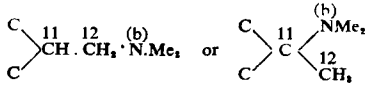
ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

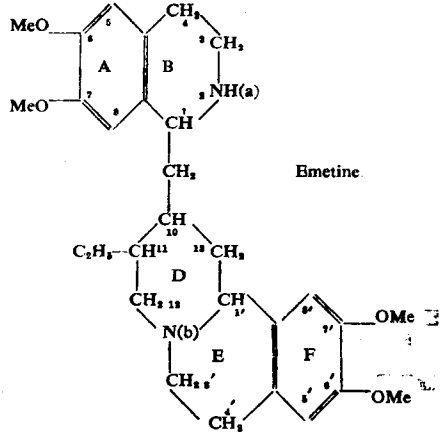
CHEMISTRY

ALKALOIDS

Emetine, Structure of. A. R. Battersby and H. T. Openshaw. (*J. chem. Soc.*, 1949, 3207.) Evidence is presented leading to an independent proof of the structure of emetine. Oxidation of des-N-(a)-emetinetetrahydromethine (I), $ArCH{=}CH[\overline{C_4H_7}].CH_2.CH(Ar)N(Me).\overline{CH_2}$ (Ar = 2-ethyl-4:5-dimethoxyphenyl-), yields 6-ethyl-veratric acid and an amino acid $HOOC.[\overline{C_4H_7}].CH_2.CH(Ar).N(Me).\overline{CH_2}$, indicating that I contains the structure $Ar.CH{=}CH-$ and the absence of a side chain at C_9 in emetine. Hydrogenation of I produced des-N-(a)emetinehexahydromethine, the methiodide of which on Hofmann degradation yielded des-N-(a)-emetinehexahydrobismethine (II), $Ar.CH_2.CH_2[C_4H_7].(CH_2.N\,Me_2)CH:CH.Ar$. The point of ring fission is indicated by the similarity between the absorption spectra of I and II and by the thermal stability of the methiodide of the latter compound. Oxidation of II gave 6-ethylveratric acid and an amino-acid, together providing a complete balance of carbon and indicating the absence of a side chain at C_{13}. Hofmann degradation of II methiodide in agreement with the results of Späth and Pailer (*Monatsch.* 1948, **78,** 348) gave the diene $Ar.CH_2CH_2[C_4H_5].(CH_3).CH:CH_2Ar.$, the absorption spectrum of which confirms its conjugated structure. Hydrogenation of II gave des-N-(a)-emetinioctahydrobismethine (III), which on exhaustive methylation yielded $Ar.CH_2CH_2[C_4H_6](:CH_2).CH_2CH_2.Ar$ (IV). Ozonolysis of IV yielded formaldehyde and a non-aldehydic carbonyl compound suggesting for III the partial structure



C_{11} is suggested as the sole remaining asymmetric centre in III on account of the optical inactivity of IV. Dehydrogenation studies of



Emetine

des-N(a)-emetinehexahydromethine showed the presence of an ethyl group attached to C_{11} and that ring D was 6-membered, confirming the structure given for emetine. J. B. S.

ANALYTICAL

Dicophane, Detection and Estimation of Traces of. M. Brandstätter and G. Breuer. (*Sci. pharm.* 1951, **2**, 87.) A trace of dicophane, dissolved in chlorobenzene, gives an intense violet colour with anhydrous aluminium chloride. This colour, which gradually disappears, is immediately destroyed by water, alcohols, ketones, etc. The actual colour depends on the concentration, and the quantity present may therefore be estimated roughly by a simple inspection (see Table). When applying the test to spray

Dicophane in 10 ml. of chlorobenzene mg.	Aluminium chloride g.	Colour after 5 minutes shaking	Colour after 1 minute in water-bath
0·025	0·1	nil	yellow, with orange shade
0·050	0·1	nil	bright orange
0·25	0·1	nil	reddish brown
0·50	0·1	nil	bright red
1·00	0·1	orange, with violet shade	strong red
2·50	0·1	rose	strong violet
12·00	0·1	wine-red to violet	deep violet blue
0·00	0·1	nil	trace of yellow

residues, about 0·1 g. of the material is warmed with 6 ml. of chlorobenzene, with strong shaking, for several minutes. The solution is filtered off, cooled and made up to 10 ml. Freshly powdered aluminium chloride is then added. For the detection in petroleum it is necessary to remove the whole of the solvent by evaporation at a temperature not exceeding 130°C. before dissolving the residue in chlorobenzene. By this reaction it is also possible to distinguish dicophane from its derivatives, which give different colours. G. M.

Sulphonamides, Acidimetric Determination of. C. G. van Arkel and A. Wuite. (*Pharm. Weekbl.* 1951, **86**, 426.) For the acidimetric determination of sulphonamides, 0·25 g. of the material is suspended in 10 ml. of neutralised acetone and, after the addition of 10 drops of phenolphthalein solution (or mixed phenolphthalein-thymol blue), titrated with 0·1 N sodium hydroxide to a distinct pink colour. The method may be applied to sulphadiazine, sulphamerazine, sulphamezathine, sulphathiazole, sulphacetamide, succinyl- and phthalyl-sulphathiazole: the last two take two equivalents of alkali, while for sulphacetamide 0·5 g. should be taken for the titration. Sulphanilamide and sulphaguanidine cannot be titrated. G. M.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

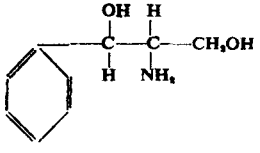
Cellulose from Marine Algae. E. G. V. Percival and A. G. Ross. (*J. chem. Soc.*, 1949, 3041.) Algal celluloses were prepared from *Laminaria cloustoni*, *L. digitata* and *Fucus vesiculosus* by successive extractions of the seaweed with dilute sulphuric acid and sodium hydroxide solutions, followed

by dissolution and reprecipitation of the residue from cuprammonium hydroxide. The product gave the characteristic blue colour with iodine and zinc chloride; a solution in Schweizer's reagent was used in a determination of fluidity. Hydrolysis to glucose by the method of Monier-Williams gave 80 per cent. of the theoretical yield, confirmed by paper chromatography and by the isolation of glucosazone. Strong evidence for the presence of 1:4-β-linkages was obtained by the isolation of cellobiose octa-acetate in 31 per cent. yield. Potassium periodate oxidation indicated a chain length of 160 units, though there is some possibility that the original cellulose may be degraded. X-ray diagrams of algal cellulose show the characteristic pattern of normal cellulose. J. B. S.

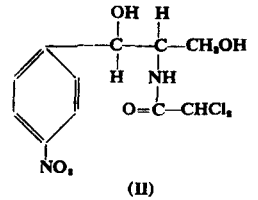
Sugars, Chromatographic Separation on Charcoal. R. L. Whistler and D. F. Dursco. (*J. Amer. chem. Soc.*, 1950, **72**, 677.) The Tiselius technique has been developed, whereby the desorption of various mono,-, di-, and tri- saccharides from columns of charcoal has been effected with various solvents. Preliminary results indicate that the desorption characteristics of these three groups are so different that they may easily be separated as sugar classes. Using 1 g. quantities in each case, monosaccharides were completely desorbed by 800 ml. of water or 200 ml. of 15 per cent. ethanol-water; disaccharides required 2 l. of water, 900 ml. of 5 per cent. ethanol-water or 400 ml. of 15 per cent. ethanol-water; trisaccharides were not desorbed by water or 5 per cent. ethanol-water, but were rapidly recovered by 15 per cent. ethanol-water. The course of desorption was readily followed polarimetrically. Neither the degree of dilution of the sugar solution initially employed nor the presence of inorganic salts such as sodium chloride, sodium bicarbonate or sodium acetate affects the desorption process. J. B. S.

ORGANIC CHEMISTRY

Chloramphenicol. A Synthetic Approach. L. M. Long and H. D. Troutman. (*J. Amer. chem. Soc.*, 1949, **71**, 2469.) Compound (I), *dl-threo*-1-phenyl-2-amino-1:3-propandiol is an important intermediate in the synthesis of chloramphenicol (II); a previous communication (*J. Amer. chem. Soc.*, 1949, **71**, 2463), gave the information that the tricetyl derivative of (I) could be nitrated in the *para* position, and the *p*-nitro derivative of (I)



(I)



(II)

could be converted to (II) by resolution and dichloroacetylation of the D-(-)-isomer. Methods for the preparation of (I) and its derivatives were investigated. α-Acylamidoacetophenones, prepared by reduction of α-nitroacetophenone and subsequent acylation were hydroxymethylated with paraformaldehyde in the presence of sodium carbonate to form α-acylamido-β-hydroxypropiophenones. The ketonic group was then converted into the secondary alcoholic group by hydrogenation in methanol with Raney nickel catalyst, and the diastereoracemates separated by crystallisation.

A. H. B.

Chloramphenicol Synthesis through *p*-Nitroacetophenone. L. M. Long and H. D. Troutman. (*J. Amer. chem. Soc.*, 1949, **71**, 2473.) *p*-Nitroacetophenone was brominated to produce *p*-nitro-α-bromoacetophenone which was converted to *p*-nitro-α-aminoacetophenone *via* the hexamethylenetetramine salt. Acetylation of this compound to give *p*-nitro-α-acetamidoacetophenone was accomplished using acetic anhydride in the presence of sodium acetate or sodium bicarbonate. The next step involved monohydroxymethylation with aqueous formaldehyde in presence of sodium bicarbonate to produce *p*-nitro-α-acetamido-β-hydroxypropiophenone, which was reduced with aluminium isopropoxide to produce *di-threo*-1-*p*-nitrophenyl-2-acetamido-1 : 3-propanediol in good yields and the corresponding *dl-erythro* racemate in much lower yields. The acetyl group was then removed by hydrolysis with dilute acid to yield *dl-threo*-1-*p*-nitrophenyl-2-amino-1 : 3-propanediol, from which the chloramphenicol intermediate aminediol may be obtained by resolution. A. H. B.

2 : 4-Dinitrothiophenol as Reagent for Organic Halogen Compounds. R. W. Best, P. K. Starnes and E. L. Wood. (*J. Amer. chem. Soc.* 1951, **73**, 1968.) The use of 2 : 4-dinitrothiophenol as a reagent for the characterisation of aliphatic halogen compounds has been examined. This method for the formation of alkyl 2 : 4-dinitrophenyl sulphides is complementary to the reaction between 2 : 4-dinitrochlorobenzene and allylmercaptans (Best *et al.*, *J. Amer. chem. Soc.*, 1932, **54**, 1985; 1933, **55**, 4956). Primary and secondary alkyl chlorides, bromides and iodides dissolved in butyl carbitol or ethanol and in the presence of potassium hydroxide react readily with this substance; in many cases the reaction is spontaneous and no heat is required. The reaction is applicable to substituted alkyl halogen compounds including halohydrins, halonitriles, haloamides, haloesters, haloketones and haloethers. Vicinal and disjunctive halogen compounds also react but not aryl halides, unless there is considerable activation of the halogen. Oxidation of the resulting alkyl 2 : 4-dinitrophenyl sulphides to the corresponding sulphones is readily accomplished, with the exception of those derived from halohydrins, haloketones and haloethers, and affords ease of access to a secondary derivative. J. B. S.

Khellin, Visnagin and Khellol-glucoside, Ultra-violet, Infra-red and Polarographic Studies on. S. D. Bailey, P. A. Geary and A. E. de Wald. (*J. Amer. pharm. Ass. Sci. Ed.*, 1951, **40**. 280.) The ultra-violet and infra-red absorption curves, and the polarographic half-wave potentials are reported for khellin, visnagin and khellol-glucoside. These three principles are found in the seeds of the plant *Ammi visnaga* Linn. The ultra-violet absorption was measured in 95 per cent. ethanol and cyclohexane in the region from 210 to 380 mμ. All three absorption bands of visnagin are displaced to longer wave-lengths with the additional methoxy group in khellin which is capable of resonating with the conjugated ketone structure. In cyclohexane this difference between khellin and visnagin is sufficient to provide a means of quantitatively determining these substances in mixtures of the two substances. The infra-red spectra of both synthetic and naturally occurring khellin and visnagin are presented, and these results confirm the structures which have been assigned to these active principles. The absorption spectra from 2 to 12 μ was measured in chloroform solution. Analytical estimations of khellin and visnagin in plant fractions were made using the range 8 to 10 μ. The infra-red absorption spectrum of khellol-

glucoside was obtained from a suspension of the solid material in a paraffin base. Khellin, visnagin and khellol-glucoside were reduced at the dropping mercury electrode, and the half-wave potentials reported. Khellin and visnagin gave a single wave step, whereas the glucoside showed a two-step reduction. The diffusion current values were found useful for the quantitative estimation of combined khellin and visnagin. A. H. B.

L-Thyroxine, a Synthesis from L-Tyrosine. J. R. Chalmers, G. T. Dickson, J. Elks and B. A. Hems. (*J. chem. Soc.*, 1949, 3424.) In preliminary experiments L-tyrosine was converted to the corresponding hydantoin and nitrated to L-5-(3 : 5-dinitro-4-hydroxybenzylhydantoin, (I); the latter treated with *p*-toluenesulphonyl chloride in pyridine followed by the action of *p*-methoxyphenol in the same solvent yielded L-5-(3 : 5-dinitro-4-*p*-methoxyphenoxybenzyl hydantoin (II). Catalytic reduction of II followed by tetrazotisation and conversion to L-5-(3 : 5-di-iodo-4-*p*-methoxyphenoxybenzyl) hydantoin yielded a product which had undergone considerable racemisation. Treatment of I with *p*-toluenesulphonyl chloride in pyridine, followed by reaction with quinol gave L-5-(3 : 5 : dinitro-4-hydroxyphenoxybenzyl) hydantoin (III), which was converted *via* the diamine to L-5-(3 : 5-di-iodo-4-*p*-hydroxyphenoxylbenzyl)hydantoin of high optical purity. The following synthetic route was finally adopted. L-tyrosine was nitrated to 3 : 5-dinitro-L-tyrosine, which after N-acetylation and esterification was converted by reaction with *p*-toluenesulphonyl chloride and *p*-methoxyphenol in pyridine into 3 : 5-dinitro-4-*p*-methoxyphenoxy-N-acetyl-L-phenylalanine ethyl ester (IV). Reduction of IV, tetrazotisation and reaction with iodine and aqueous sodium iodide yielded 3 : 5-di-iodo-4-*p*-methoxyphenoxy-N-acetyl-L-phenyl-alanine ethyl ester (V), which on hydrolysis and demethylation gave 3 : 5-di-iodo-L-thyronine (VI). Iodination of VI with iodine in ethylamine gave L-thyroxine in an overall yield of 26 per cent. J. B. S.

TOXICOLOGY

Arsenic in the Hair. H. Griffon and J. Barbaud. (*C.R. Acad. Sci., Paris.* 1951, **252**, 1455.) The presence of arsenic in the hair has been followed by exposing the hair to thermal neutrons, so that the arsenic in it becomes artificially radio-active, a correction being applied for the other elements normally present which also become radio-active. Comparison with chemical analyses carried out on the same lengths of hair showed satisfactory agreement. G. M.

Cadmium, Toxicological Determination of. R. Fabre, R. Truhaut and C. Boudène. (*Ann. pharm. franc.*, 1951, **9**, 30.) The method is based on the nephelometric determination of cadmium as brucine iodocadmiate. To avoid loss of cadmium by volatilisation, organic material is first macerated overnight with twice its weight of nitric acid, then heated until the volume is reduced to one-third. The liquid is treated with twice its volume of mixed sulphuric and nitric acids (1 + 2) and the temperature is raised gradually. A mixture of nitric and perchloric acids (1 + 2) is added cautiously in small portions. When decolorisation is complete, the liquid is evaporated, taking care to avoid loss of cadmium by volatilisation. If the residue is coloured, it is treated with a little nitric acid and reheated, but without ashing. Oxides of chlorine are removed by boiling with 10 ml. of sulphurous acid, the solution is filtered and cooled. In order to concen-

trate the cadmium, the solution is diluted to 75 ml. and treated with 2 g. of sodium citrate and 5 ml. of copper sulphate solution containing 250 μg. of copper. Bromophenol blue is added, and the *p*H is adjusted to 3 by careful addition of ammonia. The solution is treated with hydrogen sulphide for 5 minutes at the boiling-point, and for a further 5 minutes with the flame removed, then with 1 drop of 5 per cent. solution of aluminium chloride. After 24 hours the precipitate is filtered off, washed with hydrogen sulphide water, and dissolved in aqua regia. After evaporation on the water-bath, the residue is dissolved in water, adjusted to *p*H 2·5, and treated with cupferron, the copper compound being finally extracted with ether-chloroform. The aqueous solution is evaporated with a few drops of aqua regia, and the residue is taken up in a little water. The *p*H is adjusted, if necessary to about 3; the solution is filtered, and 3 ml. or a suitable quantity, is taken for the assay, and treated with 3 drops of a solution of 24 g. of brucine and 5·5 ml. of glacial acetic acid in 100 ml., then with 1 drop of potassium iodide solution (100 per cent. w/v). After 10 minutes the turbidity is determined nephelometrically. G. M.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Folic Acid, Action of Ascorbic Acid on. S. Scheindlin and I. Griffith. (*Amer. J. Pharm.*, 1951, **123**, 78.) In the presence of methyl carbitol at *p*H 3·5 folic acid is rapidly decomposed by ascorbic acid. Impurities or the methyl carbitol itself also cause decomposition and the reaction may be accelerated by the addition of citric acid. Propylene glycol (50 per cent.) has no effect on folic acid and in the presence of this solvent the degradative activity of ascorbic acid is reduced. In this case, ascorbic acid does not accelerate the reaction. At *p*H values about 6·5, in water, the reaction occurs rapidly, about 20 per cent. of the folic acid content being lost in 4 weeks whilst control solutions lost only 3 per cent. *p*-Aminobenzoic acid also reacts with aqueous ascorbic acid solutions at low *p*H values. In 50 per cent. methyl carbitol this reaction is slower, and it is slower still in water at *p*H 6·5. A. D. O.

Insulin, Terminal Peptides of. F. Sanger. (*Biochem. J.*, 1949, **45**, 563.) The method of protein end-group assay, whereby the protein, treated with 2:4-dinitrofluorobenzene, is hydrolysed to yield the *N*-2:4-dinitrophenyl derivatives of the terminal amino-acids, has been extended to determine the sequence of amino-acids in close association with the terminal amino-acids of insulin. The protein, after treatment with 2:4-dinitrofluorobenzene, is partially hydrolysed to a series of *N*-2:4-dinitrophenyl peptides, which are readily isolated from other peptides by their more strongly acidic properties. Individual *N*-2:4-dinitrophenyl peptides are separated by chromotography on silica gel. The results indicate that both the terminal phenylalanyl residues of insulin are present in the amino-acid sequence phenylalanyl-valyl-aspartyl-glutamic acid and that both the terminal glycyl residues are present in the peptide sequence glycyl-*iso*leucyl-valyl-glutamyl-glutamic acids; both the lysyl residues, in the same chain as the phenylalanyl residues are present in the peptide threonyl-prolyl-lysyl-alanine. In a critical discussion of the evidence available upon the composition of insulin, it is concluded that the molecule is built up of two closely similar polypeptide chains. J. B. S.

Vasopressin, Preparation of Pure. P. Fromageot and H. Maier-Hüser. (*C.R.Acad. sci. Paris*, 1951, **232**, 2367.) This paper describes the preparation, from the posterior lobe of the hypophysis, of a highly active preparation which probably represents a pure vasopressin. The activity is about 1,000 I.U./mg., and there is an oxytocic activity corresponding to 18 I.U. oxytocin per mg. G. M.

Vitamin B_{12}, an Analogue of. R. P. Buhs, E. G. Newstead and N. R. Trenner (*Science*, 1951, **113**, 625.) In this compound, which is biologically equivalent to vitamin B_{12}, the cyanide ion has been replaced by thiocyanate. It is prepared from vitamin B_{12} by reaction with excess of potassium thiocyanate in the molar ratio of 10:1 at room temperature for a few hours. Excess of acetone is then added and the mixture maintained at 5°C. for 24 hours. The compound crystallises in dark purple-red needles. Acute toxicity tests in mice showed that the thiocyanate analogue was not toxic at the equivalent dose of 3·2 mg. for a 70-kg. man and preliminary clinical trials have shown it to be fully active in pernicious anæmia. The ultra-violet absorption spectrum of the compound between 6000 and 2200 Å is practically identical with that of vitamin B_{12} and in the infra-red there is an absorption band at 4·70μ. It is hygroscopic and is decolourised by ascorbic acid. A. D. O.

BIOCHEMICAL ANALYSIS

Adrenaline and Noradrenaline, a Distinguishing Colour Reaction on Paper Chromatograms. A. J. Glazko and D. W. Dill. (*Nature*, 1951, **168**, 32.) Samples of adrenaline were dissolved in methanol containing the minimum quantity of hydrochloric acid and chromatographed on paper, either descending on strips or on circular discs of filter paper using Rutters' technique. Phenol saturated with water is used as a developing solvent in an atmosphere of hydrochloric acid. The chromatograms are sprayed with a freshly prepared buffered solution of sodium β-naphthoquinone-4-sulphonate. Noradrenaline slowly develops an intense blue colour (15 to 30 minutes), adrenaline gives a pink colour, and dihydroxyphenylalanine gives a yellow colour, gradually changing to blue-grey. The latter distinction between dihydroxyphenylalanine and noradrenaline is important since their R_F values are close. Careful control of *p*H is necessary to prevent discoloration of the chromatograms. J. B. S.

Potassium in Serum, Colorimetric Determination of. J. Colin. (*Bull. soc. chim. biol.*, 1951, **33**, 394.) When preparing serum or plasma for use with the following methods, oxalates must not be used: sodium polyanethol sulphonate or heparin are suitable. About 0·4 ml. of the liquid is treated with sulphuric and nitric acids in a platinum dish to destroy organic matter, and ashed. The reagent used for the estimation is a solution of 3 g. of hexanitrodiphenylamine and 1 g. of anhydrous sodium carbonate in 100 ml. of water. This solution, as also the saturated solution of the potassium salt of hexanitrodiphenylamine, is cooled in ice and filtered immediately before use. The inorganic residue from the serum is taken up in 0·6 ml. of water, and of this the largest possible aliquot is treated with 0·4 to 0·5 ml. of reagent, added drop by drop, and placed in ice. After shaking and rubbing until a definite precipitate is obtained, the mixture is allowed to stand for 20 minutes in ice, and centrifuged. The residue is

washed with 0·5 ml. of iced solution of the potassium salt, then similarly with 0·5 ml. of iced water. The precipitate is dissolved in 2 ml. of acetone, and transferred to a 50 ml. measuring flask with 0·001 N sodium hydroxide. The colour strength is then determined photometrically in blue light. G. M.

Raffinose and Sucrose, Identification of, on Paper Chromatograms, by Enzymatic Hydrolysis. K. T. Williams and A. Bevenue. (*Science*, 1951, **113**, 582.) 1 μl. of a solution containing 10 to 50 μg. of sucrose or raffinose was placed on a chromatogram strip and 5 μg. of an invertase solution was immediately superimposed on the spot. After 5 minutes it was found that hydrolysis of the sugar was complete. The chromatograms were then prepared using *n*-butanol-ethanol-water (10-1-2) as the solvent and on spraying with dinitrosalicylic reagent (0·5 per cent. of 3:5-dinitrosalicylic acid in 5 per cent. sodium hydroxide solution), melibiose and fructose from the raffinose and dextrose and fructose from the sucrose were found to be adjacent to known pure sugars used as controls. Partial hydrolysis was also effected by covering only half of the sugar spot with enzyme solution and in this case both the original substances and the products of hydrolysis were detected. A. D. O.

Spectrophotometric Assay of Ascorbic Acid with *peri* Naphthindanetrione hydrate. El Ridi, R. Moubasher and Z. F. Hassan. (*Biochem. J.*, 1951, **49**, 246.) A new spectrophotometric estimation of ascorbic acid depending on the colour reaction obtained with *peri*naphthindanetrione hydrate and its derivative 2-nitro-*peri*naphthindanetrione hydrate is described. The colours are due to the formation of dihydroxy*peri*naphthindenone and dihydroxy-2-nitro*peri*naphthindenone respectively and are stable for about 24 hours. A number of substances, reductones, reductic acid and cysteine interfere, producing the same red colour. No interference was experienced with glucose, fructose, alanine, leucine, isoleucine, phenylalanine, lactic acid, acetoacetic acid, pyruvic acid, urea, uric acid, acetone and dehydroascorbic acid. The absorption bands of the reagents are quite distinct and separate from those of the colours produced. The method is applicable to pure solutions of ascorbic acid, and to urine after preliminary precipitation with trichloracetic acid. Estimation of ascorbic acid in blood plasma is also possible, after preliminary treatment with ammonium sulphate and metaphosphoric acid. Results comparable with those by other methods were obtained. The reagents are easily prepared, inexpensive and stable. J. B. S.

Thiosemicarbazones: Estimation and Photolability of. A. Spinks. (*Brit. J. Pharmacol.*, 1951, **6**, 35.) *p*-Aminobenzaldehyde thiosemicarbazone (6198) and *p*-acetylaminobenzaldehyde thiosemicarbazone (berculon A, conteben, TB1) have been estimated simultaneously by extracting them from blood with chloroform, reading the optical densities at 320 and 342 mμ and solving simultaneous equations. Compound 6198 is acetylated by the mouse to berculon A, but deacetylation of berculon A has not been demonstrated in mouse, dog or man; the latter can therefore be estimated directly in these species by reading optical density at $\lambda_{max.}$ 330 mμ only. A graphical method of estimating berculon A or berculon B (8388; *p*-ethylsulphonylbenzaldehyde thiosemicarbazone) in blood in therapeutic concentrations of 0·02 to 0·2 mg./100 ml. is described. The direct technique cannot be used to estimate such small amounts because of the high and variable absorption of an extract of normal blood. In the mouse and dog berculon A gives blood concentra-

tions about a quarter as high as those of berculon B. The photolability of the thiosemicarbazones is described and a possible method of stabilisation is discussed. Because of their photolability thiosemicarbazones must be estimated in the darkroom. Weak, unfiltered tungsten light may be used.

S. L. W.

Trichlorethylene in Air and Biological Media, Determination of. R. Truhaut. (*Ann. pharm. franc.*, 1951, **9**, 175.) After a survey of the methods available for the determination of trichlorethylene, the author describes an improved form of pyridine reaction. For determination in air, the sample is bubbled through pyridine (10 m. and 5 ml.), contained in two tubes cooled in ice and salt, at about 10 l. per hour. The contents of the tubes are mixed, and 5 ml. of the liquid is treated with 1 ml. of a 1 per cent. alcoholic solution of sodium hydroxide. The mixture is placed in a water-bath at 70°C. for exactly 3 minutes, cooled, and treated with 3 ml. of water. After 60 to 90 seconds (but not more than 3 minutes) the colour is determined, using filter S53. For the determination in organs of the body, the trichlorethylene is removed and carried over by a current of air at 50°C. This modification is much more specific than the original form; carbon tetrachloride does not interfere appreciably, and chloroform only at comparatively high concentrations (1000 μg.) Positive reactions are given by tetrachlorethylene and tetrachlorethane. The limit of sensitivity is about 4 μg.

G. M.

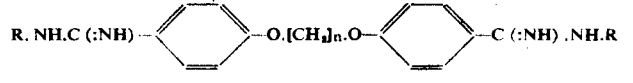
Vitamins D, Analysis of. J. Green. (*Biochem. J.*, 1951, **49**, 232, 243.) A general method is described for the chemical determination of vitamin D in irradiation products of ergosterol and 7-dehydrocholesterol. The oil in which the sample is dissolved is saponified and the resulting alkaline solution extracted with ether. The solid remaining on evaporation of the ethereal extract is dissolved in light petroleum and chromatographed on a column of floridin earth under standardised conditions and finally eluted in carbon tetrachloride. Digitonin precipitation is used to remove non-steroid and precipitable steroid material and the resulting material estimated absorptiometrically after reaction with either iodine trichloride or antimony trichloride and acetyl chloride. It has been shown that the non-saponifiable fractions of olive oil and arachis oil in the presence of vitamin D yield greater amounts of iodine from iodine trichloride than would theoretically be expected, in spite of the fact that alone these fractions yield negligible amounts of liberated iodine with vitamin D. These substances are, however, eliminated by the chromatographic procedure. Good agreement between results obtained by the above $SbCl_3$ determination and bioassay figures is recorded. Results with ICl_3 titration are slightly high. Quantitative studies have confirmed that the formation of calciferol from ergosterol occurs much more rapidly in ether than in benzene solution. The kinetics of the process have been examined and the destructive action of ultra-violet light on vitamins D has also been studied; this latter effect is slower in benzene than in ether solution. The crude destruction product yielded on extraction with light petroleum a new insoluble substance, isolated as a light yellow amorphous powder described as suprasterol III. It could not me obtained in crystalline form and did not form a crystalline 3 : 5-dinitrobenzoate. The irradiation of 7-dehydrocholesterol has been studied by similar methods. A scheme is suggested for the total analysis of the components of an irradiation product. A number of fish liver oils of high, medium and low potency have been

assayed chemically for vitamin D by this method. The results compare favourably with those obtained by bioassay. Double chromatography on floridin has been shown to be necessary for the removal of residual interference with oils in which the ratio of vitamin A to vitamin D is high. Good recoveries of vitamin D are difficult to obtain with the double chromatographic procedure on fish liver oils. The use of floridin columns up to 15 cm. in length has been investigated and appears to be quite safe. J. B. S.

CHEMOTHERAPY

Alanine Derivatives as Bacteriostatics. D. F. Elliott and Sir Charles Harington. (*J. chem. Soc.*, 1949, 1374.) Syntheses of amino acids of the β-phenylalanine series substituted in the para position with guanidino, guanidinomethyl, *p*-aminophenylsulphonyl and mercapto groups are recorded. β-*p*-Guanidinophenylamine, $H_2N.C(:NH).NH.C_6H_4.CH_2.CH(NH_2).COOH$, I, was prepared by the action of cyanamide on ethyl acetamido-4-aminobenzyl malonate, followed by acid hydrolysis. The homologue $H_2N.C(:NH).NH.CH_2C_6H_4.CH_2.CH(NH_2).COOH$, II, was obtained by the condensation of ethyl acetamido-4-aminomethylbenzylmalonate with *S*-methyl-*iso*-thiourea and hydrolysis of the product. β-*p*-(p′-Aminophenylsulphonyl)phenylalanine, $p\text{-}H_2N.C_6H_4.SO_2.C_6H_4.CH_2.CH(NH_2).COOH$, III, was synthesised from *p*-nitrophenyl-*p*-tolyl sulphide, which was oxidised to the corresponding sulphone, brominated in the ω-position and condensed with ethyl acetamindomalonate; hydrolysis of the product followed by catalytic reduction yielded the required product. A new and better method of preparation for β-*p*-mercaptophenylalanine, IV, is also described; 4:4′-dicarboxydiphenyl disulphide was reduced with sodium in liquid ammonia and condensed in the same operation with benzyl chloride to give *p*-benzylthiobenzoic acid. The latter was converted to *p*-benzylthiobenzaldehyde by the method of McFadyen and Stevens, which on condensation with hippuric acid and subsequent hydrolysis of the product yielded α-benzamido-*p*-benzylthiocinnamic acid, $Ph.CH_2S.C_6H_4.CH:C(NH\ Bz).COOH$; reduction and hydrolysis gave IV. Thianaphthen-3-aldehyde, obtained from thianaphthen-3-carboxylchloride (Rosenmund reduction) and from thianaphthen-3-carboxy-*p*-toluenesulphonylhydrazide, was condensed with hippuric acid and the resulting oxazolone reduced directly to benzoy-β-3-thianaphthenylalanine; the latter upon hydrolysis gave β-3-thianaphthenylalanine (V). None of the amino acids I-V inhibited the growth of *Staph. aureus* or of *E. coli in vitro*. V inhibited the growth of *Strep. hæmolyticus* at 1 in 20,000. The sulphone III had a slight effect against *M. tuberculosis in vitro*, but was less effective than 4:4′-diaminodiphenyl sulphone. J. B.S.

Antituberculous Compounds. Di-(p-arylamidino-phenoxy)-alkanes. M. W. Partridge. (*J. chem. Soc.*, 1949, 2683.) A series of di-(*p*-N-arylamidinophenoxy) alkanes and analogues of the type

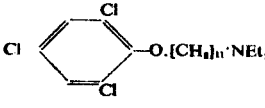


were prepared for a study of the relation between structures and activity against *Mycobacterium tuberculosis*. The compounds included those in which R = Ph and n = 2, 4, 5 and 6, and those in which R was *p*-tolyl, *p*-chlorophenyl, *p*-hydroxyphenyl, and *p*-alkoxyphenyl and n = 3 or 5. These N-substituted

diamidines formed sparingly water-soluble salts; their dilactates were, however, moderately soluble in water. When n was an even number the compounds were inactive, whereas, when the number was odd, high activities were obtained. The presence of serum did not appreciably affect the *in vitro* activity. No activity could be demonstrated against experimental tuberculosis in guinea-pigs, possibly due to the high toxicity of these compounds.

A. H. B.

Antituberculous Compounds. Halogenated ω-aryloxyalkylamines and Analogues. D. J. Drain, D. A. Peak, and F. F. Whitmont. (*J. chem. Soc.*, 1949, 2680.) A series of chloro-ω-aryloxyalkylamines and analogues of the type



were prepared and tested for activity against *Mycobacterium tuberculosis*. The effect of the following structural features was investigated: (i) the extent and position of chlorine substitution; (ii) increase of the chain length between the oxygen and nitrogen atoms; (3) replacement of the terminal basic groups by other basic groups; and (4) replacement of the phenyl by naphthyl and quinolyl nuclei, both chlorinated and unchlorinated. Although high activities were obtained *in vitro* in certain cases, *in vivo* activity could not be demonstrated.

A. H. B.

Curarising Agents, New Synthetic. R. Hazard, J. Cheymol, P. Chabrier, E. Corteggiani and F. Nicholas. (*Therapie*, 1950, 5, 129.) Of 17 new compounds synthesised, the one having the curarising action in the purest form and the most free from side reactions was the diiodoethylate of *NN'*bis(piperidylethyl)piperazine. (336H.C.). This has only 1/66 of the toxicity of curare, but its effect on the tonus of the neck muscles is 1/15th. At equal toxicities, it is proportionally more active than the natural compound with all the tests except one, on mammals and frogs. The duration of curarisation with rabbits under artificial respiration is only one-half as prolonged as with curare. The inhibiting action on cholinesterase is 4 times greater; it does not affect the arterial pressure; and the curarising action is suppressed by eserine or prostigmine.

G. M.

Papaverine Derivatives, Comparative Spasmolytic Activity and Local Anæsthetic Activity in. A. Quevauviller and S. Garcet. (*Therapie*, 1950, 5, 69.) The following table gives, for a number of derivatives of papaverine, the toxicity, spasmolytic activity, and local anæsthetic activity (calculated with reference to cocaine):

Substance	M.L.D. 50 for mice g/kg.	Spasmolytic activity	Relative local anæsthetic activity
Papaverine	0·22	1	0·5
Dihydropapaverine	0·32	1	0·25
Dihydrobromopapaverine	0·28	2	0·6
Tetraethoxybenzylisoquinoline (perparine) ...	0·66	7	7·2
Dihydroperparine	0·12	10	9·1
Dihydrobromoperparine	1·23	7	9·8

Thus the surface anæsthetic power and the spasmolytic musculotropic power vary in the same direction. This concordance between two different pharmacological actions may be explained by physico-chemical considerations. It appears that papaverine spasmolytics, like narcotics, local anæsthetics and vagotonics, show activities which run in the same order as the interfacial tension of solutions against oils, in accordance wth the theory of Traube. G. M.

PHARMACOGNOSY

Digitalis Species, Comparative Assays of. L. Fuchs, E. Soos and I. Kabert. (*Sci. pharm.* 1951, **19**, 73.) The following table summarises the results of a series of colorimetric and biological assays, applied to various species of digitalis.

Drug	Colorimetric assay, calculated as percentage of digitoxin, Method of				Biological activity, referred to International standard	
	Baljet	Legal	Kedde	Keller-Kiliani	Frog method	Guinea-pig method
D. ferruginea	0·304	0·30	0·31	0·23	1·24	1·31
D. lanata	0·33	0·37	0·34	0·24	1·43	2·53
D. orientalis	0·23	0·22	0·23	0·08	5·00	3·05
D. sibirica	0·21	0·20	0·17	0·20	0·40	0·66
D. purpurea	0·26	0·24	0·22	0·20	1·08	0·86
D. purpurea (from American seeds) ...	0·15	0·15	0·15	0·09	0·35	0·55
D. ambigua	0·16	0·15	0·15	0·23	0·91	0·86
D. lutea	0·26	0·27	0·27	0·27	1·25	0·68
D. parviflora	0·21	0·22	0·21	0·24	0·91	0·94

As expected, the first three colorimetric methods show good agreement, as they all respond to the aglycone fractions, while the fourth one, reacting with digitoxose, gives somewhat differing results. With the biological methods, *D. lanata, D. orientalis* and *D. lutea* especially show deviations. It is thus essential that every species should be tested against a standard powder of the same species, as is already done in Austria for *D. lanata.* G. M.

***Holarrhena antidysenterica* Wall., Seasonal Variation of Alkaloids in.** A. T. Dutta, B. K. Ghosh and J. C. Gupta. (*Ind. J. med., Res.*, 1950, **38**, 467.) The total content of conessine and holarrhenine in the various parts of the air-dried plant was determined at different times of the year. In the bark and root-bark the alkaloidal content was greatest during February, August, November and December (up to 3·9 per cent.) and lowest from March to July (about 3·0 per cent.). The alkaloidal content of the stem was high from July to January (about 0·5 to 0·7 per cent.) and lowest in February (0·25 per cent.) whilst the content of the leaves was low during February and March (0·58 per cent.) and at a maximum during June (1·56 per cent.). Fresh seeds obtained in May had a content of 1·82 per cent. which is higher than any previous reports. The maximum amount of alkaloid occurred in the bark of plants between 8 and 12 years old.

A. D. O.

PHARMACOLOGY AND THERAPEUTICS

Aneurine, Potentiation of Local Anæsthetics by. G. Dastugue and J. Mathon. (*Therapie.* 1950, **5**, 23.) It has been shown that by combining aneurine with a local anæsthetic, especially procaine, the dose of the latter may be reduced by one-third or even one-half. One of the authors has shown that the hydrolysis of procaine by procaine-esterase may be reduced by 30 to 50 per cent. by aneurine and it is suggested that this fact offers an explanation of the potentiation. It appears that a similar enzyme is present, though to a much smaller extent, in cerebrospinal fluid. Although this theory does not explain the case of percaine, it is possible that a similar enzyme, capable of splitting percaine, may be present in the tissues. G. M.

Dihydrostreptomycin and β-Pyridine Aldehyde Thiosemicarbazone, Synergistic Action of. C. Levaditi, A. Girard, A. Vaisman, A. Ray and H. Chaigneau-Erhard. (*C.R. acad. Sci. Paris,* 1951, **232**, 770.) β-pyridine aldehyde thiosemicarbazone (G. 469) has been shown to be more effective than *p*-acetylaminobenzaldehyde thiosemicarbazone and equivalent in activity to streptomycin in tuberculous mice. It is now shown that in tuberculous mice combined treatment with β-pyridine aldehyde thiosemicarbazone and dihydrostreptomycin is more effective than treatment with either of the two medicaments used alone in equivalent doses.

J. B. S.

Folinic Acid (Citrovorum Factor), Hæmatological Effect of, in Persons with Pernicious Anæmia. T. Jarrold, D. Horrigan, C. Thompson and R. W. Vilter. (*Science,* 1951, **113**, 688.) It is suggested that folinic acid may be a biologically important intermediate in the metabolism of folic acid, and therefore its hæmatopoietic effect was tested in persons with pernicious anæmia in relapse. Three such subjects were injected intramuscularly with folinic acid for 10 consecutive days. Hæmatological responses occurred, but folinic acid was no more effective than folic acid. Folinic acid failed to produce a local erythrocyte maturation effect on instillation into the marrow cavity, suggesting that, like folic acid, it must be altered elsewhere in the body before becoming active in hæmatopoiesis.

A. H. B.

Gallamine Triethiodide, Pharmacology and Clinical Use of. A. G. Doughty and W. D. Wylie. (*Proc. R. Soc. Med.,* 1951, **44**, 375.) Gallamine triethiodide (Flaxedil) a synthetic curarising agent, is tri-(β-diethylaminoethoxy)-benzene triethiodide. It is eliminated from the body more rapidly than tubocurarine, being excreted unchanged by the kidneys. The greater proportion of a dose is eliminated within 2 hours. In cats, a dose causing complete suppression of the gastrocnemius jerk left respiratory ventilation at 30 per cent. of normal whereas with tubocurarine respiratory ventilation was 25 per cent. of normal. Clinically, in patients undergoing upper abdominal operations, 10 per cent. needed artificial respiration after 100 to 110 mg. of gallamine triethiodide as compared with 45 per cent. after the approximately equivalent dose, namely 20 mg. of tubocurarine. When used to facilitate intubation, the percentage of easy intubations is about the same with 80 mg. of gallamine triethiodide as with 20 mg. of tubocurarine.

The muscle relaxant effect of the compound is completely and immediately suppressed by neostigmine; with tubocurarine the antidotal effect of neostigmine is only completed over a period of 10 minutes. Both drugs on injection liberate histamine but the amount is considerably less with gallamine triethiodide although some flushing of the skin and possibly a slightly increased bleeding at the site of operation may result. These effects are not abolished by antihistamines. Tachycardia follows the injection of gallamine triethiodide in conjunction with all anæsthetic agents except cyclopropane; with the latter it depends on the depth of anæsthesia. The onset is as prompt as that of the curarising effect, occurring within 1 to 1½ minutes and continuing after the curarising action ceases to be noticeable. There is no irregularity of rhythm and the tachycardia is thought to be due to vagal block unaccompanied by sympathetic block. In patients with diminished reserves of the heart the tachycardia could result in acute cardiac failure and the drug may not be the relaxant of choice for prolonged major surgery on patients with cardiac disease. In clinical use gallamine triethiodide has a greater margin than tubocurarine between the relaxant dose and the dose causing diaphragmatic paralysis. It is therefore preferred for use in conjunction with anæsthetic procedures without controlled respiration. It is also preferred for intubation and bronchoscopy. The short duration of its action makes it advantageous in short operations otherwise needing deep anæsthesia such as hæmorrhoidectomies. H. T. B.

Hexœstrol, Action of, on Rats. J. Cheymol, Y. Gay and J. P. Lavedan. (*Ann. pharm. franc.*, 1951, **9**, 59.) Hexœstrol, injected into male rats, produces atrophy of the tissues of the genital tract. This atrophy, with a partial diminution of the secretion, is more important than the hypertrophy of the musculofibrous elements which is balanced by a decrease in weight of the neighbouring glands. With castrated animals, the hypertrophic effect is due to the development of smooth muscle which results in an increase in weight of the glands. The weights of the glands of entire males and of castrated males (prostate and seminal vesicles) tend to approach one another by a decrease in the former case and an increase in the latter. The mechanism of the two cases is however different. G. M.

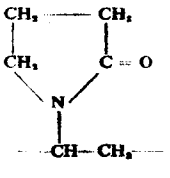
Liquorice Extract, for the Treatment of Addison's Disease. J. Groen, H. Pelser, A. F. Willebrande and C. E. Kamminga. (*New Engl. J. Med.*, 1951, **244**, 471.) A 34 year-old male patient after a course of sodium chloride and deoxycortone acetate, was treated with 1 mg. of deoxycortone daily and 15 g. of a 25 per cent. aqueous extract of liquorice. The sodium and potassium balances were maintained and because of the satisfactory conditions of the patients after 10 days the treatment was changed to 15 g. daily of liquorice extract only. After a temporary loss in weight and upset in sodium and potassium balances the patient remained in excellent condition. 9 days later, treatment was changed and the patient received sodium chloride only for the next 7 days. He relapsed but much more slowly than is normally the case with deoxycortone therapy. Treatment was recommenced at the rate of 30 g. daily, reduced to 20 g. daily after 1 week, and this dosage together with 10 g. of sodium chloride daily was sufficient to maintain the patient in good health. A.D. O.

Methylene Blue, Potentiation of Local Anæsthetics. G. Dastugue, M. M. Roche and J. Mathon. (*Therapie*, 1950, **5**, 26.) The action of local anæsthetics (cocaine, percaine, dunacaine) on Batrachian larvæ is increased

by the addition of a number of dyes (methylene blue, gentian violet, alizarin red, thionine, fluorescein, mercurochrome, Congo red). A similar effect is observed with guinea-pigs. Thus intraperitoneal injection of 7 mg./100 g. of body weight of cocaine hydrochloride, or 4 mg. of methylene blue, is not fatal; 7 mg. of cocaine hydrochloride and 3 mg. of methylene blue is always fatal. Similarly, administration of methylene blue 24 hours before the cocaine increases the mortality. As it is known that methylene blue has a powerful anti-cholinesterase action, it is suggested that the above results may be due to a similar effect on procaine-esterase. It has in fact been shown recently that methylene blue has such an action. G. M

Œstrogens, Observations on Potency and Clinical Assessment of. P. M. F. Bishop, N. A. Richards and W. L. M. Perry. (*Lancet*, 1951, **260**, 818.) A method for comparing the potency of œstrogens consists in giving the œstrogen daily by mouth in 14-day courses to amenorrhœic women and recording whether œstrogen-withdrawal bleeding takes place. The results obtained from a study of 29 patients indicate that stilbœstrol sulphate is half, œstrone one-twentieth and equilin one-third as potent as stilbœstrol. Toxicity, manifested as nausea, was not encountered with these three œstrogens in the range of doses administered (usually from 1 to 4 mg. daily). S. L. W.

Plasmosan, A Plasma Substitute. W. R. Thrower and H. Campbell. (*Lancet*, 1951, **260**, 1096.) Plasmosan is an improved modification of a solution containing polyvinylpyrrolidone which was used to some extent as a plasma substitute in Germany during the 1939-45 war. 100 ml. contains polyvinylpyrrolidone 3·5 g., sodium 361 mg., potassium 22 mg., calcium 9 mg., magnesium 0·06 mg., chloride 582 mg., bicarbonate 17 mg., dissolved carbon dioxide 75 mg. The mineral constituents make it isotonic and the carbon dioxide increases stability. The osmotic pressure is 0·033 to 0·040 atmosphere, which is comparable to the normal values for blood. The polyvinylpyrrolidone used is a white, slightly hygroscopic polymeric substance in which the repeating unit has the formula:



As with most synthetic polymers, the substance consists of molecules with a range of molecular weights. It is stable in aqueous solution, even on autoclaving. The lethal dose for laboratory animals is 8 g./kg., death being due to cardiovascular insufficiency attributable to hyperviscosity of the blood. Doses of 10 to 40 ml. of plasmosan per kg. of body-weight given to rabbits produced no macroscopic changes in tissues examined when the animals were killed 14 or 28 days later except for a small, probably agonal, hæmorrhage in one of them. The livers showed no evidence of storage. Microscopic examination of sections of lung, kidney and liver showed no changes in the rabbits killed after 28 days but some swelling of the cells of the convoluted tubules was noted in the animals killed after 14 days; if due to the plasmosan it was evidently a transitory effect. 4 human patients who had died from their injuries or disease after being given 500 to 1500 ml. of plasmosan showed at necropsy no macroscopic or microscopic changes due to the drug. About 75 per cent. of the polyvinylpyrrolidone injected can usually be recovered from the urine. It exerts no ill-effects on kidney function. After injection of 500 ml., the initial polyvinylpyrrolidone level in the plasma is about 0·35 g./100 ml.; after 50 hours the level is about 0·2 g./100 ml. It does not produce

hæmolysis nor any significant changes in erythrocytes, nor in the clotting-time, bleeding time or blood-grouping reactions. The erythrocyte sedimentation rate is raised. Polyvinylpyrrolidone can be determined in plasmosan, and in serum and urine, by measuring the intensity of the red-brown colour produced on adding a solution of iodine in potassium iodide under specified conditions. H. T. B.

Pentetrazole, Reinforcement of Oxytocic Action of Sparteine by. E. Savini. (*Therapie.* 1950, **5**, 133.) Both pentetrazole and sparteine have an oxytocic action, but differ in pharmacological mechanism, so that the two supplement one another. Sparteine sulphate alone causes a hypertonic, tetaniform contraction of the uterine muscle; pentetrazole transforms this tetaniform contraction into a regular rhythmic contraction—a reinforced physiological contraction. The pentetrazole sensitises the uterus to the action of sparteine. The advantages of the combination of the two drugs are: to obtain an oxytocic synergy; to decrease the respective doses; to correct the depressant action of the sparteine; to combine the analeptic, respiratory and circulatory properties of pentetrazole with the cardiotonic and sedative qualities of the sparteine. G. M.

Thiosemicarbazones in Corneal Tuberculosis. R. J. W. Rees and J. M. Robson. (*Brit. J. Pharmacol.*, 1951, **6**, 83.) Corneal tuberculosis, produced by an intracorneal injection in mice of about 1,000 viable organisms of a bovine strain of *Mycobacterium tuberculosis* was used to assess the chemotherapeutic activity of 4-ethylsulphonylbenzaldehyde thiosemicarbazone (TB3/1374) given alone and in combination with *p*-aminosalicylic acid or streptomycin. TB3 and other active thiosemicarbazones tested showed marked antituberculous activity, producing a prolongation of the incubation period, but over 90 per cent. of eyes eventually developed active tuberculosis. The results suggest that the action of the thiosemicarbazones is essentially bacteriostatic. A combination of *p*-aminosalicylic acid and thiosemicarbazone showed no advantage over the thiosemicarbazone alone, but a combination of streptomycin and thiosemicarbazone showed a definite additive effect, greater than that produced by a combination of streptomycin and *p*-aminosalicylic acid. S. L. W.

Vitamin B_{12} in Pernicious Anæmia, Parenteral Administration. C. C. Ungley. (*Brit. med. J.*, 1949, **2**, 1370.) Vitamin B_{12} was injected intramuscularly in 53 patients with pernicious anæmia in relapse. The rate of increase of red blood cells was found to be a better method for the evaluation of the magnitude of the hæmatopoietic response than the method of Isaacs and Friedman (*Amer. J. med. Sci.*, 1938, **196**, 718), using reticulocyte response compared with the "expected" response to liver extract. There were no unpleasant side-effects, and sensitisation to vitamin B_{12} was not encountered. A summary of 73 responses is presented. Doses of 1·25 μg. had little or no effect, 2·5 μg. produced a definite but small response, 5 to 7 μg. produced on average somewhat below, and to 10 μg. just up to expectation in respect to reticulocytes and increase of red blood cells in 15 days. Larger doses produced still greater increase of red blood cells, the mean response being roughly proportional to the logarithm of the dose in the range 5 to 80 μg. Maintenance doses of 10 μg. of vitamin B_{12} every 2 weeks were satisfactory in 18 out of 21 patients followed for 6 to 15 months. Fresh neurological symptoms did not develop. 6 women developed iron deficiency at 8 to 15 weeks, relieved by ferrous sulphate. A. H. B.