and that formed by chromic acid oxidation of β-hydroxybutyric acid and of *iso*propanol. Details of experimental procedure are given together with standard calibration curves, and a description of the method as applied to biological materials such as blood, milk, urine and rumen liquor. Recovery experiments using ethyl acetoacetate purified by distillation under reduced pressure gave results within ±4 per cent. of the theoretical amounts. Acetic acid, lactic acid, sodium chloride, cholesterol, urea, and formaldehyde did not interfere with the estimation, but acetaldehyde in concentrations as low as 3 mg./100 ml. reacted with the colour reagent to give a slightly opaque orange solution, which was sufficient to prevent an accurate determination even of total ketones. R. E. S.

CHEMOTHERAPY

Mersalyl Analogues, Synthesis of. W. O. Foyre, H. M. Kotak and J. J. Hefferren. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 273.) The following analogues of mersalyl, in which naphthalene or thiophene is substituted for the benzene nucleus and in which sulphonamide linkages sometimes replace the carbonamide type, were prepared:—(I) 2-oxyacetic acid 3-[*N*-(γ-acetoxymercuri-β-ethoxypropyl)] naphthoylamide, (II) 2-[*N*-(γ-acetoxymercuri-β-methoxypropyl)] thiophenesulphonamide, (III) 2-carbethoxy-5-[*N*-γ-(acetoxymercuri-β-methoxypropyl)] thiophenesulphonamide and (IV) 2-carboxy-5-[*N*-(γ-hydroxymercuri-β-methoxypropyl)] thenoylamide. Mersalyl was prepared by treating allyl*iso*cyanate with salicylic acid to form *N*-allylsalicylamide, reaction with chloracetic acid and subsequent treatment with mercuric acetate. The 2-hydroxy-3-naphthoic acid analogue was similarly prepared but using the acid chloride to form the amide, as a better yield was obtained than with the free acid. Compound (III) was prepared from the allylamide of 2-thiophenesulphonic acid. This compound was not soluble in alkali, and α-mercuration of the ring took place at elevated temperatures. Compound (III) was prepared because of the difficulties experienced with compound (II); it was soluble in dilute alkali, but it was not possible to hydrolyse the ester without affecting the mercury linkage. Compound (IV) was obtained from the half-ester of dicarboxythiophene, formation of the allylamide and treatment to give the mercury derivative. The ester was readily hydrolysed to the free acid which contained a solubilising group and was soluble in dilute sodium carbonate solution. The compounds exhibited diuretic activity but only compound (I) was sufficiently soluble to be administered by intravenous injection. G. B.

PHARMACY

NOTES AND FORMULAE

Ethyl Biscoumacetate (Tromexan Ethyl Acetate). (*New and Nonofficial Remedies, J. Amer. med. Ass.*, 1952, **149**, 277.) Ethyl biscoumacetate is 3:3′-carboxymethylene bis-(4-hydroxycoumarin) ethyl ester, and occurs as a white, odourless, bitter, crystalline solid, m.pt. 177° to 182°, or 154° to 157°; soluble in acetone and benzene, slightly soluble in ethanol and ether, and insoluble in water. When warmed with sulphuric acid, an orange colour forms which is unaffected by ammonia but turns yellow on the addition of sodium hydroxide. A solution in ethanol becomes reddish-brown on the addition of ferric chloride. When treated with sodium hydroxide and allowed to stand for an hour, the odour of iodoform is produced on the addition of iodine. On double extraction with ammonia, it leaves not more than 1·4 per cent. of a residue of 3:3′-carboxymethylene bis-(4-epoxycoumarin) ethyl acetate, after washing with water and drying at 105° for 2 hours. The difference between 100 and the sum of the percentage of this impurity and the percentage of ethyl biscoumacetate found in the assay is the amount of 3:3′-carboxymethylene bis-(4-hydroxycoumarin) present. Ethyl biscoumacetate loses not more than 0·3 per cent. of its weight when dried at 105° for 4 hours, and leaves not more than 0·1 per cent. of residue on ignition. It contains 96·0 to 100·0 per cent. of ethyl biscoumacetate and is assayed by distilling a solution in aqueous sodium carbonate with strong sodium hydroxide, treating the distillate with potassium dichromate and sulphuric acid and estimating the excess of potassium dichromate iodometrically. Ethyl biscoumacetate is an anticoagulant. G. R. K.

PHARMACOLOGY AND THERAPEUTICS

o-Aminophenol in Experimental Tuberculosis. B. Croshaw. (*Nature, Lond.*, 1952, **169**, 966.) Using an *in vitro* floating pellicle method with *Mycobacterium tuberculosis* var. *hominis H37Rv* and a modified Long's medium with 10 per cent. of ox serum, a dilution of 1 in 729,000 of *o*-aminophenol inhibited growth for 4 weeks. Mice were treated with 2 or 4 mg./20 g. mouse/day for 2 to 23 days after infection with Revenel strain bovine tubercle bacilli and the macroscopic extent of lung lesions examined in survivors. Guinea-pigs infected with H418 strain of human tubercle bacilli were treated with 50 mg. of *o*-aminophenol/500 g. guinea-pig/day starting 21 days after infection and continuing for 60 days. The drug did not retard tuberculosis *in vivo*, but caused tissue damage at the site of injection, which did not occur with streptomycin, used as an active drug control. G. B.

Chloramphenicol, Aplastic Anæmia Following Prolonged Administration. L. E. Wilson, M. S. Harris, H. H. Henstell, O. O. Witherbee and J. Kahn. (*J. Amer. med. Ass.*, 1952, **149**, 231.) In a study of the effect of chloramphenicol in the control of chronic bronchopulmonary suppuration, 62 patients ranging in age from 1 year to 72 years were given doses of 1 g. twice or 3 or more times weekly for periods of from 1 month to 14 months, after an initial period of more intensive antibiotic therapy, which included penicillin and streptomycin. Two of the patients, both female, developed aplastic anæmia. One of these, who subsequently died, received a total of 56 g. of chloramphenicol over a period of 7 months; the other received 52 g. over 5½ months. Early untoward symptoms included heavy epistaxis, which occurred after 5 months and 3 months respectively, profuse menstruation, and purpuric manifestations. During the administration of chloramphenicol, blood cell counts, including studies of stained smears, should be carried out twice weekly. The drug should be discontinued when the level of granulocytes, erythrocytes, or thrombocytes falls below normal. G. R. K.

Digitalis, Comparison of the Biological Activity of Leaves of Various Species. P. Duquenois. (*Ann. pharm. franç.*, 1952, **10**, 177.) Plants were grown on chalky soil of *p*H 7·2 to 7·3, without manure. Leaves of all the species were collected at the time of flowering, at the same time of day and under identical weather conditions. Drying (24 hours at 45° to 50° C.) commenced 1 hour after collection. The biological activity, determined in guinea-pigs, was compared with that of the international standard preparation of digitalis and with an average sample of wild *Digitalis purpurea* collected in the Vosges in the same year. The activity of *D. purpurea* varied from 73 per cent. of that of the international standard in 1950 to 90 per cent. in 1948, being about the same for wild and cultivated plants. *D. ambigua* and *D. lutea* had a similar activity when grown under the same conditions. The yield of leaves from *D. purpurea* was poor in calcareous soil, but was improved by manures and fertilisers. *D. lanata* gave a greater yield of material of higher activity. *D. ferruginea* yielded material twice as active as *D. purpurea* under the same conditions. This species appeared suitable for cultivation in the calcareous soils of Alsace. The yield of leaves was increased by manuring. G. B.

Nicotinic Acid Ester, Skin Response to Local Application in Rheumatoid Arthritis. J. R. Nassim and H. Banner. (*Lancet*, 1952, **1**, 699.) Trafuril, (5 per cent. tetrahydrofurfuryl nicotinic acid ester in a water miscible-base),

when rubbed into the flexor surface of the forearm in normal individuals produces an area of erythema with a sensation of tingling and heat. This effect is not produced in patients with rheumatoid arthritis, but occurs when symptomatic relief is obtained with cortisone or adrenocorticotrophic hormone. Normal responses have been observed in a few cases of osteo-arthritis and tuberculous arthritis. It is suggested that the reaction may be valuable in elucidating the action of adrenocorticotrophic hormone and cortisone and in studying abnormal peripheral circulation in rheumatoid arthritis and allied conditions. G. B.

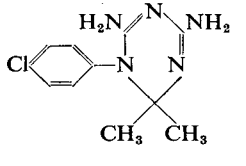
Nitrogen Mustard Treatment of Rheumatoid Arthritis. C. J. Díaz, E. L. Garćia, A. Merchante and J. Perianes. (*J. Amer. med. Ass.*, 1951, **147**, 1418.) Since a relationship can be observed between the effects produced by cortisone and corticotrophin (ACTH) and those of nitrogen mustard, two patients with rheumatoid arthritis were treated by injections of 6 mg. of nitrogen mustard. No details are given of the form, frequency or route of the injections or of the duration of the treatment. In each case after the second injection the patients became free from pain; there was considerable reduction in joint swellings, and movement of limbs, where it was not prevented by bony ankylosis, was restored. The improvement had been retained 1 month and 2 months respectively after treatment had been stopped. Subsequently 7 further cases were treated. There was complete disappearance of pain and recovery of joint movements in 4. In 2 others improvement was less obvious owing to bony ankylosis or irreversible muscular retraction, and the remaining case showed improvement in that pain disappeared and the exudative condition regressed. The treatment causes a drop in the eosinophil count from 100–200 to 20–50 by about the third injection. The sedimentation rate at first increases and then falls but does not become normal. Elimination of 17-ketosteroids is increased. Nitrogen mustard was also tried in 2 patients with prolonged status asthmaticus, the dyspnœa disappearing after the first injection. H. T. B.

Noradrenaline, Adrenaline and the Human Circulation. H. J. C. Swan. (*Brit. med. J.*, 1952, **1**, 1003.) The author reviews published work on the action of adrenaline and noradrenaline in the circulation and on their part in normal and abnormal physiology. Owing to the variable absorption time after intramuscular or subcutaneous injection, responses obtained during intravascular infusion are regarded as of most value and most of the results reviewed were obtained by using this route. The output of the heart is increased by adrenaline and either unaltered or decreased by noradrenaline. The increased output is due in part to changes in the heart rate and in part to alterations in stroke volume. Both substances increase the stroke volume. Adrenaline causes an increase in heart rate, an initial increase to 90 to 120 beats per minute being followed after 30 to 45 seconds by a decrease in the rate to 5 to 30 beats per minute above the resting value. Noradrenaline produces a bradycardia, both this and the secondary slowing of adrenaline being due to vagal influences. Adrenaline increases the systolic pressure and the pulse pressure but its effect on the diastolic pressure is variable. Noradrenaline increases systolic, diastolic, and mean pressures, the pulse pressure remaining unaltered. Adrenaline decreases the total peripheral resistance, measured by the ratio of mean blood pressure to cardiac output, while noradrenaline increases it. The action of adrenaline on the blood flow in muscles is complex. When infused into the main artery of a limb an initial dilatation lasting 45 to 60 seconds is followed, with a small dose, by a return of the blood flow to its resting level or, if the dose is

large, by constriction of the blood vessels. The initial dilatation is so transient that its physiological significance is doubtful. Intravenously the transient dilatation is followed by a sustained increase in blood flow. Noradrenaline infused into the femoral artery does not produce the transient dilatation and the dose required to produce restriction is only one-tenth to one-twentieth of the dose of adrenaline required to produce the same effect; given intravenously it does not produce sustained dilatation. Noradrenaline is now accepted as the predominant transmitter substance at sympathetic nerve endings. Only small amounts leak away into the general circulation from neurovascular junctions and they cause no significant effect on the general circulation. The nerves to the skin and the cardiac sympathetic nerves have been cited as possible exceptions but their effect could equally well be mediated by liberation of noradrenaline.

Support for the view that adrenaline functions in an emergency is given by the reports of more than 60 patients that intravenous adrenaline produces symptoms similar to what they experienced during times of alarm, while the milder symptoms caused by noradrenaline were always unfamiliar to the subjects. Experience suggests that during stress, adrenaline enters the circulation at the rate of rather less than 10 μg./minute. Noradrenaline may well play a causative role in human hypertension and it has been suggested that essential hypertension is due to a failure of methylation of noradrenaline to adrenaline. Another possibility is that hypertensive vessels may be sensitive to noradrenaline. There is no conclusive evidence that either of the compounds is concerned with the vasospastic diseases. In phæochromocytoma there is usually a tumour either in the gland itself or in the para-aortic region and the physiology of the gland changes. The total amount of pressor hormone secreted is greatly in excess of normal and it may be liberated from the gland continuously over long periods; the mixture of hormones liberated may contain a high proportion of noradrenaline so that the effect of the latter is more marked than that of adrenaline. Noradrenaline has been used therapeutically for its pressor action in doses of 5 to 40 μg. per minute, but is not yet proved superior to other pressor drugs; it would appear to be the most rational agent in the treatment of the hypertensive state due to peripheral collapse. H. T. B.

Proguanil (Paludrine), a Metabolite of, with High Antimalarial Activity. H. C. Carrington, A. F. Crowther, D. G. Davey, A. A. Levi and F. L. Rose. (*Nature, Lond.*, 1951, **168**, 1080.) A basic substance, isolated first as the picrate and then as the free base, which crystallised from a mixture of moist chloroform and ether in colourless prisms, was obtained from the urine of rabbits receiving daily doses of proguanil hydrochloride (50 mg./kg.). The same product was also isolated from the fæces of rabbits, and from the urine of human volunteers receiving proguanil. This metabolite is about 10 times as active as the parent drug against infections of *P. gallinaceum* in chicks. Analysis of the picrate and the free base showed the empirical formula to be $C_{11}H_{14}N_5Cl$. Heating the base alone or in alkaline aqueous solution gave a ready conversion to an inactive isomeric substance. The chemical evidence indicated that this active metabolite is 2:4-diamino-1-*p*-chlorophenyl-1:6-dihydro-6:6-dimethyl-1:3:5-triazine (I), and this structure has been confirmed by X-ray crystallographic analysis. An easy synthesis of this substance has been achieved.



A. H. B.