# ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

## CHEMISTRY

### ANALYTICAL

**Aneurine in Pharmaceutical Products, Determination of.** R. Patrick and J. F. H. Wright. (*Analyst*, 1949, **74**, 303.) A modification of Holman's mercuric oxide oxidation of aneurine followed by the measurement of the fluorescence in aqueous acetone solution has been used for the determination of aneurine in aneurine tablets, vitamin " B group " tablets, injectable " B group " solutions, a multi-vitamin concentrate in aqueous alcohol and chocolate-malt based granules containing vitamins A, $B_1$, C and D and vanillin. The technique was simpler than the Jansen method and the precision and accuracy were higher; all reagents were stable for long periods, the purification of *iso*butyl alcohol was eliminated, and the acetone used was prepared by heating the commercial product with activated carbon under reflux and distilling. Alterations in the concentrations of potassium hydroxide and mercuric chloride were made to prevent the precipitation of mercuric oxychloride. An aqueous solution of 1-methyl-5-aminoacridine hydrochloride was preferred to quinine sulphate as standard, because of its greater stability. Fluorimeter readings were found to be directly proportional to aneurine concentrations over the range used, the results being based on measurements with a Klett instrument using a Corning 586 filter in the exciting beam and Corning 430 and 038 filters in front of the photo-cell. Reproducible results were obtained in the assay of tablets containing aneurine, the coefficient of variation being 1·6 per cent. With the chocolate-malt granules very poor results were obtained by simple acid extraction. Separation of the aneurine from interfering substances was obtained by adsorption on synthetic zeolite, previously activated by washing alternately with boiling 3 per cent. solution of acetic acid and with a 25 per cent. solution of potassium chloride in 0·1N hydrochloric acid until no precipitate appeared when the potassium chloride washings were made alkaline; the results obtained from nine samples of chocolate malt granules containing 0·1 mg. of aneurine per g. showed a coefficient of variation of 2·0 per cent. R. E. S.

**Ephedrine, Colour Reaction of.** H. Wachsmuth. (*J. Pharm. Belg.*, 1949, **4**, 186.) By warming a slightly alkaline solution of ephedrine with 1 drop of a 1 per cent. ninhydrin solution, a violet colour is produced. This may be extracted with amyl alcohol. The colour has a maximum absorption of 550 m$\mu$. The reaction appears specific, since it is not given by some 40 alkaloids and bases which were tried. The sensitivity is about 1 in 5,000. G. M.

**Picric Acid in Picrates, Colorimetric Determination of.** R. Stöhr. (*Biochem. J.*, 1949, **44**, XXXV.) Dissolve quantities of picrates corresponding to 0·5 to 2·5 mg. of picric acid in 1 ml. of 20 per cent. anhydrous sodium carbonate solution or 1 per cent. sodium hydroxide solution in a 12·5 ml. marked test-tube, and add 5 ml. of 0·600 per cent. glucose solution (equal to 30 mg. of glucose) and water to a total volume of 11 ml. Heat in a boiling water-bath for 10 minutes, cool, and fill to the 12·5 ml. mark. Reading is made in the colorimeter against a standard solution prepared as follows: in a 12·5 ml. marked test-tube heat for 10 minutes in a boiling water-bath 5 ml. of 0·600 per cent. glucose solution, 5 ml. of water and 1 ml. of 20 per

cent. sodium carbonate, or 1 ml. of 1 per cent. sodium hydroxide. After cooling, add 1 ml. of 0·100 per cent. picramic acid (Egerer, *J. biol. Chem.*, 1918, **35,** 565) and fill with water to the 12·5 ml. mark. The author used a Klett colorimeter; height of the standard 20 mm. The method enables the determination of the molecular weight in organic basic substances in picrates of known constitution, whereas in picrates of unknown constitution the equivalent weight can be determined. S. L. W.

**Theophylline, Mercurimetric Detection and Determination of.** J. Bosly. (*J. Pharm. Belg.*, 1949, **4,** 66.) The composition of the precipitate obtained by the addition of mercuric acetate to a solution of theophylline indicates that it is a mercury salt of theophylline, $B_2Hg2H_2O$. The reaction may be used qualitatively as a test for theophylline; 1 ml. of a 0·1 per cent. solution of theophylline gives with one drop of a 5 per cent. solution of mercuric acetate a crystalline precipitate, soluble in mineral acids or in a large excess of reagent. Using the microscope it is possible to detect 1 to 2 μg. of theophylline. For the detection of theophylline in theobromine, 0·6 g. of the latter is shaken for 15 minutes with 1 ml. of water, and the mixture is then filtered on a small glass filter. The solution is treated with one drop of mercuric acetate solution (0·5 per cent.) and after several hours is examined for the characteristic crystals. It is possible to detect 0·1 per cent. of theophylline in theobromine. For quantitative work, an excess of a freshly prepared and filtered solution of mercuric acetate is added to the solution under examination, and, after filtering, the excess of mercury in the filtrate is determined by titration with 0·1N thiocyanate. For the assay of theophylline ethylenediamine and theophylline monoethanolamine it is necessary first to neutralise the solution. When determining theophylline in theobromine or caffeine, it is essential that the volume of mercuric acetate added should not be more than 50 per cent. more than that required to precipitate the theophylline. G. M.

**Thiomersalate, Polarographic Determination of.** J. E. Page and J. G. Waller. (*Analyst,* 1949, **74,** 292.) A polarographic method has been developed for the measurement of small amounts of thiomersalate (sodium ethyl mercurithiosalicylate) in pharmaceutical preparations. The substance gave, in acid or neutral solution, a characteristic polarogram with two well-defined steps. Concentrated hydrochloric acid (1·0 ml.) and 1·0 ml. of 0·1 per cent. gelatin solution were added to a volume of solution containing between 0·1 and 1·0 mg. of thiomersalate and diluted to 10 ml. A 3 ml. portion of the diluted solution was transferred to the polarograph cell, nitrogen was bubbled through it for 10 minutes to remove oxygen and it was examined over the potential range 0 to −0·8 v.; the height of the step appearing at about −0·5 v. was then measured. For solutions containing suspended matter (e.g., alum-precipitated vaccines), either the precipitate was filtered off before addition of the acid or the final solution containing suspended matter was examined in a polarograph cell connected through an agar bridge to a saturated calomel electrode, the latter being used as anode. Determinations by the two procedures on vaccines containing 0·01 per cent. of thiomersalate agreed to within 10 per cent. The recoveries of added thiomersalate from vaccine preparations were very good but those from a crude prolactin preparation and from liver extract preparations were less satisfactory; for vaccines containing about 0·01 per cent. of thiomersalate the recovery was 90 per cent. or more. Antiseptics of the phenol type, such as *p*-chloro-*m*-cresol, most organic substances and metallic ions (except those of antimony, arsenic, bismuth, cadmium, tin, titanium and vanadium) did not interfere.

Thiomersalate could be determined in vaccine preparations, but not in whole blood or in ter- and quinquevalent antimony preparations, such as stibophen and sodium antimonyl gluconate. Determinations on liver extracts containing about 0·01 per cent. of thiomersalate were usually about 20 per cent. low, but as this loss was consistent, a calibration curve could be used. Phenylmercuric nitrate and acetate, mercurochrome, mercuric nitrate and mercuric chloride were examined in acid solution at negative potentials; only the phenylmercuric salts formed similar steps. In an ammoniacal cobalt buffer solution thiomersalate gave catalytic steps of similar shape to, but of a much lower height than, those given by cysteine. R. E. S.

***d*-Tubocurarine Chloride, Determination of.** D. K l e i n and S. M. J o r d a n. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 438.) A multiple chemical assay procedure has been developed for *d*-tubocurarine chloride in dilute solution based on three different properties of the substance. Measurement of optical rotation gives a roughly quantitative value, while direct determination of the extinction at 280·5 mμ and photometric determination of the pink Reineckate in alcoholic solution at 525 mμ yield values which agree with each other and with the known concentrations. The combination of all three procedures may be used to identify and determine *d*-tubocurarine chloride in solution.

S. L. W.

## FIXED OILS, FATS AND WAXES

**Ground-Nut Oil.** K. R a m a m u r t i and B. N. B a n e r j e e. (*Indian J. med. Res.*, 1948, **36**, 371.) A study has been made of the quality and stability of ground-nut oils commercially available in India. There was no direct relationship between the colour and acidity of the oil although, broadly, they were related. As regards the extraction and refining processes it was found that the incorporation of broken, mouldy, and shrivelled grains raised the acidity from 3 to 4 times, while subjecting the seeds to heat-treatment before extraction of the oil nearly doubled the acidity. The process of refining an oil of high free fatty acid content did not increase its stability. The rate of hydrolysis of ground-nut oils using pancreatic lipase was studied in comparison with fresh cow ghee as a standard; fresh ground-nut oil showed a satisfactory hydrolysis rate and samples possessing up to 2 per cent. acidity gave a slight fall in the rate of hydrolysis, although with oils of higher acidity the fall was rapid and a highly rancid sample showed only slight hydrolysis. There was a gradual decrease in the rate of hydrolysis of the oil, with increasing acidity. The fall in the rate of hydrolysis of the oil after cooking was more marked if the acidity was higher than 2 per cent. of free fatty acids. The hydrolytic curve with pancreatic lipase showed that an acidity of less than 1 per cent. is desirable; such an oil can be prepared if damaged nuts are removed before crushing. The presence of a high free fatty acid content caused the inactivation of carotene and vitamin A. Vitamin A was more easily inactivated with fried oil than with raw oil; refining and hydrogenation of a high acidity ground-nut oil did not prevent the inactivation to any considerable extent. The addition of an anti-oxidant such as ethyl gallate was effective in prolonging the period of inactivation of vitamin A only so long as the free fatty acid content of the oil was below a certain limit; as the free fatty acid content of the oil increased the protection afforded by the anti-oxidant decreased. An oil containing less than 1 per cent. of free fatty acids caused little inactivation of carotene or vitamin A. The removal of the free acidity from a high-acidity oil did not improve the storage property and the addition of an anti-oxidant then failed to increase its

storage life. It was considered that ground-nut oils should only be used for edible purposes if containing less than 1 per cent. of free fatty acids.

R. E. S.

## GLYCOSIDES, FERMENTS AND CARBOHYDRATES

**Cardio-active Toad Poisons.** K. Meyer. (*Pharm. Acta Helvet.*, 1949, **24**, 222.) Toad venom has been used in Chinese medicine for centuries under the name of Ch'an Su. The author examined a purified extract from this material, using chromatography on alumina, and succeeded in isolating 7 compounds, as follows :

| | Substance | Yield | M. Pt. °C. | $[\alpha]_D$ in Chloroform | Probable Formula |
|---|---|---|---|---|---|
| 1 | α-Sitosterin ... ... ... | 1·5 g. | 150–152 | −39·9° | $C_{29}H_{50}O$ |
| 2 | Cinobufagin ... ... ... | 14·0 g. | 216–217 | − 3·6° | $C_{26}H_{34}O_6$ |
| 3 | Bufalin ... ... ... ... | 3·0 g. | 244–248 | − 8·7° | $C_{24}H_{34}O_4$ |
| 4 | Bufotalin (impure) ... ... | 1·0 g. | 168–180 | + 1·7° | $C_{26}H_{36}O_6$ |
| 5 | Cinobufotalin ... ... ... | 1·1 g. | 259–262 | +10·7° | $C_{26}H_{34}O_7$ |
| 6 | Gamabufotalin (as acetate) ... | 0·9 g. | 265–266 | −10·4° | $C_{28}H_{36}O_7$ |
| 7 | Telocinobufagin ... ... | 0·15 g. | 160–175<br>210–211 | + 4·4° | $C_{24}H_{34}O_6$ |

The four free bufogenins (Nos. 2, 3, 5 and 7) were tested biologically on cats, and gave values for the mean lethal dose ranging from 0.1 to 0.2 mg./kg. The identity of fraction 6 could not be confirmed with certainty, as sufficient data for comparison are not available. Compound 7, which appears to be different from all previously reported bufogenins, shows a double melting-point (from acetone). Cinobufotalin, which has also been reported in material from this source, was not obtained.

G. M.

## ORGANIC CHEMISTRY

**Phenolic Compounds, Adsorption of, on Aluminium Oxide.** C. O. Björling. (*Farm. Revy*, 1949, **42**, 588.) The adsorption on aluminium oxide of three series of compounds, di-, mono-, and non-phenolic has been studied. The first series examined consisted of adrenaline, oxedrine and ephedrine; the second consisted of alkaloids (apomorphine, morphine, codeine, and dionine); while the third consisted of simple benzene derivatives (catechol, resorcinol, hydroquinone; guaiacol, phenol; veratrole, anisole). In all the series especially in the first and third, the (ortho) diphenolic compounds were adsorbed more strongly than the mono-phenolic substances, and these, in turn, more strongly than the non-phenolic ones. Adrenaline, apomorphine, and catechol were retained by the adsorbent, both in alcoholic and aqueous solution, and were desorbed only by acid. Oxedrine, morphine, and phenol and guaiacol were desorbed slowly and incompletely by alcohol, fully by water, and in the series stated were intermediate in behaviour between the di-phenols and the non-phenolic compounds which were easily removed from the column. The adsorption of the substances investigated seemed to be independent of their basic properties. With reference to acidic properties, the more acidic apomorphine was adsorbed more strongly than morphine and both much more than the non-acidic codeine and dionine;

in series 3 no clear connection could be traced apart from the fact that the acidic phenols were adsorbed more strongly than the non-acidic compounds. Experimental details of the technique are given for both macro- and micro-scale operations, the macro scale employing about 0·5 millimol. of the substance to be tested and 10 g. of the oxide in a tube, 1 cm. wide, while the micro scale used about 0·005 millimol, of the substance and 0·4 g. of the oxide in a column, 3 mm. wide. R. E. S.

## TOXICOLOGY

**Arsenic Contents of Human Organs after Fatal Arsenic Poisoning.** F. Hansen and K. O. Møller. (*Acta Pharmacol. Toxicol.*, 1949, **5**, 135.) An account of an investigation into the relative arsenic contents, based on quantitative determinations, of isolated organs and blood from normal human subjects, from individuals treated with ordinary therapeutic doses of arsenic preparations and from individuals dying of acute arsenic poisoning. The material in the last-mentioned group consisted of organs obtained from 20 individuals who had swallowed arsenic trioxide, usually in the form of a cattle wash, either accidentally or intentionally, together with the results of analyses of a further 20 cases appearing in the literature. The figures for arsenic content in the blood of patients treated with inorganic arsenic preparations were based on those observed in 57 patients treated with neoarsphenamine, and figures were also obtained for arsenic content of blood and organs of two patients who died after treatment with organic arsenic preparations. The data obtained are set out in the following table:—

| | Arsenic concentration in mg./100 g. | | |
|---|---|---|---|
| | Liver | Kidneys | Blood |
| Normals .. .. | 0·001—0·01 | 0·001—0·01 | 0—0·002 |
| Treated with inorganic arsenic preparations .. | | | 0·01—0·025 |
| Treated with organic arsenic preparation .. | *ca.* 0·1(1) | *ca.* 0·1(1) | *ca.* 0·2(2) |
| Acute Poisoning[3] .. | 1 to 50 | 0·5 to 15 | 0·1 to 1·5 |

(1) About 4 to 14 days after conclusion of treatment.
(2) About 8 days after conclusion of treatment.
(3) Death occurred ½ to 14 days after intake of poison. S. L. W.

# BIOCHEMISTRY

## GENERAL BIOCHEMISTRY

**nor-Adrenaline from the Adrenal Gland, Isolation of.** S. Bergstrom, U. S. von Euler and U. Hamberg. (*Acta. Chem. Scand.*, 1949 **3**, 305.) The isolation of *l*-nor-adrenaline from cattle adrenals is reported. It occurred together with *l*-adrenaline in the approximate proportions 1:4, the mixture of these bases being isolated from the crude protein-free extract by mean of ion-exchangers. The bases were then separated with counter-current distribution between 0·02N hydrochloric acid and phenol; after extraction of the phenol with ether, pure *l*-nor-adrenaline was isolated as the crystalline base by addition of ammonia. Ultimate analyses are given together with the ultra-violet absorption spectra and the X-ray powder diffraction patterns of the isolated product. These properties were identical with those found for a synthetic specimen. Colorimetric and biological tests also indicated that synthetic and natural samples were identical. R. E. S.

**Gramicidin S, Tyrocidine, and Gramicidin, Diffusion Experiments on.** K. O. Pedersen and R. L. M. Synge (*Acta chem. scand.*, 1949, **2**, 408). In an attempt to evaluate the molecular weight of these peptides, the diffusion constants were measured by means of the Lamm scale method (*Nova Acta Reg. Soc. Scient. Uppsal.*, 1937, **4**, (6) 10) the results being calculated according to the "area method" and according to the "moment method." The agreement between the results in acetic acid and in ethyl alcohol solutions suggested that the molecular states of the peptides in the different solvents were not greatly different. For gramicidin S, if a spherical unhydrated molecule is assumed having partial specific volume 0·81 the diffusion constants correspond to molecular weights in the range 1080 to 1880, suggesting that gramicidin S has the cyclodecapeptide structure. If the same degree of asymmetry/solvation was assumed for tyrocidine as for gramicidin S and the partial specific volume was taken as 1·75, the diffusion data indicated a molecular weight in the range 1900 to 5100. The diffusion of gramicidin was studied only in 70 per cent. (v/v) ethyl alcohol and, making the same assumption as with tyrocidine, partial specific volume 0·80, the molecular weight range indicated was 2800 to 5000. R. E. S.

**Saponins, Rate of Hæmolysis by.** A. Sols. (*Nature*, 1949, **164**, 111.) By increasing the density of a saponin solution, a suspension of erythrocytes can be superposed without mixing; on standing, sedimentation of the erythrocytes will occur. The rate of incorporation of the erythrocytes into the saponin solution is less than that of complete reaction between saponin and erythrocytes (cholesterol), hæmolysis continuing until the drug is exhausted. There will therefore be a linear relationship between the quantity of saponin and that of liberated hæmoglobin, which can be estimated by colour measurement after discarding the residual erythrocytes. A saponin solution of density *ca.* 1·020 was used and an equal volume of the suspension of erythrocytes was placed upon it. Practical details of the procedure are given. Digitonin liberated from 20 samples of oxalated human blood 90 (84 to 95) mg. of hæmoglobin per mg. of drug, from 3 samples of rat blood 88 to 92 mg., from 3 samples of guinea-pig blood 77 to 84 mg., and from 1 sample of rabbit blood 78 mg.; irregular results were obtained with some samples of defibrinated sheep blood. Sapindus saponin (Merck) liberated from human blood 200 mg. of hæmoglobin per mg. of drug. A slow reacting saponin (Kahlbaum) liberated 60 mg. of hæmoglobin per mg., the value at 37°C. being identical with that obtained at room temperature (17°C.). The hæmoglobin/saponin relation was found to be linear within wide limits. For small liberations of hæmoglobin a blank may be necessary although a hæmoglobin result of less than 15 mg. per cent. is of doubtful value. A slow reacting saponin in an extract of *Hepatica triloba* Chaix., gave the same linear relationship as the other saponins studied. R. E. S.

**Vitamin A in Shark-Liver Oil, Protection by Anti-Oxidants.** S. M. Bose and V. Subrahmanyan. (*Ind. J. med. Res.*, 1949, **37**, 11.) The influence of acidity and moisture and of diffused and direct sunlight on the protective action of a combination of *iso*butyl gallate and citric acid for shark liver oils has been studied. The oils were stored under varying conditions in sealed bottles at 40°C., and were examined at intervals, the normal analytical figures and vitamin A content being determined. For the study of the influence of diffused sunlight on the protection of vitamin A by anti-oxidants, samples of shark-liver oil (acid value 0·68), treated with and without anti-oxidants (0·02 per cent. of *iso*butyl gallate—0·01 per cent. of citric acid), were spread out into a number of closed Petri dishes. One batch of dishes containing both

control and treated oils was stored in a dark cupboard at room temperature (30° to 35°C.) while the other batch containing similar samples was stored in diffused light; dishes were removed at intervals for examination. Results indicated that a high free acidity adversely affected the keeping properties of the oils and lowered the efficiency of added anti-oxidants; the adverse affect of moisture was more marked with oils of high acidity than of low acidity. Considerable destruction of vitamin A in shark liver oils exposed to sunlight occurred and anti-oxidants were ineffective in preventing this; the protective power of anti-oxidants was reduced in the presence of diffused light although the difference between the rates of destruction of vitamin A in the control samples stored in diffused light and those stored in darkness, was small. The destruction of vitamin A in sunlight was considered to be due to increased temperature and to ultra-violet light. R. E. S.

## BIOCHEMICAL ANALYSIS

**Adrenaline: Separation from Mixtures.** C. O. Björling. (*Farm. Revy.*, 1949, **43**, 601.) Adrenaline is preferentially adsorbed on aluminium oxide from aqueous solutions of *p*H greater than 6, and can be quantitatively eluted with hydrochloric acid. The efficiency of the method is such that the adrenaline can be estimated by the Folin-Ciocalteu reagent without risk of interference by other phenols which may be present. The sensitivity of this reagent permits the ready estimation of small amounts of adrenaline, so that only small volumes of sample are required. The method was applied to an aqueous solution of adrenaline hydrochloride (0·00385 per cent.) and procaine hydrochloride (2 per cent.) containing chlorbutol, sodium chloride and sodium metabisulphite. 0·1 g. of tri-sodium citrate was added to 10 ml. of sample to adjust the *p*H, and the solution drawn through a column of 1 g. of aluminium oxide in a tube of 5-mm. diameter. The column was carefully eluted with water to remove the procaine hydrochloride, which gives a colour with the reagent, and then with 0·1N hydrochloric acid to remove the adrenaline. Chlorbutol and sodium metabisulphite separately tested under similar conditions were found not to interfere. G. R. K.

**nor-Adrenaline in Adrenaline, Determination of.** M. E. Auerbach and E. Angell. (*Science*, 1949, **109**, 537.) A standard curve is first obtained as follows. To each of five 50 ml. glass-stoppered graduated cylinders add 1 ml. of standard adrenaline solution (prepared by dissolving 100 mg. of pure synthetic adrenaline in 2·5 ml. of borax solution (5 per cent.) and diluting to 100 ml. with water); to 4 of the cylinders add respectively 0·25, 0·50, 0·75 and 1·00 ml. of standard nor-adrenaline solution (prepared freshly by dissolving 40 mg. of pure *l*-nor-adrenaline in 5 ml. of borax solution (5 per cent.) and diluting to 200 ml. with water). To each cylinder add 1 ml. of buffer solution *p*H 9·6 (Clark and Lubs), swirl, add 0·5 ml. of solution of sodium β-naphthoquinone-4-sulphonate (0·5 per cent. and used within one hour of preparation), swirl and allow to stand at room temperature for 45 minutes. Add 0·15 ml. of aqueous solution (1 per cent.) of alkyl dimethylbenzylammonium chloride (Roccal or Zephiran) followed by exactly 10 ml. of mixed solvent (toluene 85 parts, redistilled ethylene dichloride 15 parts mixed freshly daily, washed with borate buffer and filtered through dry paper). Shake thoroughly and set aside for 45 minutes with re-shaking at intervals. The solvent layer (purplish red in the presence of nor-adrenaline) should separate clear but may be clarified if necessary by centrifuging in stoppered tubes. The extracts are transferred to colorimeter tubes and the percentage

transmission determined in a suitable photo-electric colorimeter using a green glass filter (540 mμ), the instrument being set at 100 per cent. transmission for the extract from the cylinder containing only the adrenaline. On semi-logarithmic paper, a straight line should be obtained. For examination of samples of natural adrenaline, a weighed quantity is dissolved in solution of sodium borate (5 per cent.) and diluted with water such that the solution contains borax (approximately 1 per cent.) and adrenaline (approximately 2 mg./ml.) and the solution used in place of the mixture of adrenaline and nor-adrenaline solutions in the above procedure. Samples of U.S.P. Reference Standard epinephrine examined in this way contained respectively 16·3 and 17·5 per cent. of nor-adrenaline and 4 representative samples of epinephrine U.S.P. contained 10·5 to 18·5 per cent. of nor-adrenaline. The absorption spectrum of the coloured extract showed a very broad maximum at 530 to 560 mμ. The procedure used eliminates interference by dihydroxyphenyl-alanine and dihydroxyphenylethylamine and the borate buffer used forms a complex with the catechol group thus diminishing chromogenic side reactions. The application of the method to the assay of fresh adrenal glands is being examined. F. H.

**Aureomycin, Chemical Assay of.** J. Levine, E. A. Garlock, and H. Fischbach. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 473.) This paper presents two physico-chemical methods of assay : fluorimetric, measuring the fluorescence developed by alkaline treatment, and colorimetric, measuring the intensity of the colour developed by acid treatment of aureomycin. The former works with a lower limit of 0·25 μg./ml. and the latter with a lower limit of 20 μg./ml. Time-rate studies are included for the separate steps in the procedures. The results obtained by the two methods are in very close agreement and are directly related to biological potency of the antibiotic as shown by a comparison with results obtained by the cup-plate bioassay.

S. L. W.

**Peptide-Amino-Nitrogen, Determination by the Copper Method.** H. K. Kerkkonen (*Acta chem. scand.*, 1948, **2**, 518.) The method of Pope and Stevens for the determination of amino-nitrogen based on the formation of soluble copper compounds between the amino-acid or digested protein and the excess of copper present in the form of copper phosphate, has been found to give correct figures with amino-acid mixtures and with completely hydrolysed proteins. With partially hydrolysed proteins, however, the values were too high, indicating that the soluble copper compounds of peptides formed were not similar to those of the amino-acids as suggested by Pope and Stevens. A number of determinations were carried out with synthetic peptides and with hydrolysates of zein and zein plastein. Solutions of glycyl-L-leucine, glycyl-glycine, L-leucyl-L-tyrosine and L-leucyl-glycyl-glycine were prepared containing about 10 to 20 mg. of total nitrogen per 25 ml., and on each of these solutions the amino-nitrogen was determined by the copper method and by Van Slyke's volumetric nitrous acid method (5 minutes shaking), the total nitrogen being estimated by the micro-Kjeldhal method. It was found that the peptides bind twice as much copper as the amino-acids, and, in calculating the results from the amino-nitrogen value obtained by the copper method, the factor 0.14 instead of 0.28 should therefore be used. R. E. S.

**Sugar in Urine or Milk; Colorimetric Estimation of.** Salah El-Dewi. (*Brit. med. J.*, 1949, **1**, 899.) Fine's method (*Brit. med. J.*, 1934, **2**, 167) is adapted to the colorimeter so as to give the amount of sugar in urine or milk within 2 g./l. (0·2 g. per cent.) of that determined by Folin's method.

For the determination of urine sugar mix 5 ml. of Benedict's qualitative reagent and 0·25 ml. of urine, heat in a boiling water-bath for 5 minutes, and centrifuge. If the supernatant fluid is not blue, repeat with 0·1 ml. of urine; if it is still not blue, dilute the urine 1 in 2 or more, and repeat. The procedures for the colorimetric reading of the blue colour of the supernatant solution, using either a Duboscq type of colorimeter or a photoelectric colorimeter (Lumetron) are given, and the amount of sugar (g./l.) can be directly read for the colorimetric reading of the standard from the Table or Figures set out in the paper. For the determination of milk sugar, the proteins of milk are precipitated by adding 1 ml. of sodium tungstate solution (10 per cent.), 1 ml. of 2/3 N sulphuric acid, and 2 ml. of water to 1 ml. of milk, thus diluting milk to 1 in 5. Filter through a filter paper; the filtrate should be clear. Add 0·5 ml. of the filtrate to 5 ml. of Benedict's qualitative reagent. The procedures for the colorimetric readings are given. S. L. W.

## PHARMACOLOGY AND THERAPEUTICS

**Acetaldehyde, Pharamacological Action of, on Human Organism.** E. Asmussen, J. Hald and V. Larsen. (*Acta Pharmacol. Toxicol.*, 1948, **4**, 311.) The authors confirmed that the normal concentration of acetaldehyde in blood is from 0·020 to 0·040 mg. per cent. By intravenous infusion of a 5 per cent. solution of acetaldehyde into normal human subjects they were able to show that at concentrations of 0·2 to 0.7 mg. per cent. in the blood there was a marked increase in heart rate, ventilation and dead space, and a decrease in alveolar carbon dioxide. Qualitatively and quantitatively the symptoms were the same as those seen after alcohol intake in persons previously treated with antabuse, which results in a similar increase in the blood acetaldehyde. The experimental subjects who had previously experienced the antabuse and alcohol effect spontaneously reported that the effects were very similar to those experienced after the acetaldehyde infusions and in both instances the same characteristic "hangover" feeling was noticed. Since the formation of acetaldehyde is limited by the combustion rate of alcohol in the organism, and as the rate of alcohol elimination is hardly altered after antabuse treatment, the authors consider it very probable that alcohol intake after antabuse could result in dangerous concentrations of acetaldehyde in the blood. S. L. W.

**Adrenaline, Metabolism of.** Z. M. Bacq. (*J. Pharmacol.*, 1949, **95**, 1.) A review of information recently published on the normal metabolism of adrenaline in the body (a bibliography containing 221 references is appended). From this review the author draws the following conclusions:—(1) that adrenaline may be excreted unchanged in small amounts by the kidneys and stored in the tissues and red blood cells; (2) that its deamination by amine-oxidase in the body is unlikely; (3) that an important fraction is sulpho-conjugated; (4) that another important fraction is simultaneously oxidised to indole substance; (5) that adrenochrome and its derivatives have important biochemical and physiological properties entirely different from those of adrenaline and deserving of further study. S. L. W.

***para*-Aminosalicylic Acid Therapy, Complications of.** M. Hemming and C. J. Stewart. (*Lancet*, 1949, **257**, 174.) The authors report a case of idiosyncrasy to *p*-aminosalicylic acid. Benadryl exerted a slight suppressive action on the condition and it is suggested that it was caused by a local liberation of histamine-like substances. After the administration of

6 g. of *p*-aminosalicylic acid twice daily for 24 days the patient became acutely ill and had an intense generalised skin irritation. Withdrawal of the *p*-aminosalicylic acid produced almost immediate relief of symptoms. The patient gave no history suggesting allergy. The drug was freshly prepared on alternate days as a solution in water flavoured with liquorice. In a second patient with amyloidosis, a course of 12 g. of *p*-aminosalicylic acid daily in 6 doses for 4 weeks caused an increase in the urinary protein excretion to 20 or 30 g. a day. After withdrawal of the drug, urinary protein excretion dropped to about 10 to 12 g. per day. There was no evidence that the diseased kidneys had been irreparably damaged by the *p*-aminosalicylic acid.

A. D. O.

**Antabuse and Alcohol, Effect of, on Animals.** V. Larsen. (*Acta Pharmacol. Toxicol.*, 1948, **4**, 321.) In experiments with rabbits most of the symptoms so characteristic in man after antabuse and alcohol are either lacking (peripheral vasomotor reactions and increase in heart rate) or very irregularly present (increase in ventilation). Only one symptom, the increase of acetaldehyde in blood is invariably present; this increase was about 1 to 2 mg. per cent., and was about 5 times as much as is seen after the same dose of alcohol given to untreated rabbits. S. L. W.

**Arterenol (Nor-Adrenaline) and Epinephrine (Adrenaline), Acute Toxicity of Optical Isomers of.** J. O. Hoppe, D. K. Seppelin and A. M. Lands. (*J. Pharmacol.*, 1949, **95**, 502.) The object of this investigation was to determine the acute intravenous toxicity in mice and rats of lævo, dextro and racemic arterenol in comparison with epinephrine. In rats, *l*-epinephrine was found to be 20 times and in mice 18·5 times as toxic as *d*-epinephrine, while it was 1·8 times in rats and 1·5 times in mice as toxic as the racemic mixture. The acute toxicity ratio between *l*- and *d*-arterenol is smaller than with epinephrine. *l*-Arterenol is 14 times as toxic in rats and 12 times as toxic in mice as the *d*-isomer. No significant differences were observed between the toxicities of *l*- and *dl*-arterenol in either rats or mice. There were striking species differences between the sensitivities to these amines as judged by lethal doses, the rats being approximately 50 times as sensitive to arterenol and 60 times as sensitive to epinephrine as mice. The three optical forms of epinephrine were approximately twice as toxic as the corresponding forms of arterenol. The dose-mortality curves were very steep in rats for arterenol and epinephrine and in mice for *l*- and *dl*-epinephrine, but they were flat in mice and for *d*-epinephrine and arterenol.

S. L. W.

**Chloramphenicol in Experimental Cholera.** R. L. Gould, A. S. Schlengman, E. B. Jackson. M. C. Manning, H. C. Batson and C. C. Campbell. (*J. Bact.*, 1949, **57**, 349.) Chloramphenicol (chloromycetin) was found to be an effective agent in the treatment of mice with experimental cholera provided it was given in adequate dosage within 2 hours of inoculation with *Vibrio comma*. Only two deaths occurred in 90 mice after 2·5 mg. of chloramphenicol intraperitoneally at times ranging from 1 hour before to 2 hours after inoculation, as compared with 50 deaths in 60 mice receiving no treatment. When the dose was increased to 0·5 or 2·0 mg. the number of deaths was 3 out of 178. The vomiting, diarrhoea and dehydration characteristic of cholera in man do not occur in mice infected with *V. comma* and might prevent absorption from doses given orally, so that parenteral injection would be necessary. It is suggested that the drug should be tried clinically, especially for prophylaxis, in any outbreak of human cholera. H. T. B.

**Colchicine, Pilocarpine and Veratrine, Toxicities of.** C. F. Poe and C. C. Johnson. (*Acta Pharmacol. Toxicol.*, 1949, **5**, 110.) The toxicities for white rats were determined by intraperitoneal injections of the alkaloids and the effects on bacteria were determined by observance of their action on the normal fermentative activities of *Escherichia* and *Aerobacter*. Colchicine was shown to be highly toxic for the white rat but only very slightly toxic for bacteria; pilocarpine showed only low toxicity for both bacteria and rat; and veratrine was fairly toxic for both. S. L. W.

**Decamethonium Iodide, Comparison with *d*-Tubocurarine in Controlling Electrically Induced Convulsions.** J. A. Hobson and F. Prescott. (*Lancet*, 1949, **256**, 819.) The use of decamethonium iodide and thiopentone for modifying electrical convulsions is described. Decamethonium iodide has all the advantages of other curarising drugs. The traumatic complications of convulsion therapy are avoided; there is no serious rise in blood pressure; and it enables convulsion therapy to be given to certain patients in whom otherwise convulsions would be contraindicated. It is to be preferred to *d*-tubocurarine for this purpose as it has no tendency to produce histamine-like reactions and as the curarisation passes off more rapidly; several patients were able to sit up within 10 to 15 minutes after the convulsion modified with decamethonium iodide, whereas the time after giving *d*-tubocurarine is rarely less than half an hour. Further advantages are that it is miscible with thiopentone without precipitation, and, being a synthetic preparation, it should not need biological standardisation. Efficient facilities for controlled respiration should be at hand; the injection of stimulants or analeptics cannot replace the provision of a clear airway and rhythmic insufflation of oxygen. It is probable that the fatalities which have occurred in using curarising drugs to modify electrically induced convulsions have been due to the inefficient methods of artificial respiration used.

S. L. W.

**Heparin, Biological Assay of.** M. N. Lewis and F. De Maria. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 441.) A method for the routine assay of the anticoagulant activity of heparin samples is described. Citrated beef plasma is recalcified with calcium chloride in the presence of increasing amounts of heparin and the percentage of the clot formation evaluated. As the heparin concentrations are selected so as to produce an inclusive curve, from 100 per cent. to 0 per cent. clotting, the most characteristic zone of clotting (50 per cent.) can be used in the computations. Two workers reading the same series will have practically identical curves. Experience has shown that the less calcium chloride and heparin used in the clotting system the greater the sensitivity of the method. S. L. W.

**Methyl *n*-Propyl Ether.** C. E. Sykes. (*Brit. med. J.*, 1949, **2**, 420.) Methyl *n*-propyl ether, metopryl, $C_3H_7.O.CH_3$, is a clear, colourless liquid with a characteristic but not unpleasant odour. It boils at 39°C., and has a specific gravity at 16°C. of 0·726 and an inflammability range similar to that of diethyl ether. Previous reports on its use as an inhalation anæsthetic have shown that, compared with diethyl ether, is causes less irritation of the respiratory tract and has a somewhat higher potency and wider margin of safety. In this report, its use on 20 patients undergoing a variety of operations confirmed its lack of irritation of the respiratory tract. G. R. K

**Penicillin, Concentration in Cerebrospinal Fluid.** W. P. Boger and W. W. Wilson. (*Amer. J. med. Sci.*, 1949, **217**, 593.) A study was made of the length of time necessary to demonstrated the presence of penicillin in

the cerebrospinal fluid after parenteral administration in large doses. 21 patients suffering from central nervous system syphilis were treated with a single intravenous dose of 500,000 units in aqueous solution. The penicillin diffused into the cerebrospinal fluid within 2 hours in 14 of 18 individuals, and in 3 hours in 15 out of 18. 3 patients failing to show the presence of the antibiotic in the cerebrospinal fluid after this dosage showed measurable quantities when caronamide was administered intravenously in conjunction with the penicillin. In the 18 patients the cerebrospinal concentrations of penicillin were doubled by the simultaneous administration of 3 g. of caronamide. Since significant levels of penicillin are attained in the cerebrospinal fluid of all the patients studied, in whom the barrier between the blood and cerebrospinal fluid was less permeable than is the case in patients suffering from meningitis, it is suggested that parenteral, and particularly intravenous, administration of penicillin has a place in the therapy of purulent meningitis, and that intrathecal injection is unnecessary in the majority of cases. H. T. B.

**Posterior Pituitary Injection: Evaluation of Antidiuretic Activity.** K. M. Lindquist and L. W. Rowe. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 227.) The intravenous rabbit method has been found more accurate and rapid than the previously used rat and mouse methods. 3 mg./kg. of morphine sulphate is given subcutaneously and 1·5 g./kg. of urethane intraperitoneally. The jugular vein is then cannulated for infusion of warm saline solution and the bladder or urethra for collection of the urine. To start diuresis a single injection of 25 ml. of 20 per cent. glucose solution is given intravenously 30 minutes before the first saline infusion of 50 ml. Urine is then collected for 45 minutes and measured at 5-minute intervals, immediately after which the dose of posterior pituitary injection is given intravenously and the urine again collected for 45 minutes. This permits several injections of sample and standard for comparison in the course of an 8-hour day. The modification suggested by Fugo and Aragon (*Fed. Proc.*, 1947, **6**, 330) of giving water by stomach tube (5 per cent. of the rabbit's body weight) 3 hours before the start of the antidiuretic experiment does not seem to contribute to the accuracy or uniformity of the results and its omission is desirable. The present International Reference Standard, while equal to the U.S.P. Reference Standard in oxytocic and pressor activity, was found to be only about 75 per cent. as active by the rabbit antidiuretic method. Two and often 3 doses may be directly compared in the same animal for antidiuretic activity, but 4 doses will usually give an unreliable result. S. L. W.

**Pyrogens, a Quantitative Assay Method for.** W. C. Ott. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 179.) A method for the quantitative determination of pyrogens in water and biological products, based on the relationship between the log dose and the average maximum temperature rise following intravenous injection into rabbits, is described. Evaluation is based on the standard curve having the equation "average maximum temperature rise in °C. $= 1{\cdot}00 + 0{\cdot}78$ (log. pyrogen units/kg.)," a "pyrogen unit" being the amount of pyrogen in 0·10 mg. of a standard preparation. Under the conditions of this test one pyrogen unit caused an average maximum rise of 1·0°C. in rectal temperature. The standard error of an assay value based on the average response of 4 rabbits was estimated at approximately $\pm$ 60 per cent. Tabular values are given for evaluation of assays conducted under conditions conforming to the statistics of the standard curve, and a

(*Continued on page* 127)