REVIEW ARTICLE

FOLIC ACID, VITAMIN B_{12} AND ANÆMIA

I. CHEMICAL ASPECTS*

By E. Lester Smith, D.Sc., F.R.I.C.

Senior Biochemist, Glaxo Laboratories

One of the most fascinating stories in the whole realm of Biochemistry is the story of the attack on pernicious anæmia and other macrocytic anæmias; that is to say, those anæmias characterised by red blood cells that are too large but too few, in contrast to the microcytic anæmias in which the red cells are too small, owing generally to iron deficiency.

The story begins in 1926 with the discovery by Minot and Murphy[1] that the so-far incurable pernicious anæmia would respond to large amounts of raw or lightly cooked liver given by mouth. The story has culminated in the isolation and synthesis of folic acid and the isolation of vitamin B_{12}. An interesting feature is that it represents the convergence of a large number of different researches, some having entirely different original objectives.

The discovery of the effectiveness of liver treatment in pernicious anæmia was an immediate challenge to biochemists and medical men; first to produce a less nauseating treatment, then to isolate the active principle in liver, and finally to discover its mode of action. The first objective was accomplished within a few years, but the second and third have remained for over twenty years problems that are not fully solved even now. It was soon found possible to make aqueous extracts of liver and to purify them somewhat by fractionation with alcohol and by other means. The early crude extracts had to be taken by mouth, but the more purified preparations could be given by injection. The active principle appeared to be much more efficiently utilised when given by injection, so that this treatment has remained the standard practice.

The problem of purifying these liver extracts and finally isolating the active principle itself proved exceptionally difficult. This was due not only to inherent difficulties—although it is probably true that the isolation of vitamin B_{12} has been more troublesome than that of almost any other vitamin—but mainly to the lack of a satisfactory assay method. In this country we had to rely entirely on clinical tests on cases of pernicious anæmia in relapse, although in America the final stages of the work were made easier by Shorb's development[2] of a microbiological assay technique.

Space will not permit a review of the earlier stages of this work beyond recalling the names of those concerned. The first contributions by Cohn, Minot, West, Nichols, Howe, Dakin, Jacobson and SubbaRow came from America, and this early work has been fully reviewed in the literature[3,4,5,6]. Further valuable contributions were made by Laland and

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Klem[7] in Norway, while Karrer in Switzerland worked on the problem for a time. This covers the period up to about 1938, by which time purified preparations had been obtained which were fully active in a single dose of a few milligrams.

In order to tell this complicated story in roughly chronological fashion, it is necessary next to consider another series of researches which, until they were practically complete, seemed to have no connection with human anæmias. This work was reported between the years 1939 and 1945. Several groups of American workers had prepared, from liver and from yeast, concentrates that were characterised by two tests. They enhanced the growth rate of *Lactobacillus casei* and other micro-organisms, and they promoted normal growth of chicks on certain deficient diets. These investigations were carried out by research teams in two American industrial laboratories, Parke Davis and Co. and Lederle, and in several academic institutions[3,4,5,6]. A little later a similar or possibly identical factor was isolated in a nearly pure state from spinach, and was named folic acid because it was obtained from leaves. In 1943 the Parke Davis group also obtained crystals from liver, for which they retained their own nomenclature vitamin B_C. Almost simultaneously the Lederle team obtained a crystalline substance from liver, for which they retained the name *L. casei* factor. It soon appeared that these products were identical and a little later it was shown that the same substance could be obtained in crystalline form from yeast.

These commendable achievements did not entirely resolve the intense confusion in this field. It had arisen through the use by different groups of investigators of different animal and microbiological assay techniques; they also used different procedures for the isolation of their factors, which appeared to be distinct, though possibly related, entities. Thus among the animal assays in use were those involving growth rate, anæmia, and feathering in chicks, and anæmia in monkeys, while two different organisms were used in the microbiological work. In addition to folic acid, vitamin B_C and the *L. casei* factor, all the following names have been coined: vitamin B_{10}, vitamin B_{11}, vitamin M, Norit eluate factor, factor S, factor R and factor U.

Many of the discrepancies were cleared up with the discovery of the vitamin B_C conjugates. It was shown that from yeast in particular could be obtained preparations fully active in chick tests, but nearly inactive against *L. casei*. Microbiological activity was released, however, on treating these preparations with enzymes obtained variously from rat liver, hog kidney, chicken pancreas and almond. However, this advance was complicated by the fact that often only part of the total *in vivo* activity was released by some enzyme preparations. This difficulty was later traced to the presence in some materials like yeast extract of an inhibitor for the enzyme.

In 1944 Hutchings, Stokstad[8] and others prepared one of the conjugates in crystalline form from a new source material described as a fermentation residue. It was later shown that it could be broken down enzymatically to folic acid and two molecules of glutamic acid.

Pfiffner and others[9] isolated another conjugate from yeast which could similarly be broken down to folic acid plus six molecules of glutamic acid.

The procedures used for the preparation of these substances were not published until several years after their isolation had been announced. The methods used by the two independent teams were rather similar. Pfiffner and others[10] prepared an aqueous extract of the source material after autolysis or enzymatic digestion. The active principle was then adsorbed on the ion exchange resin Amberlite IR-4 and eluted with aqueous ammonia. The product was readsorbed on Super Filtrol and then on Norit SG-11, elution from each being with aqueous or alcoholic ammonia. An aqueous solution was then extracted with butyl alcohol, first at *p*H 5·6 to remove impurities, and then at *p*H 3 to extract the folic acid. Further purification was effected through the barium and zinc salts. Stokstad and others[11] also employed adsorption on Norit A and Super Filtrol. They also purified the product via the barium salt, but their next step was the preparation of the methyl ester which was then purified by chromatography on Super Filtrol. The ester was finally hydrolysed and the folic acid was crystallised from water.

The crowning achievement in this field was the synthesis of folic acid[12,13], announced in 1945, only two years after its isolation. The intense effort put into this project by the Lederle group is indicated by the fact that this paper had no less than sixteen authors. In the following year the constitution of the substance was revealed. It contains a pteridine nucleus (found also in xanthopterin—a yellow pigment of butterflies' wings) linked to a molecule of *p*-aminobenzoic acid, which is linked in turn to a molecule of glutamic acid. This elucidation of the structure was marked by the introduction of yet another name for the substance—pteroylglutamic acid. Subsequently the conjugates have also been synthesised and it has been proved that the successive glutamyl residues are all linked in the γ position[13,14,15,16,17]. Finally it was concluded that folic acid, vitamin B_C, or *L. casei* factor, whether isolated from yeast, liver, or spinach, was identical with the synthetic product, while all the other factors showing different microbiological activities were almost certainly glutamic acid conjugates.

It was only after the synthesis of folic acid had been accomplished that evidence of its value in human macrocytic anæmias was published[18]. In other words, all this intensive research was directed towards substances appearing at the time to be of value only to micro-organisms, chicks and monkeys. However, the demonstration of its value in human anæmias created a considerable stir in medical and other scientific circles. It seemed at first that it would soon displace liver extract. It was quickly evident, however, that folic acid could not be identical with the active principle in liver because already at that time liver concentrates had been obtained that were far more active, weight for weight, than pure folic acid. Then gradually evidence accumulated as to the clinical shortcomings of folic acid. Naturally this revived interest in

the liver factor and research on its isolation was intensified in this country and America.

Further stages of purification were effected by my own colleagues Hurran, Emery and Parker[19,20], at Glaxo Laboratories, and reported in 1945 and 1946. They carried the work to the stage where a clear-cut and generally maximal response in pernicious anæmia followed a single injection of only 1 mg. of material. At the time this seemed a rather remarkable achievement, but we now know that their product contained only about 1 per cent. of vitamin B_{12}. Nothing further appeared in the literature until 1948 when the Merck team[21], directed by Karl Folkers, dropped a bombshell by announcing their isolation of the anti-pernicious anæmia factor of liver in the form of a deep red crystalline substance; this they christened vitamin B_{12}. In the following week there appeared my own publication[22] on the isolation from liver of two highly active red amorphous preparations, while a few weeks later Parker and I[23] announced the isolation of crystalline material, probably identical with vitamin B_{12}.

Other papers appeared in quick succession on both sides of the Atlantic. The most interesting concern the simultaneous announcement[21,22] of the presence in vitamin B_{12} of an atom of cobalt, an element not previously found in any organic substance of biological origin. On the clinical side[26] it became clear that vitamin B_{12} along with our related red substance represented the true liver principle, active in pernicious anæmia and its associated neurological complications. Moreover this substance set up a new record among the vitamins and hormones by its great potency; the human requirement was assessed at something under 1 μg. per day.

The method used for isolating vitamin B_{12} from liver has now been published by our team[27]. It follows from the unexpectedly high potency of vitamin B_{12} that the proportion present in liver is extremely small. In fact it is of the order of one part per million. Another reason for the difficulty of the isolation was that the substance appears to have no chemical properties that can be utilised (such as the acidity of folic acid, for example). Accordingly our methods were almost exclusively physical. We relied upon repeated adsorption on charcoal and elution with either 80 per cent. phenol or with aqueous alcohols, and adsorption chromatography on alumina and silica. Another invaluable step was partition chromatography on damp silica from butyl alcohol or other solvents. It was desirable to interpose at some stage enzymic proteolysis to break down persistent peptide impurities. The vitamin was finally crystallised in a hydrated form from aqueous acetone.

The structure of vitamin B_{12} has not yet been fully elucidated. Its empirical formula[28] is approximately $C_{61-64}H_{86-92}N_{14}O_{13}PCo$, its molecular weight about 1300. The substance is not a peptide as earlier work with impure preparations had suggested. There is some indication that it may be related structurally to chlorophyll and the pigment of hæmoglobin. It appears to have nothing in common with folic acid. Two fragments have been "chipped off" the molecule, according to

recent reports from England and America; one was thought to be β-aminopropanol, but its true identity remains unknown, and the other a dimethylbenzimidazole[29,30,31,32].

Many vitamins and antibiotics are now known to occur in two or more varieties, closely related chemically. Thus there are several forms of vitamins A. D and E, several varieties of penicillin and two of streptomycin. Vitamin B_{12} is no exception; we have already published evidence for the existence of one and probably two additional closely related red biologically active substances. It has just been announced that one of these has been obtained in crystalline form by Jukes and his colleagues at the Lederle laboratories[33]. It has been named vitamin B_{12B}, the name B_{12A} having already been used for an artificially produced hydrogenation product of vitamin B_{12}.

It is now time to turn to still another group of researches that originally appeared to have no connection with human anæmia. During the war-time scarcity of animal feeding stuffs it was found that chicks raised on vegetable diets grew poorly. Since this trouble was cured or prevented by giving adequate amounts of animal protein, the term animal protein factor was coined. Hammond and Titus[34] showed that dried cow manure was a rich source of this factor. Rubin and others[35,36] showed that hens on an inadequate soya bean meal diet produced eggs of low hatchability, a phenomenon prevented by a purified fraction from cow manure. Independently Zucker[37] in 1948 presented evidence that a factor in animal proteins, which he called zoopherin, improved the growth of rats on a deficient diet. Robblee and others[38] presented evidence for a chick growth factor in fish solubles and described some of its chemical properties. The work of Cary and others[39] had shown that liver extracts active in pernicious anæmia contained a rat growth factor, and by 1948 it seemed probable that all these factors were identical with one another and with the anti-pernicious anæmia factor. Meanwhile it had been observed that chicks seemed to have less need for the factor during the summer months when they were out in the open and had access to food materials contaminated with their own fæces. This observation, along with the activity of cow manure, suggested that the factor might be synthesised by micro-organisms. Finally Stokstad and others[40] showed that the factor could indeed be synthesised by an organism isolated from hen fæces. They showed in addition that this bacterial preparation was effective in cases of pernicious anæmia.

This discovery immediately gave rise to the idea that the pernicious anæmia factor might be obtained by a fermentation process similar to those used for making antibiotics. This was duly followed by the even more exciting idea that the factor might be produced incidentally in some fermentations already in progress industrially. This proved to be so and late in 1948 the Merck team announced the isolation of crystalline vitamin B_{12} from the fermentation products of *Streptomyces griseus*, the organism used to make streptomycin[41].

The discovery that vitamin B_{12} contains cobalt appeared to link up this work with yet another group of researches carried out in Australia in

1935 and onwards. I refer to the independent discoveries by Filmer and Underwood[42], and by Marston[43], that cobalt is a necessary trace element for sheep and cattle. These ruminants when kept on certain pastures in Australia, and indeed in many other parts of the world, develop a fatal sickness that can only be cured by frequent ingestion of minute amounts of cobalt. On some Australian pastures there is an additional requirement for traces of copper. Our first guess was that the animals needed cobalt in order to synthesise vitamin B_{12}. Accordingly we provided Marston with a little of the crystalline vitamin B_{12} for trials on sheep. He has recently reported that the results were entirely negative[44]. The same conclusion was reached independently by Becker and others[45], so it seems that after all this work on cobalt had no direct connection with vitamin B_{12}. Various lines of evidence supported the view that the cobalt was not required by the animal directly, but rather by the rumen bacteria that help it to digest its food. Thus injected cobalt was found to be useless, while on the other hand Albert[46] had demonstrated the unique requirements of some bacteria for cobalt, and more directly Gall[47] and others had very recently shown that oral cobalt administration increases the number, diversity and viability of rumen bacteria in cobalt deficient sheep.

Nevertheless these researches may be linked in another fashion. Vitamin B_{12}, or the animal protein factor, has so far been found only in natural products of animal origin, and yet there is no evidence that animals can synthesise it for themselves; indeed the fact that several species require the substance in their diet is evidence that these at least cannot synthesise it. We are led to suggest, therefore, that vitamin B_{12} originates in nature only through bacterial synthesis, and that it finds its way into the liver and other tissues of herbivorous animals as a result of bacterial synthesis in the rumen or intestine.

The fact that intestinal synthesis of a vitamin is going on within an animal or human being does not necessarily imply no requirement for an external source of that vitamin; if the synthesis occurs too low down in the intestinal tract, then the vitamin may either not be released from the bacterial cells or if released, it may not be absorbed. It has lately been shown, for example, that some pernicious anæmia patients excrete in their fæces amply sufficient vitamin B_{12} to keep them in health if only it could find its way into the blood-stream[48].

It is now known that folic acid is also synthesised by the intestinal flora. Deficiencies of both anti-anæmic factors can be induced, in pigs for example, by giving deficient diets along with suitable sulphonamides to keep down bacterial fermentation in the intestine; folic acid antagonists have sometimes been added as well. In this way there has been demonstrated the need for both vitamin B_{12} and folic acid for normal growth and red cell regeneration. It is probable that both factors are needed by other species and by humans.

The problem of the biogenesis of vitamin B_{12} calls to mind another early group of investigations on pernicious anæmia which I am deliberately taking out of their proper chronological order; I refer to the

work of Castle and his colleagues[49]. They showed conclusively that pernicious anæmia can be treated without having recourse to liver preparations. The alternative treatment consisted in giving by mouth, along with suitable foodstuffs, either normal gastric juice or a preparation from the lining of a hog's stomach. These materials were supposed by Castle to contain an intrinsic factor, which reacts with an extrinsic factor present in certain foodstuffs to produce either the liver factor itself or something equally effective. The value and mechanism of this therapy was rapidly confirmed[50]. Ungley[51], in particular, showed that it was useless to give the hog's stomach extract alone between meals; it was effective only when given simultaneously with the sources of extrinsic factor, although previous incubation of the two was not necessary. It was argued that if the product of the interaction of these factors was identical with the anti-pernicious anæmia factor of liver, then it should prove possible to work up the product from incubating the two factors (using techniques known to be effective with liver) to make a preparation active by injection. Many attempts were made to do this but without success. Castle's suggestion that some intermediate product was formed and required further digestion to convert it into the liver factor could also not be proved experimentally.

There the matter remained until recently, but meanwhile evidence accumulated that some at least of the known sources of extrinsic factor like meat and milk contained low concentrations of vitamin B_{12} itself. When the crystalline vitamin became available it was tested orally and found to be relatively ineffective. On giving it orally, however, along with normal gastric juice, vitamin B_{12} proved to be nearly as effective as when given by injection[52]. This led to the idea that extrinsic factor and vitamin B_{12} are one and the same thing. If this conclusion is accepted, then it solves one mystery—only to pose another; that is, to explain by what means the intrinsic factor renders vitamin B_{12} effective by the oral route. Presumably it must in some fashion facilitate its passage through the wall of the intestinal tract, but it is hard to imagine the details of the mechanism.

This is only one of the mysteries still remaining to be elucidated. In the field of animal nutrition some recent investigations[53] have thrown doubt on the complete identity of the animal protein factor and vitamin B_{12}. In addition another apparently related factor[54] has been isolated from distillers' solubles and christened vitamin B_{13}.

In 1931 Lucy Wills and her colleagues[55] were working on nutritional macrocytic anæmia in India and on a related anæmia in monkeys induced by giving deficient peasant diets. They found that these conditions could be successfully treated with liver fractions that did not appear to contain the anti-pernicious anæmia factor. Independently Ungley[51] has shown that pernicious anæmia could be treated with large oral doses of yeast extracts without intrinsic factor. In the light of subsequent knowledge various reviewers have sought to explain away these findings on the grounds that the effective preparations contained either some vitamin B_{12}, or some folic acid or its conjugate. They very likely did, but the question

remains whether they contained enough of these factors to explain their efficacy. A critical review of the data makes this seem improbable, in which event the nature of the Wills factor still remains to be elucidated.

Finally it remains to be explained how and in what cells of the body this peculiar cobalt compound vitamin B_{12} accomplishes its work of regenerating the red blood cells. This includes the problem of how an injection of only some 10 µg. finds it way to the proper site of action and produces such rapid and dramatic results. Alongside remains also the problem of how larger amounts of a totally different substance, folic acid, achieve, in part at least, the same distant ends.

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RESEARCH PAPERS

STUDIES IN SYNTHETIC ANALGESICS

PART I

BY A. H. BECKETT AND W. H. LINNELL

From the Pharmaceutical Chemistry Research Laboratories of the School of Pharmacy, University of London

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THE reports[1,2] published after the end of the 1939-45 war revealed that German workers had found that compounds of type I possessed pronounced analgesic activity.

$$\text{(I)}\ R_2N.CHR'.CH_2.CPh_2.COR''$$

R=alkyl; R'=H or methyl; R''= alkyl or $-O$ alkyl.

One of the compounds of this class, namely amidone (I; $R=CH_3$, $R'=CH_3$ and $R''=C_2H_5$), was reported to possess 5 to 10 times the analgesic activity of pethidine. These reports led to extensive investigation of related types of compounds. The basic group has been altered; the alkyl chain connecting tertiary nitrogen with quaternary carbon has been varied; the phenyl groups have been reduced, substituted and replaced by other groups; different types of ketones have been investigated. More recently, the ketonic group has been modified to $-CH_2OH$, $-CH_2O.CO.R$, $-CONH_2$, $-C(=NR).C_2H_5$, $-CH(O.CO.R).C_2H_5$, etc. Some of these changes have resulted in producing compounds with increased analgesic activity and reduced side effects.

When this work was commenced, the latter modifications of the ketonic group had not been reported. The present investigation led to the preparation of an alcohol, and a series of esters (II) related to the amidone type of compound, in an attempt to reduce the toxicity of the ketone and provide further information concerning the relationship between structure and analgesic activity.

$$\text{(II)}\ O\langle C_4H_8\rangle N.CH_2.CH_2.C(C_6H_5)_2-CH(OR)C_2H_5 \qquad R = \text{H or acyl}$$

These changes are attended by the production of an asymmetric centre in the molecule, but attempts to resolve any of the compounds have not been made. After the completion of this portion of the work, Speeter *et al.*[3] reported the preparation and analgesic activity of the above alcohol (II; R = H) and its acetyl ester (II; $R = -CO.CH_3$).

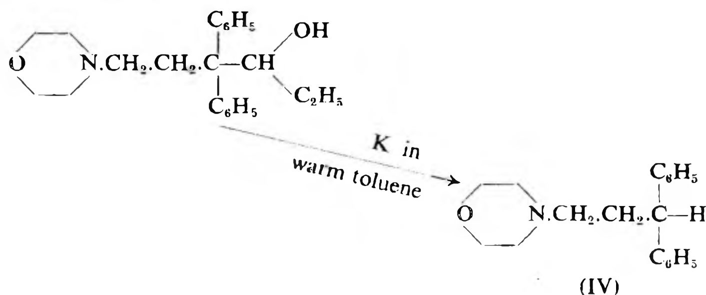
The ketone (III), from which the alcohol (II) was obtained by reduction, was prepared according to the method described by Hems *et al.* (private communication, now published[4]).

$$\text{(III)}\ O\langle C_4H_8\rangle N.CH_2.CH_2.CPh_2.CO.C_2H_5$$

This reduction did not proceed readily, and it was finally accomplished by aluminium *iso*propoxide, using the method recommended for ketones resistant to reduction[5]. The reaction required 100 hours, and the alcohol (II; R = H) was obtained in 80 to 90 per cent. yields. In the course of this reduction, it was discovered that a solution of sodium nitroprusside, used as described in the experimental section, was a more reliable test for a trace of acetone in solution in toluene than the test using 2:4-dinitrophenylhydrazine described in "Organic Reactions"[6], May and Mosettig[7] have reported that amidone resisted reduction by aluminium *iso*propoxide, and accomplished their reduction using hydrogenation with platinum oxide as catalyst. More recently, Speeter *et al.* (*loc. cit.*) found that lithium aluminium hydride was very effective for reducing the above type of ketone.

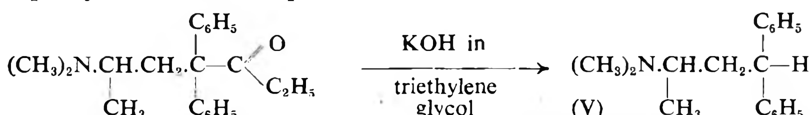
The esters from the alcohol (II; R = H) were prepared by refluxing a toluene solution of the alcohol, under anhydrous conditions, with the appropriate acid chloride for 10 hours, and then diluting with dry ether to produce, upon cooling, the hydrochlorides of the esters on 80 to 90 per cent. yields. The picrates of the esters proved to be very suitable for characterisation. The pharmacological results are reported at the end of this account. Further work to produce related alcohols and esters was discontinued when the publications by May and Mosettig *(loc. cit.)* and Speeter *et al.* (*loc. cit.*) appeared.

Attempts have been made to prepare a series of ethers from the alcohol (II; R = H) but, up to the present time, these efforts have not met with success. A toluene solution of the alcohol (II; R = H) failed to react with the sodium or sodamide. In some reactions potassium "emulsified" in benzene has been found to be more useful than sodium in the preparation of alcoholates preparatory to reaction with alkyl halides[8]. When a warm solution of the alcohol (II; R = H) was treated with "emulsified" potassium, reaction occurred readily, a gas was evolved, and subsequent treatment with benzyl bromide or ethyl iodide yielded an identical product. This was proved to be 3-morpholino-1:1-diphenylpropane (IV) by analyses, and by the preparation of a hydrochloride and picrate with melting-points and mixed melting-points identical with those of authentic samples. Because of the identity of the product when either benzyl bromide or ethyl iodide was used, the reaction between the alcohol (II; R = H) and potassium was investigated, and the following reaction has been established:—



3-Morpholino-1 : 1-diphenylpropane (IV) was isolated as the picrate in 85 per cent. yields from the reaction. The mechanism involved in the cleavage of this portion of the molecule from the quaternary carbon atom is being investigated further and the results will be communicated later.

A similar type of reaction has recently been reported by May and Mosettig (*loc. cit.*). In attempting to reduce the ketonic group of amidone by the Huang-Minlon[9] modification of the Wolff-Kishner reaction, the entire ketone chain was lost and 3-dimethylamino-1:1-diphenylbutane (V) was produced as follows:—



Small[10] has suggested that this reaction proceeds through the decarboxylation of an intermediate carboxylic acid. This explanation does not appear to be applicable to the production of (IV) from the alcohol (II; R = H), because it is difficult to see how the necessary conditions for oxidation of the alcohol to the intermediate carboxylic acid could obtain in the reaction mixture.

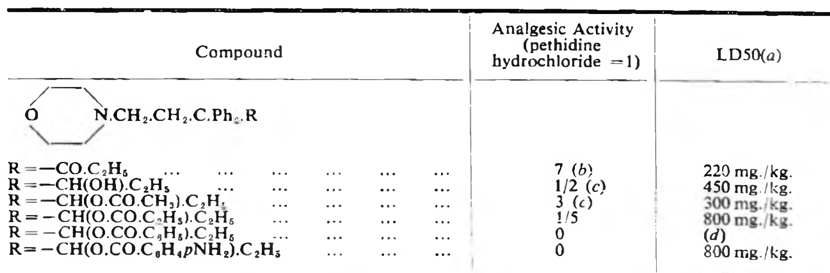
Other attempts to prepare a series of ethers from the alcohol (II, R = H) included the treatment of a toluene solution of the alcohol with lithium-phenyl to produce a white precipitate, which was presumed to be the lithium derivative of the alcohol, but this failed to yield the ether upon treatment with benzyl bromide and only the unchanged alcohol and the benzyl quaternary ammonium bromide of the alcohol were isolated. The replacement of the hydroxyl group by halogen, and then treatment with the sodium salt of benzyl alcohol also proved abortive.

PHARMACOLOGICAL RESULTS

The authors express their thanks to Professor G. A. H. Buttle, Dr. G. F. Somers and Glaxo Laboratories Ltd., for the pharmacological results recorded in Table I. For pharmacological testing the hydrochlorides were used. Analgesic activity was measured in rats against that of

TABLE I

ANALGESIC ACTIVITIES AND TOXICITIES



Compound	Analgesic Activity (pethidine hydrochloride = 1)	LD50(*a*)
O(morpholine ring) $N.CH_2.CH_2.C.Ph_2.R$		
$R = -CO.C_2H_5$	7 (*b*)	220 mg./kg.
$R = -CH(OH).C_2H_5$	1/2 (*c*)	450 mg./kg.
$R = -CH(O.CO.CH_3).C_2H_5$	3 (*c*)	300 mg./kg.
$R = -CH(O.CO.C_2H_5).C_2H_5$	1/5	800 mg./kg.
$R = -CH(O.CO.C_6H_5).C_2H_5$	0	(*d*)
$R = -CH(O.CO.C_6H_4pNH_2).C_2H_5$	0	800 mg./kg.

(*a*) LD50 was obtained by subcutaneous injection into mice.
(*b*) See Hems *et al* (*loc. cit.*).
(*c*) Compare with Speeter *et al* (*loc. cit.*).
(*d*) The hydrochloride of this substance was not sufficiently soluble in water to give a strong enough solution to kill the mice.

pethidine hydrochloride, two methods being used:—(a) the thermal radiation method described by Thorp[11], and (b) the electric grid method of Dodds *et al.*[12].

Experimental

All melting-points are uncorrected.

6-Morpholino-3 : 3-diphenylhexan-3-one (III). This was prepared according to details supplied by Dr. Hems (now published, Hems *et al.*[4]). The authors are indebted to Glaxo Laboratories Ltd., for supplies of diphenylacetonitrile.

6-Morpholino-3 : 3-diphenylhexan-3-ol (II ; R =H). The ketone III (56 g.) and aluminium *iso*propoxide (135 g.) were heated in dry toluene (250 ml.) in a bath maintained at 140° to 150°C. and slow distillation allowed to occur at intervals. More dry toluene was added as required. When the sodium nitroprusside test for acetone in the distillate gave a negative result (after about 100 hours), the solution was cooled, shaken with hydrochloric acid solution (10 per cent.) to decompose the alcoholates, made alkaline with sodium hydroxide solution (10 per cent.), and extracted with benzene. The combined benzene extracts were dried (anhydrous sodium sulphate) and most of the solvent removed under reduced pressure. Upon cooling, a white crystalline mass separated, and this was recrystallised from ethyl alcohol (97 per cent.) as white crystalline granules of 6-morpholino-3:3-diphenylhexan-3-ol (47·5 g., 84 per cent.), m.pt. 129° to 130°C. Speeter *et al. (loc. cit.)* record m.pt. 130° to 131°C. Found: C, 77·8; H, 8·7; N, 4·2 per cent. calc. for $C_{22}H_{29}O_2N$: C, 77·9; H, 8·6; N. 4·1 per cent. The *picrate* crystallised from acetone/ethyl alcohol in yellow prisms, m.pt. 200° to 201°C. Found: C, 59·5; H, 5·7; N, 10·1 per cent. Eq. Wt. (by titration) 565. $C_{22}H_{29}O_2N$, $C_6H_3O_7N_3$ requires C, 59·2; H, 5·6; N, 9·9 per cent. Eq. Wt. 568. The *hydrochloride* crystallised from ether/ethyl alcohol as white clusters, m.pt. 167°C. (with effervescence. Found: C, 69·1; H, 8·0; N, 3·6; Cl, 9·3 per cent. $C_{22}H_{29}O_2N$, HCl requires C, 70·3; H, 8·0; N, 3·7; Cl, 9·4 per cent.

Test for Acetone in the Distillate (where only a very small percentage of acetone is present in solution in toluene).

Acetone test reagent. Sodium nitroprusside solution (4 per cent.) in water. This was discarded if more than 1 week old.

Method. 5 drops of the distillate were collected in a small tube containing 5 drops of water, and a solution of sodium nitroprusside (0·1 ml. of 4 per cent.) added and the mixture shaken. Sodium hydroxide solution (0·1 ml. of 20 per cent.) was added and the mixture shaken again, allowed to stand for 1 minute, and then acidified with acetic acid solution (0·3 ml. of 33 per cent.). The appearance of a cherry-red colour after the addition of the sodium hydroxide solution, followed by a conversion to a much darker red upon acidification with the acetic acid solution, indicated the presence of acetone. The toluene layer did not interfere with this test.

3-Acetoxy-6-morpholino-4 : 4-diphenylhexane hydrochloride
(II; $R = -CO.CH_3$).

The alcohol (II; $R = H$) (2 g.) was dissolved in dry toluene (30 ml.) and acetyl chloride (10 ml.) added. The solution was boiled gently under anhydrous conditions for 10 hours, and then poured into dry ether (250 ml.). Upon cooling, the hydrochloride of the ester separated as rosettes of white crystals (2·27 g., 92 per cent.), m.pt. 250° to 252°C. Recrystallisation from ethyl alcohol gave clusters of colourless prisms, m.pt. 253° to 255°C. (with decomposition). Speeter *et al. (loc. cit.)* record m.pt. 242° to 243°C. Found: C, 69·1; H, 7·7; N, 3·2 per cent. calc. for $C_{24}H_{31}O_3N$, HCl: C, 69·0; H, 7·7; N, 3·4 per cent. The *picrate* (i.e., of the base II; $R = -COCH_3$) was obtained from the hydrochloride, using acetone/ethyl alcohol as solvent, as yellow crystals m.pt. 192° to 193°C. Found: C, 58·9; H, 5·65; N, 9·3 per cent. $C_{24}H_{31}O_3N$, $C_6H_3O_7N_3$ requires C, 58·9; H, 5·6; N, 9·2 per cent.

The following esters of the alcohol (II; $R = H$) were prepared in a similar manner:—

3-Propionoxy-6-morpholino-4 : 4-diphenylhexane hydrochloride
(II; $R = -CO.C_2H_5$).

This was recrystallised from dry ether/ethyl alcohol as rosettes of small white needles, m.pt. 225° to 227°C. (with decomposition). Found: C, 68·7; H, 7·9; N, 3·2; Cl, 8·3 per cent. Eq. Wt. (by titration) 428. $C_{25}H_{33}O_3N$,HCl requires C, 69·5; H, 7·9; N, 3·2; Cl, 8·2 per cent. Eq. Wt. 431·5. The *picrate* (i.e., of the base II, $R = -COC_2H_5$) was obtained from acetone/ethyl alcohol as yellow crystals, m.pt. 181° to 182°C. Found: C, 58·9; H, 5·8; N, 9·0 per cent. Eq. Wt. (by titration) 629. $C_{25}H_{33}O_3N$, $C_6H_3O_7N_3$ requires C, 59·6; H, 5·8; N, 9·0 per cent. Eq. Wt. 624.

3-Benzoxy-6-morpholino-4 : 4-diphenylhexane hydrochloride
(II; $R = -CO.C_6H_5$).

This *amino-ester* was recrystallised from dry ether/ethyl alcohol as small white crystals, m.pt. 256° to 258°C. (with decomposition). Found: C, 71·8; H, 7·3; N, 2·9; Cl, 7·4 per cent. Eq. Wt. (by titration) 488. $C_{29}H_{33}O_3N$, HCl requires C, 72·3; H, 7·1; N, 2·9; Cl, 7·4 per cent. Eq. Wt. 479·5. The *picrate* (i.e., of the base II, $R = -COC_6H_5$) was obtained from acetone/ethyl alcohol as yellow crystals, m.pt. 161° to 162°C. Found: C, 61·9; H, 5·5; N, 8·5 per cent. Eq. Wt. (by titration) 667. $C_{29}H_{33}O_3N$, $C_6H_3O_7N_3$ requires C, 62·5; H, 5·4; N, 8·4 per cent. Eq. Wt. 672.

3-p-Nitrobenzoxy-6-morpholino-4 : 4-diphenylhexane
(II; $R = -CO.C_6H_4p.NO_2$).

The treatment of the alcohol (II; $R = H$) (2 g.) in dry toluene (10 ml.) with *p*-nitrobenzoyl chloride (1·5 g.), in the manner described for the acetoxy derivative above, yielded a gum when poured into ether. This gum was separated, decomposed with sodium hydroxide solution (10 per cent.) and the liberated base extracted with benzene. The benzene extracts were dried (anhydrous sodium sulphate) and the solvent removed under reduced pressure. The yellow-coloured solid which

separated on cooling was recrystallised from absolute alcohol as pale yellow needles of the *amino-ester* (2·16 g., 75 per cent.), m.pt. 161° to 162°C. Found: C, 71·7; H, 6·4; N, 5·9 per cent. $C_{29}H_{32}O_5N_2$ requires C, 71·3; H, 6·6; N, 5·7 per cent. The *picrate* was crystallised from acetone/ethyl alcohol as yellow sandy crystals, m.pt. 237° to 238°C. Found: C, 58·4; H, 4·9; N, 9·9 per cent. Eq. Wt. (by titration) 726. $C_{29}H_{32}O_5N_2, C_6H_3O_7N_3$ requires C, 58·6; H, 4·9; N, 9·8 per cent. Eq. Wt. 717.

3-p-Aminobenzoxy-6-morpholino-4 : 4-diphenylhexane (II; R = $-CO.C_6H_4p\text{-}NH_2$).

Finely powdered 3-*p*-nitrobenzoxy-6-morpholino-4:4-diphenylhexane (1·5 g.) was added to a solution of stannous chloride (20 g.) in concentrated hydrochloric acid (20 ml.), and the mixture heated in a bath at 100°C. for 3 hours with vigorous stirring. After cooling, the tin complex was filtered off, decomposed with sodium hydroxide solution (10 per cent.) and the liberated base extracted with benzene. The benzene extracts were washed with water, dried (anhydrous sodium sulphate), and the solvent removed under reduced pressure. A little ethyl alcohol was added to the oil which remained, and after standing in the ice-chest overnight, a mass of crystals of the *amino-ester* separated and were recrystallised from ethyl alcohol as glistening white plates (1·26 g., 90 per cent.), m.pt. 110°C. (softening at 106°C.). Repeated recrystallisations did not improve the melting-point of the substance which proved to be the monohydrate of the amino-ester. Found: C, 73·2; H, 7·9; N, 5·8 per cent. $C_{29}H_{34}O_3N_2, H_2O$ requires C, 73·1; H, 7·6; N, 5·9 per cent. The loss of weight at 90°C. for 1½ hours under *vacuo* (10 mm.) was 3·6 per cent.; the loss of 1 molecule of water requires a loss of 3·8 per cent. The *dihydrochloride* crystallised from dry ether/ethyl alcohol at small white crystals m.pt. 234° to 237°C. Found: C, 61·8; H, 7·1; N, 4·9 per cent. Eq. Wt. (by titration) 572. $C_{29}H_{34}O_3N_2$, 2HCl, $2H_2O$ requires C, 61·4; H, 7·1; N, 4·9 per cent.. Eq. Wt. 567.

ATTEMPTED PREPARATION OF ETHERS OF 6-MORPHOLINO-4:4-DIPHENYLHEXANE-3-OL. (II; R = H).

A. Using the approach ROH→RO Metal, and then reaction with aralkyl or alkyl halides.

(a) *With potassium and benzyl bromide.* Potassium (0·13 g., 0·0033 mole) was emulsified in dry toluene (15 ml.) and the alcohol (II; R=H) (1·13 g., 0·0033 mole) added and the mixture heated under anhydrous conditions for 4 hours at 70°C. The potassium reacted, small bubbles were evolved, and a little yellowish-brown precipitate was produced. The mixture was cooled, benzyl bromide (0·57 g.) dissolved in dry toluene (10 ml.) added, and the contents of the flask heated at 90°C. for 6 hours with vigorous stirring. A white precipitate (0·55 g.), containing chiefly potassium bromide, separated and, after cooling, was filtered off. The filtrate was washed with water, dried (anhydrous sodium sulphate) and the solvent removed under reduced pressure to yield a red oil (1 g.). Distillation under reduced pressure gave a main fraction, b.pt. 135° to

140°C./0·1 to 0·2 mm. (air-bath temperature) $n_D^{22°C.}$ 1·564. Redistillation of this fraction gave a colourless oil (=A, see below), of weight 0·4 g., b.pt. 135° to 140°C./0·1 to 0·2 mm. (air-bath temperature), $n_D^{22°C.}$ 1·565.

(b) *With potassium and ethyl iodide.* The application of the above method using " emulsified " potassium (0·26 g.), the alcohol (II; R = H) (2·2 g.) and ethyl iodide (1·05 g.) yielded a yellow oil (2·2 g.) which was distilled under reduced pressure to give, as the main fraction, a colourless oil (0·95 g.) of b.pt. 128°C./0·04 mm. (air-bath temperature), $n_D^{22°C.}$ 1·561 (=B, see below).

Identification of Oils A and B. These oils were proved to be identical by analysis, by the preparation of their respective hydrochlorides and picrates which possessed identical melting-points and mixed melting-points, and by the equivalent weight of their respective hydrochlorides. These identical products were proved to be 3-*morpholino-1 : 1-diphenylpropane* (IV). Found: C, 80·7; H, 8·2; N, 4·9 per cent. $C_{19}H_{23}ON$ requires C, 81·1; H, 8·2; N, 5·0 per cent. The hydrochloride was obtained from dry ether/ethyl alcohol as small white platelets, m.pt. 207° to 208°C. and mixed m.pt of 207° to 208°C. with an authentic sample of the hydrochloride kindly supplied by Glaxo Laboratories Ltd. Adamson[13] records m.pt. 208° to 209°C. Found: C, 71·0; H, 7·8; N, 4·3; Cl, 11·2 per cent. Eq. Wt. (by titration) 316. Calc. for $C_{19}H_{23}ON$, HCl: C, 71·8; H, 7·6; N, 4·4; Cl, 11·2 per cent. Eq. Wt. 317·5. The *picrate* crystallised from acetone/ethyl alcohol as yellow prisms, m.pt. 158° to 159°C. and mixed m.pt. of 158° to 159°C. with a sample of picrate prepared from the authentic sample of 3-morpholino-1 : 1-diphenylpropane hydrochloride. Found: C, 58·9; H, 5·3; N, 11·1 per cent. Eq. Wt. (by titration) 516. $C_{19}H_{23}ON$, $C_6H_3O_7N_3$ requires C, 58·8; H, 5·1; N, 11·0 per cent. Eq. Wt. 510.

(c) *Quantitative studies of the reaction between potassium, the alcohol* (II; R = H) and benzyl bromide. The reaction was performed as in (a) but on double the scale, and the product treated as follows. The toluene solution, containing a suspension, was extracted with water until free from ionised bromide. The bromide in the aqueous washings was precipitated as silver bromide (1·186 g.), which demonstrates a quantitative conversion of the benzyl bromide to ionised bromide. The toluene solution was dried (anhydrous sodium sulphate), most of the solvent removed under reduced pressure and dry ether (50 ml.) added. A white precipitate separated (0·06 g.) and was filtered off and found to be the *benzyl quaternary ammonium bromide* of the alcohol (II; R=H) with m.pt. and mixed m.pt. with authentic sample of 204° to 205°C. The solvent was removed from the filtrate under reduced pressure to yield a yellow oil (2·58 g.), which was dissolved in absolute alcohol (50 ml.), heated to boiling, and a solution of picric acid (1·48 g.) in boiling absolute alcohol (20 ml.) added. Large rosettes of yellow prisms of 3-morpholino-1 : 1-diphenylpropane picrate (2·75 g.) m.pt. 158° to 159°C. separated upon cooling, and a further crop (0·1 g.) was obtained after reducing the filtrate to 10 ml. This weight (2·85 g.) corresponds to an 87·6 per cent. conversion of the alcohol (II; R = H) to 3-morpholino-1 : 1-diphenyl-

propane picrate by the above treatment. The filtrate was poured into water (300 ml.) containing sodium hydroxide solution (5 ml. of 20 per cent) to remove the picric acid, and the non-acidic material extracted with benzene. The benzene extracts were washed with water, dried (anhydrous potassium carbonate) and the solvent removed under reduced pressure to yield a yellow oil (0·9 g.). Fractional distillation gave a colourless oil (0·5 g.), b.pt. 130°C./0·1 mm. which has not yet been identified. Found: C, 81·5; H, 8·1 per cent.

(d) *Reaction between the alcohol (II; R = H) and potassium.* Because of the isolation of identical products from the above experiments using benzyl bromide or ethyl iodide, the following was performed. Potassium (0·26 g.) emulsified in dry toluene (20 ml.) was heated at 60° to 70°C. with the alcohol (II; R = H) under anhydrous conditions, with stirring, for 4 hours. After cooling, a portion (2·5 ml.) of the solution was added to hot absolute alcohol (10 ml.), and a hot solution of picric acid (0·5 g.) in absolute alcohol (10 ml.) added. A yellow precipitate separated immediately, and was filtered off and washed with alcohol and water to remove excess picric acid and potassium picrate to yield a residue (0·395 g.) of m.pt. 155° to 157°C. Recrystallisation from acetone/ethyl alcohol gave yellow prisms (0·322 g.) m.pt. 158° to 159°C. of 3-morpholino-1:1-diphenylpropane picrate. The weight of crude picrate indicates at least an 85 per cent. conversion of the alcohol (II; R = H) to 3-morpholino-1:1-diphenylpropane upon heating with emulsified potassium in toluene.

Other attempts to prepare the ethers of the alcohol (II; R =H) are described briefly as follows: Sodium and benzyl bromide in toluene, or potassium carbonate and benzyl bromide in boiling xylene yielded unchanged alcohol and its benzyl quaternary ammonium bromide. The same products were obtained by the reaction of benzyl bromide upon the white precipitate (presumed to be the lithium derivative of the alcohol) obtained by the treatment of an ethereal solution of the alcohol with lithium-phenyl solution.

(e) *Benzyl quaternary ammonium bromide of the alcohol* (II; R = H).

A solution of the alcohol (0·3 g.) in dry toluene (4 ml.) was refluxed with benzyl bromide for 30 minutes. The resulting white precipitate (0·3 g.) was filtered off, m.pt. 199° to 201° C., and recrystallised from ether/ethyl alcohol as rosettes of white needles, m.pt. 205° to 206°C. Found: C, 67·4; H, 7·2; N, 2·9 per cent. $C_{22}H_{36}O_2N$ Br requires C, 68·2; H, 7·1; N, 2·7 per cent.

B. Using the attempted approach ROH→RX and then treatment with R'ONa.

Thionyl chloride reacted with a chloroform solution of the alcohol (II; R = H) and phosphorus pentachloride reacted with a benzene solution of the alcohol. The analyses of the products indicated that they were mixtures of the desired chloro-compound and the dehydrated alcohol. Attempted distillation under reduced pressure led to decomposition, and attempted separation via the hydrochlorides also failed. The

crude mixture was heated with the sodium derivative of benzyl alcohol, but the desired ether was not obtained.

The attempted preparation of the *p*-toluenesulphonyl ester of the alcohol by the Schotten-Baumann reaction also failed. When the preparation of this ester was attempted by refluxing a solution of the alcohol (1 g.) in dry toluene (10 ml.) with *p*-toluenesulphonyl chloride (1 g.) for 6 hours, and then diluting with dry ether (40 ml.), an oil separated and quickly solidified. This was recrystallised from alcohol/ether to give rosettes of white needles (0·4 g.) of 6-*morpholino*-4:4-*diphenylhexan*-3-*ol p-toluene sulphonate* of m.pt. and mixed m.pt. with authentic sample of 182° to 183°C. Found: C, 68·3; H, 7·4; N, 2·9 per cent. $C_{22}H_{29}O_2N$, $C_7H_8O_3S$ requires C, 68·1; H, 7·2; N, 2·7 per cent.

6-Morpholino-4 : 4-diphenylhexan-3-ol p-*toluene sulphonate.*

To a solution of the alcohol (II; R = H) (0·15 g.) and *p*-toluene sulphonic acid (0·08 g.) in hot absolute alcohol (1 ml.), dry ether (10 ml.) was added. Upon cooling, rosettes of white needles separated, m.pt. 181° to 182°C. Recrystallisation from ether/ethyl alcohol gave rosettes of white needles, m.pt. 182° to 183°C.

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FLUORIMETRIC AND MICROBIOLOGICAL ASSAYS OF RIBOFLAVINE IN MALTED PREPARATIONS

By Chloe Klatzkin, F. W. Norris and F. Wokes

This Journal, 1949, **1,** 915-930

Correction

Page 928, lines 40-42, *should read :* The low solubility of the blue fluorescent substance in chloroform prevented its efficient removal by the latter, which has been recommended for purifying extracts[10].

STUDIES IN SYNTHETIC ANALGESICS

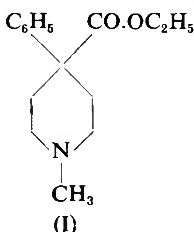
By A. H. Beckett and W. H. Linnell

Part II

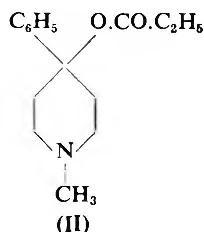
From the Pharmaceutical Chemistry Research Laboratories of the School of Pharmacy, University of London

Received May 19, 1950

It has been shown by Jensen *et al.*[1] and Foster and Carman[2] that the replacement of the $-CO.R$ or $-CO.OEt$ groups of pethidine-type compounds by the $-O.CO.R$ (" reversed " ester) group was attended with increased analgesic activity. According to the data presented by Foster and Carman (*loc. cit.*) for instance, the compound (II) is about 30 times as active as pethidine (I).

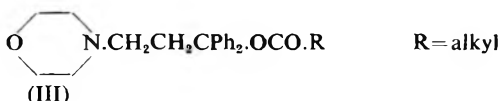


(I)



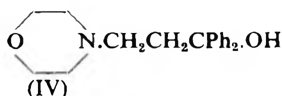
(II)

The authors in the present investigation therefore decided to introduce a similar change in the amidone-type of compounds (i.e. replace the $-CO.R$ group by $-O.CO.R$), to observe the effect upon analgesic activity. A number of compounds of type (III) have been prepared, in order that their activities might be compared with those described in Part I[3] of this work.

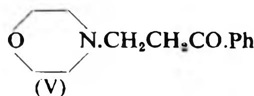


(III)

The reaction of phenylmagnesium bromide upon ethyl β-morpholino propionate produced the tertiary alcohol, 3-morpholino-1:1-diphenyl-propan-1-ol (IV) in 50 per cent. yields, along with the ketone, ω-morpholinopropiophenone (V).



(IV)



(V)

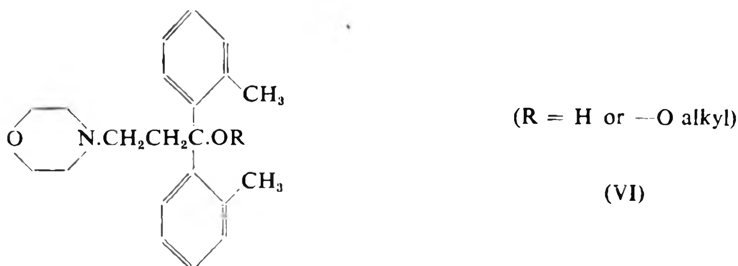
Although it has been shown, in some reactions, that organolithium compounds are more effective than Grignard reagents for the preparation of tertiary alcohols[4], the use of phenyl-lithium in the above reaction did not improve the yield of (IV). This result supports the recently published observation of Adamson[5].

A number of esters (III ; $R=CH_3$, C_2H_5 and n-C_3H_7) were prepared by the reaction of the alcohol (IV), in ether/benzene solution, with ethylmagnesium bromide to produce the Grignard complex of (IV), and then

stirring this suspension overnight at room temperature with the appropriate acid chloride or anhydride. The esters were obtained in 50 per cent. yields.

The application of the method of preparing esters described in Part I[3], by heating a toluene solution of the amino-alcohol with the acid chloride and then diluting with ether to obtain the hydrochloride of the amino-ester, caused dehydration of the alcohol (IV), and the product consisted of a mixture of the hydrochlorides of unchanged alcohol (IV) and the corresponding unsaturated derivative.

The alcohol (IV) and the esters (III ; $R=CH_3$, C_2H_5, and n-C_3H_7) proved to be inactive as analgesics. It was conjectured that perhaps the replacement of the $-CO.C_2H_5$ attached to the quaternary carbon atom bearing the two phenyl groups by $-O.CO.R$ had caused increased freedom of movement of these groups in the molecule. Thus the steric compactness which may be associated with analgesic activity had been upset. In order to reduce the freedom of movement of these groups, *ortho* methyl substituents were introduced into the phenyl rings by the preparation of compounds of type (VI).



Steric effects were undoubtedly exhibited, because the reaction of ethyl β-morpholinopropionate with the Grignard reagent prepared from o-bromotoluene gave the alcohol (VI ; R=H) in 13·5 per cent. yields, compared with the preparations of the alcohol (IV) in 50 per cent. yields using phenylmagnesium bromide under comparable conditions. After chromatographic separation of the large amount of viscous oil which remained after the isolation of the alcohol (VI ; R=H), a picrate was prepared, which gave the correct analytical figures for β-morpholinoethyl-o-tolyl ketone picrate, and the weight obtained indicated at least a 50 per cent. yield of this ketone.

The alcohol (VI ; R=H) proved to be difficult to acylate. The conditions applied with success to produce esters of the alcohol (IV) failed with the alcohol (VI ; R=H). Finally the acylation was accomplished by preparing the Grignard complex of the alcohol in ether/benzene solution, removing the ether by distillation, and then refluxing for 24 hours with the appropriate acid anhydride.

The alcohol (VI ; R=H) and its acetyl and propionyl esters were inactive as analgesics.

The authors thank Professor G. A. H. Buttle, Dr. G. F. Somers and Glaxo Laboratories Ltd. for the pharmacological testing. These tests

were performed according to the methods described by Thorp[6] and by Dodds *et al.*[7]

Conclusions

The replacement of the $-CO.C_2H_5$ group by the $-O.CO.R$ group, in the type of amidone compound tested, leads to a complete loss of analgesic activity. This is completely different from the results of this change in the pethidine type of compound. The introduction of *o, o'* methyl groups into the phenyl rings to increase the steric effect in these "reversed" esters of the amidone-type does not lead to substances with analgesic activity.

Experimental

All m.pts. are uncorrected.

Ethyl β-morpholinopropionate. This ester was prepared by the addition of morpholine to acrylonitrile according to the method of Whitmore *et al.*[8], followed by hydrolysis and esterification with ethyl alcohol and sulphuric acid[9]. In some experiments with large batches, this latter process did not go to completion readily. The following method for the preparation of the ester was found to give better results. A mixture of morpholine (218 g.) and ethyl acrylate (250 g.) was refluxed for 6½ hours and then fractionally distilled under reduced pressure to yield ethyl β-morpholinopropionate (428 g., 91 per cent.), b.pt. 112°C./ 9 mm., $n_D^{24°C.}$ 1.451 (picrate m.pt. 106° to 107°C.). Whitmore *et al.*[9] record b.pt. 138° to 140°C./25 mm., $n_D^{20°C.}$ 1.457 (picrate m.pt. 106° to 107°C.).

3-*Morpholino*-1 : 1-*diphenylpropan*-1-*ol.* (IV).

Method A—A solution of ethyl β-morpholinopropionate (125 g.) in dry toluene (500 ml.) was added slowly with vigorous stirring to the Grignard reagent prepared from bromobenzene (314 g.) and magnesium (48·6 g.) in ether (300 ml.). During this addition, a white precipitate separated. The product was heated at 100°C. for 2 hours, cooled, and then poured slowly with stirring into dilute sulphuric acid solution containing ice. A precipitate of crude 3-morpholino-1 : 1-diphenyl-propan-1-ol sulphate separated in the aqueous layer and, after separating the organic layer and further extracting the aqueous layer with ether (organic extracts rejected), the precipitate (=A) was filtered off and washed with water and ether. The aqueous filtrate and washings were retained (=B).

Treatment of Precipitate A.

The base was liberated with aqueous sodium hydroxide, extracted with chloroform, the chloroform extracts dried (anhydrous sodium sulphate), the solvent removed under reduced pressure, and absolute alcohol (50 ml.) added to the oil which remained. Upon cooling, there were obtained white crystals (78 g.) of almost pure 3-morpholino-1 : 1-diphenylpropan-1-ol, m.pt. 103° to 104°C. A brown viscous oil (=C) was obtained from the mother liquor.

Treatment of filtrate and washings (=B).

These were made alkaline with sodium hydroxide solution, extracted

with chloroform, and treated as above to yield almost pure 3-morpholino-1 : 1-diphenylpropan-1-ol (23·5 g.), m.pt. 101° to 103°C. and a brown viscous oil (=D).

The total yield of almost pure 3-morpholino-1 : 1-diphenylpropan-1-ol was thus 101·5 g., 51 per cent. Recrystallisation from ethyl alcohol gave rosettes of white needles, m.pt. 105° to 106°C. Adamson[10] records m.pt. 106°C. Found : C, 76·8 ; H, 7·7 ; N, 4·8 per cent. ; calc. for $C_{19}H_{23}O_2N$: C, 76·8 ; H, 7·7 ; N, 4·7 per cent. The hydrochloride crystallised from ethyl alcohol as white needles, m.pt. 230° to 231°C. (with decomposition). Adamson (*loc. cit.*) records m.pt. 231°C. Found : C, 68·9 ; H, 7·3 ; N, 4·35 ; Cl, 10·3 per cent. Eq. Wt. (by titration) 334 ; calc. for $C_{19}H_{23}O_2N, HCl$: C, 68·4 ; H, 7·2 ; N, 4·2 ; Cl, 10·6 per cent. Eq. Wt. 333·5. The *picrate* crystallised from acetone/ethyl alcohol as rosettes of yellow crystals, m.pt. 138° to 139°C. Found : C, 56·7 ; H, 4·8 ; N, 10·9 per cent. $C_{19}H_{23}O_2N$, $C_6H_3O_7N_3$ requires C, 57·0 ; H, 4·9 ; N, 10·6 per cent.

Treatment of Oils C and D.

The total weight was 56 g. A portion (12 g.) was distilled under reduced pressure to yield *ω-morpholinopropiophenone* (5 g.), b.pt. 84° to 88°C./0·07 mm. The constitution of this compound was proved by the preparation of the following.

(*a*) The hydrochloride, m.pt. 176° to 177°C. (This hydrochloride, m.pt. 177°C. was prepared by Harradence and Lions[11] by the use of the Mannich reaction).

Found : C, 61·0 ; H, 6·9 ; N, 5·8 ; Cl, 14·1 per cent. Eq. Wt. (by titration) 260. Calc. for $C_{13}H_{17}O_2N,HCl$; C, 61·1 ; H, 7·1 ; N, 5·5 ; Cl, 13·9 per cent. Eq. Wt. 255·5.

(*b*) The picrate, m.pt. 198° to 200°C. (with decomposition). Harradence and Lions (*loc. cit.*) record m.pt. 195° to 196°C. Found : C, 50·2 ; H, 4·4 ; N, 12·2 per cent. Calc. for $C_{13}H_{17}O_2N,C_6H_3O_7N_3$; C, 50·9 ; H, 4·5 ; N, 12·5 per cent.

Method B. (using phenyl-lithium and ethyl β-morpholinopropionate). A solution of ethyl β-morpholinopropionate (47 g.) in ether (100 ml.) was added with vigorous stirring, under anhydrous conditions and in an atmosphere of nitrogen, to a solution of phenyl-lithium prepared from freshly distilled bromobenzene (158 g.) and lithium (13·8 g.) in ether (1000 ml.). The mixture was heated at 60°C. for 2 hours, cooled, and worked up as in Method A to yield 3-morpholino-1 : 1-diphenylpropan-1-ol (31·5 g., 42 per cent.) and a brown viscous oil (32 g.) containing ω-morpholinopropiophenone.

3-*Morpholino*-1-*propionoxy*-1 : 1-*diphenylpropane* (III ; $R=C_2H_5$).

A solution of IV (7 g.) in dry toluene (40 ml.) was added slowly to ethylmagnesium iodide solution prepared from magnesium (1 g.) and ethyl iodide (6·3 g.) in ether (10 ml.), while vigorous stirring was maintained. A grey solid separated. The suspension was heated at 100°C. for 30 minutes, cooled to room temperature, and then addition drop by drop of propionic anhydride (8 ml.) in dry toluene (10 ml.) made with vigorous stirring, and the stirring continued overnight at room temperature. The product was poured into dilute hydrochloric acid solution

containing ice, and the solid (crude hydrochloride of the amino-ester) which separated in the aqueous layer was filtered off and washed with water and ether. From the crude hydrochloride, the base was liberated with sodium carbonate solution, extracted with chloroform, the chloroform extracts dried (anhydrous sodium sulphate) and the solvent removed under reduced pressure to yield a solid which was crystallised from ethyl alcohol as rosettes of colourless prisms (4·6 g., 55 per cent.) of 3-*morpholino*-1-*propionoxy*-1 : 1-*diphenylpropane*, m.pt. 108° to 109°C.

Found : C, 74·8 ; H, 7·5 ; N, 4·2 per cent. $C_{22}H_{27}O_3N$ requires C, 74·8 ; H, 7·65 ; N, 4·0 per cent. The *hydrochloride* crystallised from absolute alcohol as rosettes of white needles, m.pt. 209° to 210°C. (with decomposition). Found : C, 66·7 ; H, 7·5 ; N, 3·6 ; Cl, 9·0 per cent. Eq. Wt. (by titration) 401. $C_{22}H_{27}O_3N,HCl, \frac{1}{2}H_2O$ requires C, 66·3 ; H, 7·3 ; N, 3·5 ; Cl, 8·9 per cent. Eq. Wt. 398·5. The *picrate* crystallised from acetone/ethyl alcohol as small yellow prisms, m.pt. 176° to 177°C. Found : C, 58·5 ; H, 5·3 ; N, 9·6 ; $C_{22}H_{27}O_3N$, $C_6H_3O_7N_3$ requires C, 57·7 ; H, 5·2 ; N, 9·6 per cent.

The following esters were prepared in a similar manner and in similar yields :—

1-*Acetoxy*-3-*morpholino*-1 : 1-*diphenylpropane* (III ; $R{=}CH_3$) crystallised from ethyl alcohol as colourless prisms, m.pt. 107° to 108°C. Found : C, 74·7 ; H, 7·1 ; N, 4·1 per cent. $C_{21}H_{25}O_3N$ requires : C, 74·3 ; H, 7·4 ; N, 4·1 per cent. The *hydrochloride* was obtained from ethyl alcohol as white needles, m.pt. 205° to 206°C. (with decomposition). Found : C, 65·8 ; H, 7·1 ; N, 3·7 ; Cl, 9·0 per cent. Eq. Wt. (by titration) 389. $C_{21}H_{25}O_3N$, HCl, $\frac{1}{2}H_2O$ requires C, 65·6 ; H, 7·0 ; N, 3·6 ; Cl, 9·2 per cent. Eq. Wt. 384·5. The *picrate* crystallised from acetone/ethyl alcohol as yellow prisms, m.pt. 176° to 177°C. Found : C, 56·6 ; H, 5·0 ; N, 9·9 per cent. $C_{21}H_{25}O_3N$, $C_6H_3O_7N_3$ requires C, 57·0 ; H, 4·9 ; N, 9·9 per cent.

1-*n*-*Butyroxy*-3-*morpholino*-1 : 1-*diphenylpropane* (III ; $R = n\text{-}C_3H_7$) crystallised from ethyl alcohol as colourless prisms, m.pt. 90°C. Found : C, 76·0 ; H, 7·8 ; N, 4·0 per cent. $C_{23}H_{29}O_3N$ requires C, 75·2 ; H, 7·9 ; N, 3·8 per cent. The *hydrochloride* crystallised from ethyl alcohol as white needles, m.pt. 180° to 181°C. (with decomposition). Found : C, 67·6 ; H, 7·6 ; N, 3·4 ; Cl, 8·9 per cent. Eq. Wt. (by titration) 413. $C_{23}H_{29}O_3N$, HCl, $\frac{1}{2}H_2O$ requires C, 67·0 ; H, 7·5 ; N, 3·4 ; Cl, 8·6 per cent. Eq. Wt. 412·5. The *picrate* crystallised from acetone/ethyl alcohol as yellow crystals, m.pt. 160° to 161°C. Found : C, 58·1 ; H, 5·1 ; N, 9·5 per cent. $C_{23}H_{29}O_3N$, $C_6H_3O_7N_3$ requires C, 58·4 ; H, 5·4 ; N, 9·4 per cent.

Attempted preparation of the above esters by other methods.

Propionyl chloride (1 ml.—freshly distilled from a sample to which 10 per cent. of dimethylaniline had been added) was added to a solution of IV (1 g.) in warm dry toluene (10 ml.). A white precipitate was immediately produced. After heating at 100°C. for 30 minutes, dry ether (40 ml.) was added, and the crude product was filtered off (1·09 g.), m.pt. about 185°C. Recrystallisation from ethyl alcohol gave the hydrochloride of IV (0·55 g.), m.pt. and mixed m.pt. with authentic

sample of 230° to 231°C. The analytical figures obtained from the crude product from a subsequent experiment indicated that it was a mixture of the hydrochloride of IV and the corresponding dehydrated alcohol hydrochloride. Found: C, 70·4; H, 7·2; N, 4·3 per cent. Eq. Wt. (by titration) 327. $C_{19}H_{21}ON$ requires C, 72·5; H, 7·0; N, 4·4 per cent. Eq. Wt. 315·5. $C_{19}H_{23}O_2N$ requires C, 68·4; H, 7·2; N, 4·2 per cent. Eq. Wt. 333·5.

The reaction between propionyl chloride and the alcohol in pyridine solution failed to produce the ester.

3-*Morpholino*-1 : 1-*di*-o-*tolylpropan*-1-*ol* (VI : R=H).

From the reaction between ethyl β-morpholinopropionate (120 g.) and the Grignard reagent prepared from magnesium (45·5 g.) and *o*-bromotoluene (318 g.), using the conditions described for the preparation of IV, was isolated 3-*morpholino*-1 : 1-*di*-o-*tolylpropan*-1-*ol* as small white needles (28 g., 13·5 per cent.), m.pt. 162° to 163°C., from ethanol. Found: C, 77·6; H, 8·4; N, 4·2 per cent. $C_{21}H_{27}O_2N$ requires C, 77·5; H, 8·3; N, 4·3 per cent. The *hydrochloride* crystallised from ethyl alcohol as colourless prisms, m.pt. 238° to 239°C. (with effervescence). Found: C, 69·3; H, 7·5; N, 3·8; Cl, 9·7 per cent. Eq. Wt. (by titration) 362. $C_{21}H_{27}O_2N$, HCl requires C, 69·7; H, 7·8; N, 3·9; Cl, 9·8 per cent. Eq. Wt. 361·5. The *picrate* crystallised from acetone/ethyl alcohol as yellow prisms, m.pt. 191° to 192°C. Found: C, 57·8; H, 5·5; N, 10·3 per cent. $C_{21}H_{27}O_2N$, $C_6H_3O_7N_3$ requires C, 58·5; H, 5·4; N, 10·1 per cent.

A brown viscous oil (95 g.) was the main product from the above reaction. A portion of this oil (4 g.) was dissolved in benzene (200 ml.) completely adsorbed on a column of alumina (2 cm. diameter and 38 cm. long), and benzene used as the eluent. A brown band remained at the top of the column and a yellow band moved down as the development proceeded. The yellow fraction, and subsequent colourless fractions, all gave oils when the solvent was removed (total weight of oil, 1·9 g.), and these oils gave identical picrates, m.pt. 175°C. Recrystallisation of these picrates from acetone/ethyl alcohol gave yellow needles, m.pt. 180° to 181°C. of a substance which, from the analytical figures and by analogy with the production of ω-morpholinopropiophenone by the action of phenylmagnesium bromide upon ethyl β-morpholinopropionate, was considered to be *β-morpholinoethyl-o-tolyl ketone picrate.* Found: C, 52·2; H, 4·9; N, 11·9 per cent. $C_{14}H_{19}O_2N$, $C_6H_3O_7N_3$ requires C, 51·7; H, 5·2; N, 11·9 per cent. A yellow oil (1·4 g.), which was not identified, was obtained by the treatment of the column with an eluent of benzene containing 10 per cent. of ethyl alcohol.

The reaction between the lithium derivative derived from *o*-bromotoluene (171 g.), and ethyl β-morpholinopropionate (47 g.), using the conditions described for the reaction of phenyl-lithium with this ester, yielded 3-morpholino-1 : 1-di-*o*-tolylpropan-1-ol (12 g., 14·7 per cent.).

3-*Morpholino*-1-*propionoxy*-1 : 1-*di*-o-*tolylpropane* (VI : R=—COC_2H_5).

A solution of 3-morpholino-1 : 1-di-*o*-tolylpropan-1-ol (4·9 g.) in dry benzene (100 ml.) and dry ether (200 ml.) was added to a 2M solution (30 ml.) of ethylmagnesium bromide in ether, with vigorous stirring.

After refluxing gently for 2 hours, the ether was removed by distillation, and propionic anhydride (30 ml.) added. The resulting suspension was refluxed, with stirring, for 24 hours.

The product was cooled, poured into dilute hydrochloric acid solution containing ice, ether (200 ml.) added and, after stirring well, the precipitate was filtered off, added to sodium carbonate solution, and the liberated base extracted with chloroform. After drying (anhydrous sodium sulphate), the solvent was removed under reduced pressure from the chloroform extracts, and ethyl alcohol (5 ml.) added to the resulting oil. Upon cooling, small rectangular plates of 3-*morpholino*-1-*propionoxy*-1 : 1-*di*-o-*tolylpropane* (1·2 g., 21 per cent.), m.pt. 123° to 126°C. separated. Recrystallisation from ethyl alcohol raised the m.pt. to 127° to 129°C. Found : C, 75·6 ; H, 8·1 ; N, 3·9 per cent. $C_{24}H_{31}O_3N$ requires C, 75·6 ; H, 8·1 ; N, 3·7 per cent. The *hydrochloride* crystallised from ether/ethyl alcohol as colourless prisms, m.pt. 181° to 182°C. (with decomposition). Found : C, 69·0 ; H, 8·0 ; N, 3·5 per cent. $C_{24}H_{31}O_3N$, HCl requires C, 69·0 ; H, 7·7 ; N, 3·3 per cent. The *picrate* was obtained from acetone/ethyl alcohol as yellow crystals, m.pt. 179° to 180°C. Found : C, 59·6 ; H, 5·9 ; N, 9·1 per cent. $C_{24}H_{31}O_3N$, $C_6H_3O_7N_3$ requires C, 59·0 ; H, 5·6 ; N, 9·2 per cent.

1-*Acetoxy*-3-*morpholino*-1 : 1-*di*-o-*tolylpropane* (VI ; R = $-COCH_3$).

This was obtained, by the method used for the corresponding propionoxy compound, as colourless plates, m.pt. 133° to 134°C., from ethyl alcohol. Found : C, 75·2 ; H, 8·0 ; N, 4·0 per cent. $C_{23}H_{29}O_3N$ requires C, 75·2 ; H, 7·9 ; N, 3·8 per cent. The *hydrochloride* crystallised from ethyl alcohol as white needles m.pt 200° to 201°C. Found : C, 68·1 ; H, 7·4 ; N, 3·3 ; Cl, 9·2 per cent. $C_{23}H_{29}O_3N$, HCl requires C, 68·4 ; H, 7·4 ; N, 3·5 ; Cl, 8·8 per cent. The *picrate* crystallised from acetone/ethyl alcohol as yellow needles, m.pt. 164° to 165°C. Found : C, 58·3 ; H, 5·5 ; N, 9·2 per cent. $C_{23}H_{29}O_3N$, $C_6H_3O_7N_3$ requires C, 58·4 ; H, 5·4 ; N, 9·4 per cent.

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THE OCCURRENCE OF METHYL COMPOUNDS IN GALENICALS

By G. A. Sergeant

From the Department of the Government Chemist, The Laboratory, Custom House, London, E.C.3

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In a recent paper in this journal Brookes and Johnson[1] described an investigation into the occurrence of methyl compounds in certain galenical preparations. This is a subject of considerable importance to the Department of the Government Chemist and to all pharmaceutical houses that prepare galenicals containing spirit, since the presence of methyl alcohol is often regarded as *prima facie* evidence of the presence of Industrial Methylated Spirits. The suggestion was made in the paper and also in the ensuing discussion that in certain cases insufficient allowance was made for naturally occurring methyl alcohol, or methyl compounds which could give rise to methyl alcohol in the course of analysis.

In particular, the case of senega was examined in some detail, and it appeared that the addition of acid in the distillation of the root or its preparations could increase considerably the yield of methyl alcohol, presumably by hydrolysis of methyl compounds present in the material. This was significant, as acid distillation, with the object of " fixing " free ammonia, is prescribed in the British Pharmacopœia for the estimation of spirit in senega tinctures, and the expectation that this procedure would give a higher yield of methyl alcohol than that arising from distillation with no addition of acid was given experimental support by the authors. In this connection, it may be noted that the Government Laboratory method for senega tinctures does not follow the B.P. procedure in this respect, but, instead, employs a preliminary distillation with no addition of acid followed by acidification and redistillation of the distillate, so that our practice is here in accordance with that recommended by Brookes and Johnson. At our request, a number of samples connected with the above investigation, including some of the original materials, were kindly made available to the Government Laboratory, and the results of some independent experiments are given in the present paper.

Two methods of estimation of methyl alcohol were employed: (a) the modified Denigès test as described in the B.P. (p. 49), applied quantitatively by matching with standards, and (b) as a check on this method, one based on the colour reaction of chromotropic acid with formaldehyde as described at the end of this paper. It will be seen from Tables II and III that very considerable differences in the estimated methyl alcohol content of distillates are given in certain cases by the two methods, and that sometimes the Denigès test was found to give a definite positive reaction when the chromotropic acid test showed no appreciable reaction. As a precaution against the possible inhibition of the chromotropic acid colour reaction in such circumstances, repeat determinations were made

after making small measured additions of methyl alcohol to test-portions of the distillates, and the normal development of the colour was observed. Chromotropic acid appears, therefore, to be a more specific reagent for formaldehyde than is Schiff's reagent, and there seems to be no doubt that, in the case of relatively impure distillates, the Denigès test can be misleading. As Brookes and Johnson used the modified Denigès test exclusively, this is of great importance in its bearing on the validity of some of their experimental figures.

For example, while the figures obtained here on samples 1 to 5 (concentrated compound infusion of gentian—see Table I) show fair agreement with those given by Brookes and Johnson, both by the Denigès and

TABLE I

Concentrated Compound Infusion of Gentian	Methyl Alcohol per cent. v/v in Galenical		
	By Denigès test	By Chromotropic acid test	Brookes and Johnson
(1) No. 1	0·06	0·08	0·05
(2) No. 2	Trace	Trace	0·025
(3) No. 3	0·02	0·05	0·05
(4) No. 4	0·02	0·04	0·05
(5) No. 5	0·02	0·04	0·05

chromotropic acid tests, this is not the case with sample No. 6 (concentrated infusion of senega). In Table II it will be seen that experiments 2, 3, 4, 5 and 8 show very different results as between the Denigès and chromotropic acid methods. The apparently large yields of methyl alcohol obtained by acid distillation, and from the redistillation of residues with acid, which are indicated by the former method are not con-

TABLE II

Sample No. 6 Concentrated Infusion of Senega	Methyl Alcohol per cent. v/v in Galenical	
	By Denigès Test	By Chromotropic Test
(1) Distillation with no addition of acid	0·12	0·13
(2) Residue from above redistilled after adding water, alcohol and 2 ml. of concentrated sulphuric acid	0·19	Trace
(3) Above residue again distilled after adding water and alcohol ...	0·14	Trace
(4) Acid distillation	0·36	0·16
(5) Residue from above redistilled after adding water and alcohol	0·19	Trace
(6) Distillation with ammonia (5 ml. of concentrated solution) the distillate being acidified and redistilled	0·15	0·16
(7) D acid D (G.L. method—see text)	0·12	0·12
(8) Residue from above redistilled after adding water, alcohol and 3 ml. of concentrated suphuric acid	0·19	Trace

firmed by the latter. This is again demonstrated in Table III, and the marked differences may be attributable mainly or entirely to interfering volatile substances that react with Schiff's reagent under the conditions of the Denigès test; the findings of Brookes and Johnson in connection with liquid extract of senega need re-examination in this light. Thus, they reported in Table III of their paper 1·40 per cent. of methyl alcohol

(Denigès) by acid distillation in a sample of the liquid extract and only 0·01 per cent. by distillation with no addition of acid. Further, successive acid distillations of the residue left after plain distillation were reported to yield a total of 1·36 per cent. of methyl alcohol (Denigès).

TABLE III

Senega Root	Methyl Alcohol per cent. w/w in Root	
	By Denigès Test	By Chromotropic Acid Test
(1) 20 g. of root distilled with water and alcohol	Trace	Trace
(2) Residue from above redistilled after adding water, alcohol and 3 ml. of concentrated sulphuric acid	1·12	0·50
(3) 20 g. of root distilled with water, alcohol, and 3 ml. of concentrated sulphuric acid	0·95	0·42
(4) Residue from above redistilled after adding water and alcohol	0·55	0·37
(5) The same residue again redistilled after adding water and alcohol	0·30	0·15
(6) The last operation repeated	0·19	0·04
Total	1·99	0·98
(7) 20 g. of root distilled with water, alcohol and 5 ml. of concentrated solution of ammonia, the distillate being acidified and redistilled	0·50	0·50

In view of our findings given here in Table II, these figures are clearly in need of verification, and this applies also to the figures of 0·36 per cent. of methyl alcohol (Denigès) reported by Brookes and Johnson as arising from acid distillation of a sample of concentrated infusion of senega against 0·04 per cent. by distillation with no addition of acid. It is suggested that, had the more specific chromotropic acid test been applied here, much lower figures for methyl alcohol by acid distillation would have been obtained. In this connection it is interesting to observe (cf. Table II expt. 6 and Table III expt. 7) that with distillation in the presence of excess of ammonia, the two methods give practically identical results. As might be expected, our routine procedure (distillation, acidification, redistillation) gives substantially the same figure by either test (see Table II—7) and agrees with that found by distillation with no addition of acid (see Table II—1).

Repeated acid distillation of senega root (Table III) yielded a total of about 1 per cent. w/w of methyl alcohol as indicated by the chromotropic acid test (or apparently about 2 per cent. by the Denigès test), and it was confirmed that ammonia acts similarly to acid in increasing the yield of methyl alcohol (7). However, there is no reason to believe that more than a small fraction of this potentially large amount of methyl alcohol could appear in the galenicals as prepared by the process of extraction prescribed in the B.P., especially if acid hydrolysis is avoided in the analysis.

Thus, Brookes and Johnson found, by distillation with no addition of acid, 0·04 per cent. and 0·09 per cent. of methyl alcohol in samples of the infusion (a 40 per cent. w/w preparation) which, it is stated, were

prepared by a modification of the B.P. method involving the presence of ammonia during the percolation and, therefore, the possibility of some generation of methyl alcohol by alkaline hydrolysis. Further, distillation with no addition of acid yielded only 0·01 per cent. of methyl alcohol from a sample of the liquid extract (which is a 1 : 1 preparation).

Summary

(1) Methyl alcohol or substances that can give rise to methyl alcohol can occur as natural constituents in certain galenicals, and only where amounts of methyl alcohol are detected in excess of normal limits should they be regarded as evidence of the presence of Industrial Methylated Spirits. This has long been a guiding principle at the Government Laboratory.

(2) It is recognised that, in the case of senega preparations, a preliminary distillation with no addition of acid should precede the methyl alcohol test. This is recommended by Brookes and Johnson; it is preferable to the acid distillation prescribed in the B.P., and is in accordance with Government Laboratory practice.

(3) Some of the findings of Brookes and Johnson concerning the extraction of relatively large amounts of methyl alcohol from senega preparations have been shown to be of doubtful validity owing to the lack of specificity of the Denigès test.

(4) From their figures obtained by distillation with no addition of acid, Brookes and Johnson have not shown that concentrated infusion of senega can have a methyl alcohol content of more than about 0·1 per cent., and they found only 0·01 per cent by this method in a sample of the liquid extract.

Over many years we have examined many samples of concentrated infusion of senega by our routine method; a fair proportion of them indicate complete absence or only a trace of methyl alcohol. It seems probable that slight variations exist between different batches of senega root due to age, origin or variety, and that these variations may give rise to senega preparations containing varying but very small amounts of methyl alcohol.

Description of Chromotropic Acid Method

This procedure was developed for the special purposes required for this investigation and is based on a method described by Boos[2].

To 1 ml. of the test-solution (containing 1 per cent. v/v of ethyl alcohol and not more than 0·1 mg. of methyl alcohol) contained in a 5 × $\frac{3}{4}''$ test-tube, add 8 drops of Denigès permanganate solution (3 g. of potassium permanganate and 15 ml. of 85 per cent. phosphoric acid per 100 ml. of solution) and shake to mix. After standing for 10 minutes at room temperature, add sufficient freshly-prepared saturated sodium bisulphite solution drop by drop (about 2 drops) to reduce excess of permanganate, and transfer the tube to an ice-bath. Cool for about a minute and pour 4 ml. of concentrated sulphuric acid gently down the

side of the tube. Cool for a further 2 minutes and then shake to mix, continuing the cooling for a short time. Add 1 drop of 2 per cent. chromotropic acid solution, shake to mix, and heat in a water-bath for 15 minutes at 60°C. Transfer the tube to the ice-bath and, after cooling, dilute the solution with 3 or 4 ml. of water, cool and rinse into a Nessler tube to a total volume of about 15 ml. Compare the colour of the solution, viewed vertically against a white background, with that of standards similarly prepared from solutions containing 0 to 0·1 mg. of methyl alcohol in 1 per cent. ethyl alcohol.

Chromotropic acid reagent. Dissolve 0·1 g. of chromotropic acid in 5 ml. of cold water and filter the solution. This solution darkens on standing and is usable for a few days only, after which, matching of the violet reaction colour becomes difficult. For this reason, although the violet colour is stable, fresh standards should be prepared on the same day as the test.

Sensitivity. 0·01 mg. of methyl alcohol is readily detected in 1 ml. of test solution. The violet colour increases in depth for amounts of methyl alcohol up to about 0·5 mg., but beyond this approximate limit there is a diminution in intensity.

Acknowledgment is made to the Government Chemist for permission to publish this paper.

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THE ESTIMATION OF SODIUM GENTISATE IN TABLETS AND INJECTIONS

By M. J. H. Smith

From the Department of Chemical Pathology, King's College Hospital Medical School, Denmark Hill, London, S.E.5

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Sodium salicylate is widely used in the treatment of acute rheumatic fever and usually controls the pain and swelling associated with the disease. It has been emphasised that adequate therapy with the drug demands a high plasma-salicylate level of between 30 and 40 mg. per 100 ml.[1,2]. Graham and Parker[3] demonstrated that plasma-salicylate levels greater than 35 mg. per 100 ml. are associated with the well-known toxic effects of salicylates on the gastro-intestinal tract and on the special senses. This must be considered one of the serious drawbacks of prolonged salicylate therapy.

Normal adults excrete 4 to 8 per cent. of ingested salicylate as gentisic acid[4] (2:5-dihydroxybenzoic acid) and it has been reported that in patients with acute rheumatic fever this fraction is appreciably increased[5]. The action of salicylates may be due to formation of gentisate, the latter substance acting as an inhibitor of the enzyme hyaluronidase[6]. This enzyme depolymerises hyaluronic acid which acts as an interfibrillar cement in the tissues and it has been suggested that there is increased hyaluronidase activity in rheumatic disease[7]. The sodium salt of gentisic acid has been used in the treatment of rheumatic fever[8] and seems to be at least as therapeutically active as sodium salicylate and has the advantage that it produces few, if any, toxic effects even in doses up to 18 g. per day[9].

Sodium gentisate may be administered orally as cachets or tablets or by intramuscular injection. Two independent methods of estimation of the substance in these preparations have been devised. One method is based on the photometric measurement of the blue colour produced when gentisate reacts with an aqueous solution of the Folin-Ciocalteu phenol reagent in alkaline solution. The colour production depends on the reduction of hexavalent molybdenum and tungsten in the reagent to coloured products of lower valency. The other method utilises the absorption given by gentisate in the ultraviolet region. The ultraviolet spectra of aqueous solutions of sodium gentisate show a maximum at 3200Å and the optical densities at this wavelength were found to be directly proportional to the concentration of gentisate ion.

Gentisic acid has been estimated in urine, after a preliminary extraction with ether, by means of the blue colour it gives with ferric chloride[9] but this colour is too transient for accurate work. It has also been assayed by the reduction of alkaline cupric solutions, carried out according to the Shaffer-Hartmann method for blood sugar, and by a bromine

consumption procedure, but this latter reaction has to be performed at 0°C. to make it quantitative[4].

Experimental

Method I.

A blue colour having an absorption maximum of 6600Å develops when sodium hydroxide is added to a mixture of a solution of sodium gentisate and the Folin-Ciocalteu phenol reagent[10]. The colour reaches a maximum after 1 minute but begins to fade after 20 minutes (Fig. 1); a

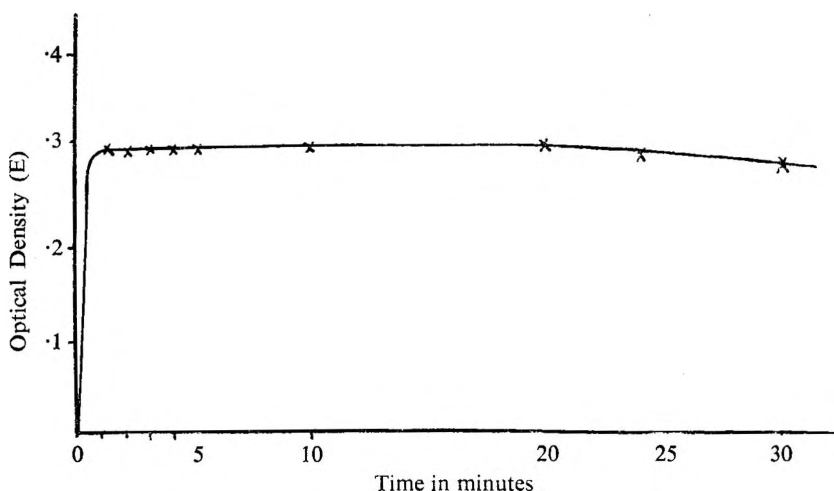

Fig. 1.—Rate of development and fading of Folin-Ciocalteu blue colour at room temperature.

similar behaviour has been reported for salicylates[11]. The absorption density of the coloured solution was found to be directly proportional to the concentration of gentisate ion up to 100 mg. per 100 ml.

Preparation of solutions of sodium gentisate for analysis. a. Powder. About 50 mg. of the powder is accurately weighed, dissolved in distilled water and made up to 100 ml.

b. Tablets. 2 tablets, each containing 0·25 g. of sodium gentisate, are dissolved in distilled water and made up to 1 l.

c. Injection. Solutions for intramuscular injection are prepared which contain 1 g. of sodium gentisate in 10 ml. of distilled water and are sterilised by autoclaving. 5 ml. of the injection is made up to 1 l. with distilled water.

The method of estimation is as follows: 1 ml. of sodium gentisate solution prepared from the powder, tablet or injection is added to 9 ml. of distilled water, 10 ml. of Folin-Ciocalteu reagent (diluted 1 to 3 with distilled water) are added and well mixed. 1 ml. of the mixture is removed and 5 m. of aqueous 0·5N sodium hydroxide added to it, the

solution is allowed to stand at room temperature for 5 minutes and the absorption density measured against distilled water in a photo-electric absorptiometer using a 1 cm. cell and a filter transmitting maximally above 6600Å. A Hilger Spekker photo-electric absorptiometer and an Ilford spectrum red filter No. 608 have been used in the present work. A calibration curve (Fig. 2) may be constructed from dilutions of anhydrous sodium gentisate in distilled water.

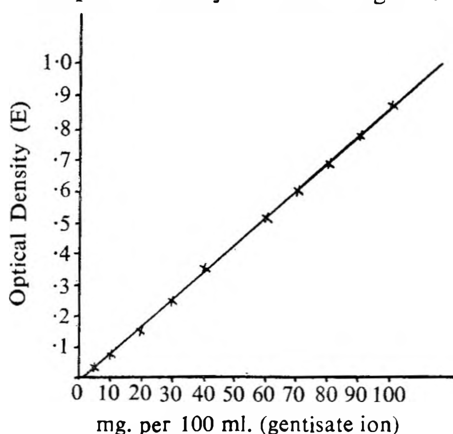

FIG. 2.—Calibration curve of gentisate in distilled water using the blue colour given by the Folin-Ciocalteu reagent in alkaline solution. The absorption densities were measured in a Hilger Spekker absorptiometer using an Ilford spectrum red filter, No. 608.

Method II.

The ultraviolet absorption spectrum of an aqueous solution of sodium gentisate (Fig. 3) shows a maximum at 3200Å, the *p*H of the solution being 5·6. The molecular extinction coefficients, $\epsilon_{(mol.)}$ at 3200Å of sodium gentisate and gentisic acid are 4312 and 3773 respectively, a value of 3750 ± 115 at 3225Å has been

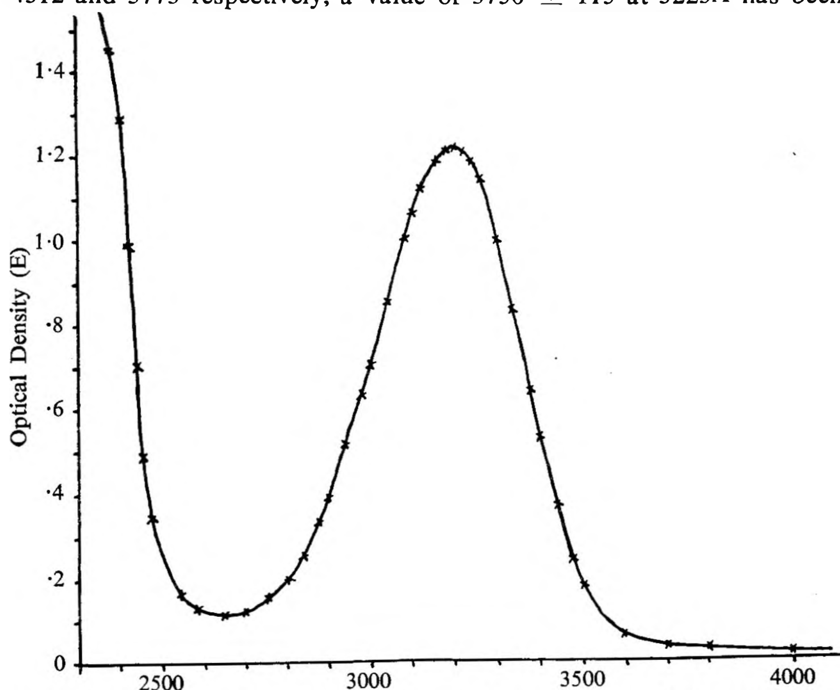

FIG. 3.—Ultra violet absorption spectrum of an aqueous solution of sodium gentisate at *p*H 5·6, the concentration of the solution was 5 mg./ml. per 100.

reported for gentisic acid[4]. It was found that the optical densities, measured at 3200Å, of solutions of sodium gentisate were directly proportional to the concentration up to 5 mg./100 ml.

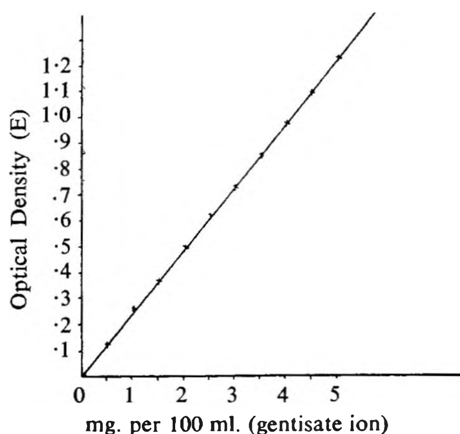

FIG. 4.—Calibration curve of gentisate in distilled water. The optical densities were measured at 3200Å in a Hilger Uvispek spectrophotometer.

Preparation of solutions. The solutions prepared for method I are accurately diluted 1 to 20. The method of estimation is as follows. The optical density of the solution is measured at 3200Å against distilled water using a 1 cm. cell in an ultraviolet spectrophotometer. The Hilger Uvispek ultraviolet spectrophotometer has been employed in the present work. A calibration curve may be constructed from dilutions of sodium gentisate in distilled water (Fig. 4). The results obtained in the estimation of sodium gentisate in various preparations are given in Table I; they are expressed as anhydrous sodium gentisate $C_7H_5O_4Na$.

TABLE I

Preparation	Method I	Method II
(1) Aqueous solution...	27·1	26·9
(2) Aqueous solution...	40·0	40·5
(3) Aqueous solution...	17·2	17·5
(4) Aqueous solution...	34·5	34·3
(5) Powder ...	95·2	95·4
(6) Powder ...	92·8	93·1
(7) Powder ...	93·4	93·2
(8) Powder ...	92·7	93·0
(9) Tablet ...	0·236	0·245
(10) Tablet ...	0·232	0·240
(11) Tablet ...	0·215	0·220
(12) Tablet ...	0·226	0·230
(13) Injection ...	9·42	9·50
(14) Injection ...	9·60	9·65
(15) Injection ...	9·24	9·40
(16) Injection ...	9·06	9·10

Nos. 1-4 are expressed as mg. per 100 ml. of sodium gentisate ($C_7H_6O_4Na$)
Nos. 5-8 are expressed as g. per cent. w/w of sodium gentisate
Nos. 9-12 are expressed as g. of sodium gentisate per tablet
Nos. 13-16 are expressed as g. per 100 ml. of sodium gentisate.

SUMMARY

1. Two independent methods of estimation of sodium gentisate are described, one method uses the blue colour which develops when gentisate reacts with the Folin-Ciocalteu phenol reagent in alkaline solution, the other method is based on the measurement of the optical densities of aqueous solution of gentisate at 3200Å.

2. The application of these methods to the assay of sodium gentisate in powder form, tablets and solutions for injection is described.

The Spekker photo-electric absorptiometer used in the work was purchased from a grant to Professor C. H. Gray from the Central Research Fund of the University of London.

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THE OPTICAL CRYSTALLOGRAPHIC PROPERTIES OF CRYSTALS FORMED WITH NITRIC ACID FROM PETHIDINE, NICOTINAMIDE AND CINCHOPHEN

By Alf Wickstrøm

From the Department of Pharmaceutical Chemistry, Institute of Pharmacy, University of Oslo

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The author has on an earlier occasion reported that pethidine forms with nitric acid characteristic crystals which may be useful for the micro-identification of this substance[1]. As pethidine is in many countries subject to the restrictions of the Opium Convention, its micro-identification may have significance. In the opinion of Professor B. Samdahl optical crystallography is useful in the analysis of crystalline pharmaceutical substances, and for this reason he urged me to examine the crystalline compound of pethidine and nitric acid by means of the polarising microscope and ascertain its optical data.

The optical characters have also been observed in respect of crystals obtained with nitric acid from other substances listed in the Scandinavian Pharmacopœias. Among these substances the following have been stated in the literature as giving colourless crystals with nitric acid: pethidine, nicotinamide and cinchophen. Among substances not listed in the Scandinavian Pharmacopœias, sulphaguanidine forms a crystalline complex with nitric acid, the optical characters of which have been examined by G. L. Keenan[2]. The purpose of this paper is to report the microscopical characters in polarised light of the crystals formed with nitric acid from the other three substances.

The refractive indices were determined for yellow light by the immersion method (all $\pm$ 0·002). The principal indices were determined statistically in contused material in order to ensure that the particles lay in random formation. Significant intermediate refractive indices, observed in a preferred orientation of the crystals, are reported when they may be useful in the identification of the substance.

Pethidine

The crystals are precipitated on a microscope slide from a drop of an aqueous solution of pethidine hydrochloride (5 to 10 per cent.) with 5 M nitric acid. Solid pethidine may also be added to a drop of nitric acid.

Crystal habit. Lance-shaped crystals of variable length. Sometimes small crystals of rhombic silhouette.

Characters shown in parallel polarised light. (Crossed Nicols). Usually no interference colours, some larger crystals having low order colours. Extinction usually unsymmetrical. Elongation negative. The crystals of rhombic silhouette have symmetrical extinction: $\frac{1}{2}(43° \pm 1)$.

Characters shown in convergent polarised light. (Crossed Nicols). An off-centred biaxial optic axis figure is commonly found. Optic sign: positive.

Refractive indices. $\alpha = 1{\cdot}572$, $\gamma = 1{\cdot}590$, $1{\cdot}575 < \beta < 1{\cdot}582$.

Distinctive optical characters. $n_i = 1{\cdot}575$ frequently found lengthwise, and $n_i = 1{\cdot}585$ less frequently found crosswise, are useful in the identification of the substance.

System. Monoclinic.

Nicotinamide

2 ml. of an aqueous solution of nicotinamide (5 per cent.) is mixed with 1 ml. of 5 M nitric acid (Swedish Pharmacopœia 1946). The precipitation is performed on a microscope slide with a drop of each reagent. Solid nicotinamide may be added or a more concentrated solution may be used.

Crystal habit. Elongated rods with rectangular, parallelogramatic or irregular silhouette. They occur isolated or linked together at one of their elongated planes.

Characters shown in parallel polarised light. (Crossed Nicols). White of higher order commonly shown. Some rods have brilliant interference colours and positive elongation. Extinction usually oblique. Extinction dispersion sometimes observed. Some of the rods with brilliant interference colours have parallel extinction. The rods linked together prove to be a characteristic polysynthetic twin formation with extinction angle $5° \pm 1$.

Characters shown in convergent polarised light. (Crossed Nicols). On the rods having parallel extinction the interference figure of an acute positive bisectrix is always observed.

Refractive indices. $\alpha = 1{\cdot}410$, β not determined, $1{\cdot}730 < \gamma < 1{\cdot}741$.

Distinctive optical characters. γ is frequently observed and may be useful in the identification of the substance. The polysynthetic twin formation is also characteristic.

System. Monoclinic. α parallels the β crystallographic axis. $2V =$ 40 to 60°. Plane 010 well developed.

Cinchophen

A few mg. of the substance are dissolved in 1 drop of 10 per cent. ammonia on a microscope slide and 1 or 2 drops of 5 M nitric acid are added, Wagenaar[3].

Crystal habit. Small needles occurring isolated or arranged in rosettes.

Characters shown in parallel polarised light. (Crossed Nicols). Brilliant interference colours. Extinction parallel or nearly parallel. Elongation positive.

In convergent polarised light no interference figures can be obtained on the small needles. The particles in contused material will be extremely small and the principal refractive indices cannot be exactly determined.

Distinctive optical characters. $1{\cdot}730 < \gamma < 1{\cdot}741$ is frequently observed lengthwise. Crosswise the index 1·492 is frequently found.

Summary

Optical data are presented for the crystals obtained by the microchemical reaction with nitric acid from pethidine, nicotinamide and cinchophen. The results are of value in the rapid identification of these substances.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Morphine and Codeine, 2-Anthraquinonesulphonate Derivatives of. M. Feldstein, N. C. Klendshoj and A. Sprague. (*Anal. Chem.*, 1949, **21**, 1580.) A solution of sodium 2-anthraquinonesulphonate (1 g. in 20 ml. of water containing 2 ml. of 3N hydrochloric acid) formed characteristic crystals with morphine and codeine, the melting-points of these derivatives being sufficiently far apart to afford a means of identification. Out of 19 alkaloids tested (cocaine, pontocaine, procaine, metycaine, atropine, homatropine, aconitine, scopolamine, strychnine, brucine, caffeine, narceine, dilaudid, quinine, ergamine, pseudopelletierine and nicotine) morphine and codeine only gave a crystalline precipitate. In performing the test about 1 to 2 mg. of the substance was placed on a slide and dissolved in one drop of 6N sulphuric acid. One drop of the reagent was added and the preparation was allowed to stand for 10 to 15 minutes or until crystallisation occurred. Excess of solution was decanted from the preparation by means of a capillary pipette and the decantation and washing repeated; the slide was dried at 100°C., and the melting-point determined. The morphine derivative melted at 198° to 199°C.; its solubility in water at 20°C., was 0·85 mg. per ml. The codeine derivative melted at 175° to 176°C.; its solubility in water at 20°C., was 0·87 mg. per ml. Both derivatives were deep yellow in colour when prepared in large amounts but appeared colourless in minute amounts on a slide. Photographs of the crystals formed with morphine and codeine are given. R. E. S.

ANALYTICAL

Aconite, Assay of. H. Mühlemann and R. Weil. (*Pharm. Acta Helvet*, 1949, **24**, 419.) 5 g. of powdered aconite tuber is mixed with 10 ml. of water, 15 ml. of 25 per cent. ammonia and 100 g. of ether. After shaking for 30 minutes, 5 g. of tragacanth is added, and the ethereal solution is filtered and weighed. The ether is shaken out 5 times with 10 ml. quantities of 0·1 N sulphuric acid, and the acid solution is made alkaline with 2·5 ml. of 25 per cent. ammonia. This solution is shaken 5 times with 20 ml. quantities of ether, and the ethereal solution is evaporated on the water-bath. The residue is again evaporated down with 5 ml. of ether, then with 5 ml. of alcohol. After dissolving, with the aid of gentle heat, in 10 ml. of alcohol, the residue is treated with 10 ml. of 0·1 N sulphuric acid and titrated back with sodium hydroxide, using methyl red-methylene blue indicator. 1 ml. of 0·1 N acid is equivalent to 0·064538 g. of total alkaloids, calculated as aconitine. The titrated solution is then made alkaline with 10 ml. of 5 per cent. sodium hydroxide, and heated under a reflux condenser for 30 minutes on the water-bath. The solution is transferred to a flask, treated with 35 ml. of phosphoric acid (d = 1·7) and 3 ml. of liquid paraffin, and distilled in steam, the distillate being collected in water, previously neutralised to phenolphthalein, containing 1 drop of 0·1 N sodium hydroxide in excess. When about 1200 ml. of distillate has been collected, it is titrated with 0·1 N sodium hydroxide. Calculation proceeds from the fact

that aconitine gives on saponification one molecule each of acetic and benzoic acids, while benzoylaconine gives one molecule of benzoic acid only.

G. M.

Antihistamine Drugs, Colorimetric Estimation of. H. M. Jones and E. S. Brady. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 579.) N-(2-pyridyl) substituted antihistamines give coloured compounds when treated with cyanogen bromide and aniline which obey Beer's Law and can be quantitatively determined. To 3 ml. of the solution to be assayed, containing 0·5 to 3 mg. of antihistamine, 1 ml. of a 4 per cent. aqueous solution of cyanogen bromide and 5 ml. of a 2 per cent. aqueous solution of potassium acid phthalate are added, followed after 15 minutes by 1 ml. of a 4 per cent. solution of aniline in alcohol. The colour is measured in a photo-electric colorimeter using a suitable filter. The method was successfully adapted to the assay of creams, elixirs, ointments and tablets, and was also applied to urine, although here interference by other pyridine compounds such as nicotinic acid must be guarded against. Compounds for which the method is suitable include tripelennamine (pyribenzamine), thenylpyramine (histadyl), methapyrilene (thenylene), pyranisamine (neo-antergan), prophenpyridamine (trimeton) and doxylamine (decapryl). G. R. K.

Iodides, Detection of Traces of. G. Denigès. (*Bull. Trav. Soc. Pharm. Bordeaux,* 1949, **87,** 65.) The reagent is prepared by mixing 20 ml. of water, 2 ml. of ammonia and 0·5 ml. of 0·1 N solution of mercuric chloride. The mixture contains a precipitate of aminomercuric chloride, and should be shaken before use. If to 5 ml. is added 10 ml. of a solution containing a trace of iodide, and a similar tube is prepared without the iodide as a control, then, after shaking, a yellowish tint may be perceived in the tube containing the iodide, when observed along the axis of the tubes. The difference is more conspicuous if the mixtures are allowed to settle for 1 hour, and the precipitate is observed. A distinct reaction is obtained at a concentration of 1 mg. of iodide per l. G. M.

Ketohexoses, Specific Qualitative Colour Test. H. Tauber. (*J. biol. Chem.,* 1950, **182,** 605.) To 0·5 ml. of sulphuric acid, 0·2 ml. of a 2·5 per cent. aqueous solution of aminoguanidine sulphate monohydrate is added without mixing, followed by 0·2 ml. of the test solution containing 0·4 mg. of fructose, sorbose or inulin. The liquids are well mixed and a bright reddish purple colour forms in about 1 minute and increases in intensity for some time. Sucrose, 0·8 mg., or raffinose, 1·2 mg., give an identical colour. The following substances produce no colour under similar conditions: arabinose, xylose, ribose, glucose, galactose, mannose, lactose, maltose, melibiose, dextrin, starch, glycogen, formaldehyde, pepsin, trypsin and chymotrypsin. Furfural gives a yellow colour, furfuryl alcohol a brown colour and acetone, methyl ethyl ketone and lævulinic acid a very slight yellow colour. G. R. K.

Kurchi Bases, Labile Nature of, and the Assay of Kurchi Bismuth Iodide. N. K. Basu and N. N. Battacharya. (*Indian J. Pharm.,* 1949, **11,** 157.) The alkaloidal content of an extract of Kurchi Bark was determined by dissolving in standard acid and back titration with alkali. A further quantity of the extract was dissolved in alcohol, the solvent evaporated on a water-bath, the residue dissolved in chloroform and the solvent again evaporated on a water-bath. On titration after this treatment, the alkaloidal content was found to be less. A similar result was obtained with a more highly purified sample consisting mainly of conessine. Assay methods involving evaporation of solutions of the bases are therefore not reliable. The following

method, in which evaporation of the chloroform extract is avoided, is recommended for the assay of Kurchi bismuth iodide. Triturate 0·3 g. of Kurchi bismuth iodide with 5 ml. of sodium hydroxide solution and extract with successive 10 ml. quantities of chloroform, washing each chloroform solution with the same 10 ml. of water. Filter the mixed chloroform solutions and shake with 0·1N sulphuric acid. Wash the chloroform solution with two 10 ml. quantities of water, add these to the aqueous acid liquid and titrate with 0·05N sodium hydroxide, using methylene blue-methyl red solution as indicator. Each ml. of 0·1N sulphuric acid is equivalent to 0·01657 g. of alkaloids. G. B.

Senna Leaves, Spectrophotometric Method of Assay for. B. V. Christensen and I. A. Abdel-Latif. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 589). 10 g. of powdered and dried leaves is refluxed for 30 minutes with 75 ml. of 10 per cent. alcoholic potassium hydroxide. The liquid is filtered, the marc washed and the filtrate adjusted to 100 ml. with alcohol. 25 ml. of this solution is diluted with 25 ml. of water and sufficient dilute hydrochloric acid to adjust the reaction to *p*H 2, and extracted with one 30-ml. and five 20-ml. quantities of ether. The ether extracts are combined and washed with a mixture of 5 ml. of dilute hydrochloric acid and 10 ml. of water. The wash-liquid is itself washed with 15 ml. of ether and the total ether extract and washing adjusted to 200 ml. with ether. 30 ml. of the ether solution is shaken with 10 ml. of ammonia and centrifuged. The ammonia layer is removed and the red colour which develops in it measured spectrophotometrically at 670 mμ. The extinction varies directly with the concentration of the anthraquinone derivatives. The method is rapid, accurate and sensitive and may be used comparatively until reference standards are set up. It should be applicable to all emodin-containing drugs. G. R. K.

Sodium *p*-aminosalicylate, Determination of. H. A. M. van Steenbergen. (*Pharm. Weekbl.*, 1949, **84**, 797.) In view of the ease of decarboxylation of *p*-aminosalicylic acid, it is essential that any method of estimation should not be affected by *m*-aminophenol. Moreover, decomposition during the estimation must be avoided by use of a low temperature and low degree of acidity. Details of the method recommended are as follows. A quantity of solution, corresponding to about 0·3 g. of the acid, is placed in a stoppered flask, diluted, if necessary, to 10 ml., and treated with 40 ml. of ether and sufficient 0·5 N sulphuric acid to bring the *p*H to between 1 and 2 (indicator paper). The mixture is transferred to a continuous extractor, being washed in with several quantities of ether. After extraction, the ether is distilled off, care being taken that the temperature of the residue is not allowed to exceed 40°C. The residue is dissolved in 25 ml. of alcohol and titrated with 0·1 N sodium hydroxide, using phenol red as indicator. The first adjustment of the *p*H may also be carried out before the addition of the ether, but in this case the coarser precipitate of the acid requires vigorous shaking to cause it to dissolve. G. M.

Thiourea as Reagent in Inorganic Analysis. G. Denigès. (*Bull. Trav. Soc. Pharm. Bordeaux.*, 1949, **87**, 67.) Thiourea may be used for the formation of sulphides in inorganic analysis. If, for example, about 5 cg. of thiourea is mixed with 1 to 2 cg. of an arsenite and heated until the reagent fuses and begins to decompose, the mass assumes a strong orange colour owing to formation of red arsenic sulphide (As_2S_2). By extraction with water, the sulphide may be separated and identified by its solubility in ammonia. With arsenates, the product obtained is yellow (arsenious

sulphide) and also soluble in ammonia. Antimonious compounds, treated in a similar manner, give a black residue, while with potassium antimonate the colour, after lixiviation with water, is a brownish green. Calomel, triturated with thiourea in the cold, gives a black mass, but mercuric chloride does not react. G. M.

FIXED OILS, FATS AND WAXES

Lard, Preservation of. E. Sandell and B. Spross. (*Svensk farm. Tidskr.* 1950, **54,** 61.) Lard, if freshly prepared and of low peroxide content, may be effectively preserved by the addition of 0·005 per cent. of hydroquinol, nordihydroguaiaretic acid, or propyl gallate. Benzoin, at a concentration of 0·3 per cent., is also satisfactory, and better than guaiacum resin at the same concentration. The preservative action of diphenylamine, hardened arachis oil, or of α-tocopherol was only slight, while α-tocopherol, which showed a preservative action under accelerated test, was found to give, under storage conditions, no better stability than lard without any addition. When water was incorporated with the lard a larger proportion of anti-oxidant was necessary (at least 0·03 per cent.). In alkaline solution (e.g., in presence of borax), these anti-oxidants cannot be used, as hydroquinonol is ineffective and nordihydroguaiaretic acid and propyl gallate, although giving some protection at high concentrations, cause discoloration. In this investigation the question of possible toxicity of the stabilisers was not considered. G. M.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Blood and Blood Plasma, Chemical Sterilisation of. F. W. Hartmann, G. H. Mangun, N. Feeley and E. Jackson. (*Proc. Soc. exp. Biol., N.Y.*, 1949, **70,** 248.) Plasma, serum or whole blood which has been contaminated with *Bact. coli, Pseudomonas æruginosa,* hæmolytic streptococci or vesicular stamatitis, is sterilised by methyl-bis(β-chloroethyl)amine hydrochloride in a concentration of 500 mg. per litre, provided that the *p*H lies between 6·7 and 7·2. After 5 days, no toxic products remain, unless the *p*H is too low to permit degradation of the ethylammonium ion. There is no evidence of antigenic or other toxic reactions. Complement, immune bodies, phosphatase, fibrinogen and red cell fragility are only slightly altered by the treatment but there is a marked increase in the prothrombin time. G. B.

Progesterone, Solubility in Water and in Saline Solution. A. L. Haskins, Jr. (*Proc. Soc. exp. Biol., N.Y.*, 1949, **70,** 228.) Synthetic α-progesterone m.pt. 128°C. has a characteristic ultra-violet absorption band with a maximum at 240 mμ in alcohol (95 per cent.) and at 248 mμ in aqueous solution. The solubility at room temperature is 16·8 μg./ml. in water and 15·1 μg./ml. in 0·9 per cent. sodium chloride solution. These solubilities have been determined by evaporating a small quantity of an alcoholic solution, warming the residue with the solvent, allowing to stand and measuring the concentration of progesterone in the supernatant liquid spectrophotometrically at intervals. Under these conditions, solution is complete in 72 hours. G. B.

Vitamin B_{12}, Synthesis of, in the Digestive System of the Sheep. P. H. Abelson and H. H. Darby. (*Science,* 1949, **110,** 566.) In an experiment to determine the possibility of a relationship between cobalt deficiency disease in sheep and the fact that vitamin B_{12} contains cobalt, the authors

administered radioactive cobalt in tracer doses to sheep. Examination of the fæces showed more than half the traced cobalt had been incorporated into an organically bound form, and treatment with acid enabled most of the active cobalt to be extracted. Biological assay, using *Lactobacillus lactis* Dorner, and *Lactobacillus leichmannii* indicated the presence in the fæces of large amounts of vitamin B_{12}. It is known that the growth of certain bacteria in the rumen of sheep is stimulated by the administration of cobalt and it is possible that these rumen bacteria synthesise the B_{12}. E. N. I.

BIOCHEMICAL ANALYSIS

Adrenaline—norAdrenaline Mixtures, Estimation of. J. H. Burn, D. E. Hutcheon and R. H. D. Parker. (*Brit. J. Pharmacol.*, 1950, **5**, 142.) The authors describe a modification of the nictitating membrane method which has a much greater accuracy and does not require a cat with a denervated nictitating membrane. The contractions of a normal nictitating membrane and the blood pressure are recorded in a spinal cat. The ratio of the height of the membrane contraction to the rise in blood pressure bears an almost linear relation to the percentage of adrenaline present, provided that the solutions injected cause about the same rise of blood pressure. The method, which has a high degree of accuracy, is applicable to total amounts of adrenaline and noradrenaline not less than 10 to 30 μg. in 1 to 3 ml. S. L. W.

norAdrenaline in presence of Adrenaline, Colorimetric Estimation of. U. S. von Euler and U. Hamburg. (*Science*, 1949, **110**, 561.) The colorimetric method is based on the formation of noradrenochrome and adrenochrome on oxidation with iodine. At *p*H 4·0, adrenochrome formation is complete in 1½ minutes after treatment with iodine, but only about 10 per cent. of the noradrenaline is transformed into noradrenochrome under the same conditions. On 3 minutes treatment with iodine at *p*H 6·0, maximal formation of noradrenochrome and adrenochrome is attained. The procedure is as follows. The extract, buffered to *p*H 4 is treated with 0·1N iodine. After precisely 1½ minutes, excess of iodine is removed by means of sodium thiosulphate. The colour is read within 5 minutes against a blank without iodine at wave-length 529 mμ. The procedure is repeated with a second sample buffered to *p*H 6 and 3 minutes iodine treatment. Standard readings are made with 100 μg. of adrenaline and noradrenaline at *p*H 4 (1½ minutes) and *p*H 6 (3 minutes), giving the calibration factors for both substances and the percentage of noradrenaline oxidised at *p*H 4 in 1½ minutes. At 529 mμ, the adrenochrome figure is the same on oxidation at *p*H 4 and *p*H 6. Results agree well with those obtained by biological methods. E. N. I.

Calcium and Magnesium in Small Amounts of Biological Material, Colorimetric Determination of. G. D. Michaels, C. T. Anderson, S. Margen and L. W. Kinsell. (*J. biol. Chem.*, 1949, **180**, 175.) The determination depends on the formation and colorimetric determination of calcium and magnesium phosphate; the total phosphate is determined (calcium and magnesium), the calcium is precipitated as oxalate, and the magnesium is determined (after separation) as magnesium ammonium phosphate; the calcium is then redissolved and reprecipitated as calcium phosphate. Both phosphates are then determined and the sum of the two should equal the

total phosphate found previously. For urine a sample is just acidified with hydrochloric acid, ammonium phosphate and ammonium hydroxide solutions are added, the precipitate is removed by centrifuging, washed with alcohol and dissolved in 1 : 4 hydrochloric acid, the solution being made up to a standard volume. An aliquot portion of this solution is taken for the determination of total phosphate; to a further aliquot is added oxalic acid and sodium acetate to *p*H 4·0 thus precipitating calcium oxalate but not magnesium. The calcium oxalate precipitate is washed with dilute ammonia solution and the supernatant liquid and washings are combined for the determination of magnesium. The calcium oxalate precipitate is dissolved in hydrochloric acid and reprecipitated as phosphate with ammonium hydroxide and ammonium phosphate; the calcium phosphate precipitate is then washed with alcohol and redissolved in hydrochloric acid for the determination of phosphate. To the supernatant liquid (containing the magnesium) ammonium phosphate and ammonia solution are added and the precipitated magnesium ammonium phosphate is washed with alcohol and redissolved in hydrochloric acid for the determination of phosphate. The actual phosphate determinations were performed colorimetrically by the standard method of Fiske and Subbarow (*J. biol. Chem.*, 1925, **66**, 375). Analysis of known mixtures of calcium and magnesium in water gave the constant ratio calcium-phosphorus of 1·62 : 1 and magnesium-phosphorus of 0·782 : 1, indicating the formation of magnesium ammonium phosphate, whereas, in the case of calcium, apparently an equal mixture of tricalcium phosphate and ammonium calcium phosphate results. Recovery of total phosphate ranged from 100·0 to 99·3 per cent.; experiments with known amounts of calcium and magnesium in urine gave equally good recoveries. R. E. S.

Chloride in Blood, Polarographic Microdetermination of. W. J. Zimmerman and W. M. Layton, Jr., (*J. biol. Chem.*, 1949, **181**, 141.) A polarographic method for the determination of blood chloride was developed, depending on the fact that, using 0·1M potassium nitrate as supporting electrolyte, the diffusion current of the anodic depolarisation wave produced at the dropping mercury electrode is directly proportional to chloride concentrations between 10^{-4} and 2×10^{-3} equivalent per l. 0·05 ml. samples of whole blood, serum or plasma are delivered into 4·0 ml. of approximately 0·15M phosphoric acid in a test-tube, and 1·0 ml. of 3 per cent. sodium tungstate solution is added to precipitate proteins; the solution is mixed, centrifuged for approximately 1 minute at 2,000 r.p.m., and the clear supernatant solution is transferred to the electrolytic cell. The current is measured at a single fixed, applied voltage and in the procedure described a setting of $-0·6$ volt against the saturated mercurous sulphate reference electrode was found suitable (0·34 volt versus the saturated calomel electrode); it was unnecessary to remove oxygen from the solutions since it is reduced at potentials negative to the chloride wave. Minimum and maximum galvanometer readings were recorded on sample solutions and a sufficient number of standard chloride solutions in phosphotungstic acid to cover the range of chloride values encountered were run simultaneously. Details of the electrolytic cell used and the electrode assembly are given. In nine experiments in which 16·0 m.eq. per l. of chloride as potassium chloride was added to 0·05 ml. samples of plasma and whole blood, the mean error in recovery was 0·9 m.eq. per l., the maximum error being 1·5 m.eq. per l. The values obtained by this method when applied to 0·05 ml. samples of serum, plasma, and whole blood were compared with the values obtained by titration; 28 parallel determinations in duplicate gave a mean deviation

between the values obtained by the two methods of 1·0 m.eq. per l.; 86 per cent. of the polarographic values agreed with iodimetric values within 2·0 m.eq. per l., and none differed by as much as 3·0 m.eq. per l. R. E. S.

Citric Acid, Microcolorimetric Determination of. H. H. Taussky. (*J. biol. Chem.*, 1949, **181,** 195.) A method is described which avoids the use of hydrazine sulphate in the procedure based on conversion of citric acid to pentabromoacetone, and on the subsequent reaction between pentabromoacetone and alcoholic sodium iodide with the development of a yellow colour complex; hydrazine sulphate had been utilised at two stages in this procedure for the reduction of free bromine and manganese dioxide. The previous method (*J. biol. Chem.*, 1947, **169,** 103) was therefore modified so that ferrous sulphate replaced hydrazine sulphate. Preliminary bromination is carried until the point at which excess of free bromine has to be removed by reduction. After the mixture has reached room temperature, the bromine fumes are removed by suction, 2 ml. of saturated ferrous sulphate solution is added and the mixture is shaken; the procedure is then continued as previously described (*ibid.*). In the oxidation of citric acid to pentabromoacetone, 2·5 ml. of potassium permangenate solution is added instead of 2·0 ml. as originally described, the larger amount resulting in a more rapid formation of the manganese suspension. After standing in the water-bath at about 18°C. for 30 minutes, bromine fumes are removed by suction and 6 ml. of ferrous sulphate solution is added, the mixture being shaken and allowed to stand for about 3 minutes. Similar recovery results were obtained with ferrous sulphate as with hydrazine sulphate, although in blood and urine lower values were found with ferrous sulphate, suggesting that the latter is more specific. R. E. S.

Copper, Determination of traces of, by means of *Penicillium*. J. Keilling, A. Camus, P. Foulet and J. Burdin-Steeg. (*C.R. Acad. Sci., Paris,* 1949, **228,** 2059.) For the determination of traces of copper, *Penicillium glaucum* is to be preferred to *Aspergillus niger* on account of the greater ease of appreciation of the scale of dosage from the colouration of the conida. The material under examination, or the ash, is sterilised and added, in increasing quantities, to flasks containing 40 ml. of nutrient medium, free from copper, which is then inoculated with a suspension of the conidia. Four days is allowed for growth. The minimum quantity which ensures a green colouration of the mould contains 1 μg of copper. Alternatively the colour may be compared with standards. It should be noted that apparatus should only be flamed with a glass burner. The appearances observed are as follows:

Copper, μg per litre	Appearance
0	white.
0·2	very few green points.
0·5	slight green stain.
1·0	definite green

G. M.

Dicoumarol in Biological Fluids, Estimation of. J. Axelrod, J. R. Cooper and B. B. Brodie. (*Proc. Soc. exp. Biol., N.Y.*, 70, **4,** 693.) A simple and sensitive spectrophotometric method is described. 1 to 3 ml. of plasma or urine, containing up to 50 μg. of dicoumarol and 0·5 ml. of 3N hydrochloric acid are shaken with 20 ml. of heptane for 30 minutes, after adjusting the aqueous volume to about 3·5 ml. The mixture

is centrifuged, 15 ml. of the heptane phase shaken with 4 ml. of 2·5N sodium hydroxide for 5 minutes and the organic phase removed. 3 ml. of the aqueous phase is transferred to a quartz cuvette and the optical density determined in a spectrophotometer (Beckman) at 315 mμ. The distribution of dicoumarol in a heptane-acidified water system is such that at room temperature, with volumes of 20 ml. and 3·5 ml. respectively, about 95 per cent. of dicoumarol is in the organic phase. Known amounts of dicoumarol gave optical densities proportional to the concentration. The method does not include metabolic products of the drug. Basic organic drugs do not interfere since they are not extracted at an acid *p*H. Of a wide range of acidic and neutral substances tested, only pentothal and salicylates interfered. E. N. I.

Digitoxin, Polarographic Determination of. J. G. Hilton. (*Science*, 1949, **110**, 526.) A study of the polarographic properties of digitoxin showed that it could be determined in amounts as low as 0·1 μg. in 50 per cent. alcohol solution, and could also be extracted by means of suitable solvents from complex mixtures and determined in similar low concentrations. Varying amounts of pure digitoxin in 0·5 ml. of 0·2N tetraethylammonium hydroxide in a Heyrovsky reaction vessel were diluted to 5 ml. total volume with a 50 per cent. alcoholic solution in order to study the half-wave potential and height of break at different concentrations. Nitrogen was bubbled through the prepared solutions for a period of 15 to 20 minutes and the polarogram recorded, the process being repeated until a curve of satisfactory height and concentration had been determined and the average half-wave potential calculated. Digitoxin in blood was extracted by shaking with 2·5 times the total volume of light petroleum followed by careful separation. After evaporation the residue was dissolved in 2·5 ml. of absolute alcohol and decanted to remove all alcohol-insoluble components; this solution was diluted to 5 ml. with distilled water and 2·5 ml. was placed in a Heyrovsky reaction vessel with 2·0 ml. of a 50 per cent. alcohol solution and 0·5 ml. of 0·2N tetraethylammonium hydroxide. Nitrogen was bubbled through the solution for 15 to 20 minutes and the polarogram recorded. Graphs of height and concentration are given and it is claimed that digitoxin can be determined in concentrations as low as 0·1 μg. with an error of ±0·02 μg. between values from 0·1 to 0·4 μg. of digitoxin. The average half-wave potentials were found to be −1·965 in alcoholic solution and −1·958 when extracted from blood. R. E. S.

Œstrogens in Urine: Determination by a Micro-fluorimetric Method. M. Finkelstein. (*Proc. Soc. exp. Biol., N.Y.*, 1948, **69**, 181.) The method permits of the accurate determination of the œstriol fraction and of the œstradiol-œstrone fraction in as little as 10 ml. of urine. The sample (10 ml.) is hydrolysed under a reflux condenser for 1 hour with 0·7 ml. of hydrochloric acid. After cooling 5·6 g. of sodium chloride and 10 ml. of water are added and the liquid extracted 5 times with 20 ml. of benzene. The benzene extracts are washed with 3 ml. of 9 per cent. sodium bicarbonate solution, evaporated to 30 ml. and again washed with 30, 15 and 15 ml. of 9 per cent. sodium carbonate solution. The œstradiol-œstrone fraction remains in the benzene while the œstriol fraction is almost quantitatively removed by the sodium carbonate solution. The pooled carbonate extract is adjusted to *p*H6, extracted three times with 35 ml. of ether, and the ether extract washed twice with 10 ml. of 9 per cent. sodium bicarbonate solution and twice with 10 ml. of water. The ether is then evaporated and the residue

taken up in 10 to 20 ml. of alcohol (96 per cent.), aliquots of which are used for fluorimetry. If the ether residue is coloured a further purification procedure can be applied. For the œstradiol-œstrone fraction the benzene extract is washed with a little dilute sulphuric acid and then twice with 15 ml. of water. It is then extracted 4 times with an equal volume of N/1 sodium hydroxide. The alkaline extract is acidified to a *p*H of less than 5 and extracted three times with 50 ml. of ether. The ethereal extract is concentrated to about 50 ml., washed with 10 ml. of dilute sulphuric acid, then twice with 20 ml. of 9 per cent. sodium carbonate solution, and twice with 20 ml. of water, and evaporated to dryness, the residue being taken up in alcohol. To make the determination, an aliquot of the alcohol solution is evaporated to dryness at 120°C. and the residue cooled. 7 ml. of phosphoric acid is added and the mixture heated in a boiling water-bath in the dark for 30 minutes. After cooling, the fluorescence is measured in a Coleman fluorimeter using filters P_2 and PC_2. The instrument is calibrated against the pure œstrogens. The best recoveries (above 80 per cent.) in the extraction process are obtained with œstradiol, and as little as 1 µg. added to 10 ml. of urine could be demonstrated, with 60 per cent. recovery. The recoveries of œstrone were about 70 per cent. and of œstriol 50 to 60 per cent. The latter should be determined only in dilute solution. H. T. B.

Organic Acids, Qualitative Analysis of, by Filter Paper Partition Chromatography. K. Fink and R. M. Fink. (*Proc. Soc. exp. Biol., N.Y.*, 70, 654.) The separation of both volatile and non-volatile organic acids, with chain lengths of about 8 carbon atoms or less, is described. The hydroxamate derivatives are prepared by reacting the methyl ester of the acid with about a two-fold excess of potassium hydroxide and hydroxylamine in methyl alcohol. The chromatogram is developed by suitable solvents and then sprayed with ferric chloride solution to make the derivatives visible as purple spots on a yellow background. The colour reaction will detect acid present in the order of 10^{-7} mol., and a rough quantitative estimation may be made of the amount present by judging from the size of the spot and the intensity of the colour. Isobutyric acid and phenol were the best solvents tried for two-dimensional chromatograms of the dicarboxylic acids. R_F values for a number of hydroxamate derivatives are given for several solvents. E. N. I.

Vitamin A in Milk. A. E. Sobel and A. A. Rosenberg. (*Anal. Chem.*, 1949, **21**, 1540.) A new method has been devised for the determination of vitamin A and carotene in milk using activated glycerol dichlorhydrin. One ml. of milk is pipetted into a test tube, 1 ml. of N potassium hydroxide in ethyl alcohol (90 per cent.) is added, the contents of the tube are mixed and the tube is placed in a water bath at 60°C., for 35 minutes. The tube is removed, allowed to cool to room temperature, 2 ml. of light petroleum is added, and the tube is stoppered with a stopper pre-extracted with light petroleum. After shaking for 10 minutes the tube is centrifuged for approximately 30 seconds, or until complete separation has occurred, when the supernatant light petroleum extract is removed with a fine tipped rubber bulb dropper. The saponified milk sample is shaken with two succeeding 1 ml. aliquots of light petroleum allowing 5 minutes for each shaking, the supernatant ether layer being collected as before and bulked with the previous extract in a small test-tube. The mixed light petroleum extracts are evaporated to dryness in a water-bath at 40° to 50°C. in a slow stream of nitrogen. One ml.

of chloroform is added to bring the dried extract into solution and 4 ml. of activated glycerol dichlorhydrin is added at 25°C. After 2 minutes the solution is transferred to a cell and the absorption read at 550 mμ against a blank consisting of 1 ml. of chloroform and 4 ml. of reagent; following this a reading is taken at 800 mμ. The absorption at 800 mμ will give the carotene content from a carotene calibration chart, while this permits the evaluation of the interference due to carotene at 555 mμ from a carotene interference chart; the optical density due to carotene interference at 555 mμ is then subtracted from the total optical density at this wavelength the resulting optical density giving the vitamin A content per ml. from a vitamin A calibration curve. Methods are given for the preparation of the various calibration graphs. Values agreed closely with those obtained using the Carr-Price reagent. In the estimation of serum vitamin A, approximately the same results are obtained with saponification as without; saponification of a milk sample is a necessary step, however, since without saponification practically none of the vitamin A is extracted with light petroleum. It is suggested that vitamin A-containing lipids in fresh milk are bound more firmly to proteins than in blood. In experiments with added vitamin A the precision of the recovery was 100 ± 3·5 per cent. of the calculated value. R. E. S.

Vitamin B_6, Some Reactions of. H. Laubie. (*Bull. Trav. Soc. Pharm. Bordeaux*, 1949, **87,** 119.) The following reactions are given. 1. A few drops of Denigès sulpho-titanic reagent added to 1 mg. of the vitamin gives a yellow colouration. 2. By adding 6 drops of 3 per cent. solution of copper sulphate to 1 ml. of a solution of the vitamin, followed by 1 drop of saturated solution of ammonium thiocyanate, a green precipitate insoluble in chloroform is obtained. 3. To 1 ml. of an alkaline solution a few drops of copper sulphate solution are added, a green precipitate is obtained. 4. To 1 ml. of a solution of the vitamin 2 drops of 5 per cent. solution of uranium acetate is added; an intense yellow colour is produced. G. M.

Vitamin B_{12}, Microbiological Assay of. C. E. Hoffmann, E. L. R. Stokstad, B. L. Hutchings, A. C. Dornbush and T. H. Jukes. (*J. biol. Chem.*, 1949, **181,** 635.) The use of two strains of *Lactobacillus leichmannii,* designated respectively strains 313 (ATCC 7830 and ATCC 4797) for the assay of vitamin B_{12} in liver extracts is described. Both strains gave satisfactory results; the former grows more rapidly, enabling the use of a short assay period. Details of the medium are given, the assays being carried out in 12 × 100 mm. tubes in a total volume of 2·0 ml.; crystalline vitamin B_{12} was used for obtaining standard response curves. In preliminary experiments the response obtained was increased when the period of autoclaving the assay tube was prolonged although, in contrast, little growth was obtained when the medium was sterilised by steaming even though large amounts of vitamin B_{12} were added; an investigation of this finding by adding the samples to the medium *after* autoclaving showed that vitamin B_{12} was partially destroyed by autoclaving and that a second growth factor was produced in the medium during the autoclaving process. When thioglycollic acid was included in the medium, similar results were obtained when the liver extract samples were added before autoclaving and when the samples were added aseptically to autoclaved medium, indicating that thioglycollic acid protected vitamin B_{12} during the assay autoclaving process. An investigation of the growth factor produced in the medium during autoclaving revealed that it was due to interaction

between the glucose and the other constituents of the media with the production of a reducing agent. Glucose did not form a reducing agent unless it was autoclaved in contact with the rest of the medium, and it was found that sucrose did not form a reducing agent even when autoclaved together with the medium. Further results indicated that ascorbic acid and cysteine could replace thioglycollic acid as reducing agents. It was found that the addition of the basal medium to a liver extract accelerated the decrease in potency and the addition of thioglycollic acid to a diluted liver extract did not completely protect the vitamin B_{12} as indicated by the microbiological activity of the samples; the addition of thioglycollic acid to liver extract diluted with the basal medium, however, gave good protection. An extract of asparagus was found to have an effect similar to thioglycollic acid. It was found that vitamin B_{12} was destroyed by heating with 0·2 N sodium hydroxide at 100°C. for 30 minutes and that under these conditions the desoxyribosides of thymine, guanine and hypoxanthine were not affected; liver extract was assayed before and after this treatment with alkali, and the difference between the two assay values was used to express the vitamin B_{12} content; usually about 97 per cent. of the total microbiological activity of liver extract was found to disappear upon treatment with alkali, indicating that desoxyribosides were responsible for only a small fraction of the microbiological activity of the extracts. R. E. S.

CHEMOTHERAPY

Analgesics, New Series of. D. W. Adamson and A. F. Green. (*Nature*, 1950, **165**, 122.) A series of potent analgesics, as active as morphine in the rat, has been discovered in the 3-tertiary-1 : 1-(2′-thienyl)-but-1-enes, the analgesic/toxicity ratios being not greatly different from that of amidone. The analgesic dose in dogs was 5 to 10 mg./kg. compared with 1 to 2 mg./kg. for amidone, but the effect was not accompanied by the gastro-intestinal disturbances common with amidone or morphine. As with other analgesics, however, analgesia was accompanied by rise of temperature and respiratory depression and by sedation. An excitatory phase was manifest, particularly in the cat, and high doses cause spasticity of the muscles. The compounds also exhibited considerable antihistamine, spasmolytic and local anæsthetic activity. F. H.

Benzene Derivatives and Simple Phenols in the Chemotherapy of Tuberculosis. F. A. French and B. L. Freedlander. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **38**, 343.) 37 compounds were tested *in vitro* against *M. tuberculosis* H 37 Rv. The 4-alkyl ethers of *p*-hydroxybenzoic acid and 2:4-dihydroxybenzoic acid showed a rise in bacteriostatic activity with increase in the weight of the ether group. Simple phenols were mildly inhibitory. Some of the compounds also inhibited the growth *in vitro* of *M. tuberculosis* 607, *M. leprae*, and *Trichophyton gypseum*. All compounds tested were devoid of *in vivo* activity in guinea-pigs, except for 2:2′-dihydroxybenzophenone and 2-hydroxy-3:5-dichlorobenzophenone which gave low positive results. Lack of *in vivo* activity was probably due to unfavourable absorption-distribution-excretion characteristics. *p*-Anisic acid, which is a constituent of the lipoid fraction of virulent tubercle bacilli, appears to stimulate growth slightly. Replacement of the methyl group by higher alkyl groups gives rise to inhibitors, and the analogous *p*-alkyl ethers of 2:4-dihydroxybenzoic acid inhibit at almost exactly the same concentrations.

Vinyl analogues shows the same or somewhat increased activity as compared with the parent compound, for example, umbelliferone and β-methylumbelliferone compared with 2:4-dihydroxybenzoic acid, and hydroxybenzophenones compared with salicylic and benzoic acids. Following the observation that salicylic acid, after administration, is partly converted into gentisic acid, which can enter into rapid reversible oxidation-reduction systems, it is suggested that the presence of drugs having quinoid oxidation-reduction systems may interfere with the growth of tubercle bacilli, and that this may be the mode of action in such cases as *p*-aminosalicylic acid and *p*-alkoxybenzenes. G. B.

PHARMACY

DISPENSING

Phenobarbitone, in Ethyl alcohol-water systems, Solubility of. E. E. Levallen. (*J. Amer. pharm. Ass., Sci. Ed.*, 1949, **10**, 722.) Incompatibility between phenobarbitone sodium and acids in dispensing mixtures can be avoided by adding alcohol to the mixture to effect solution of the precipitated phenobarbitone. The author has determined the solubility of phenobarbitone in a series of water-alcohol systems, and has expressed the results in the form of solubility curves. By reference to these curves the amount of alcohol required to dissolve any given amount of phenobarbitone can be calculated, within the limits of the curves. Examples of the calculations which are involved when other substances, likely to influence the solubility of phenobarbitone are present in the mixture, are also given. E. N. I.

Pills, Preparation of, by the Drop Method. F. Ernefeldt and E. Sandell. (*Farm. Revy*, 1950, **49**, 41.) The method is based on dropping melted fat (m.pt. 38° to 40°C.) into alcohol (about 65 per cent.), the density of the latter being so adjusted that the molten fat falls slowly while solidifying. In the apparatus described the melted fat (e.g. hardened arachis oil) is kept at constant level in a small beaker fed from a constant level device. This beaker is connected, by means of a small syphon, with a second beaker to ensure a greater constancy of level. From this last beaker the liquid drops from a syphon on to a funnel and thence to the dropping tube. The latter consists of a 2 ml. ampoule with two holes blown near the lower end, and the upper end cut off. The liquid passes through the holes and drops from the base of the ampoule. The whole of this apparatus is kept in an air bath at 60°C. It is important that the lower surface of the ampoule should be horizontal. A drop rate of about 90 drops per minute is used, and in the manufacture of a large number of pills the relative standard deviation in weight was 0·6 per cent. The temperature of the oil should be about 58°C. as if too low the shape of the pills is unsatisfactory. G. M.

Sulphadiazine-Penicillin Mixtures, Sterilisation of. O. Lennert-Petersen. (*Dansk. Tidsskr. Farm.*, 1950, **24**, 33.) Sterilisation of mixtures of sulphadiazine and penicillin is important because such materials may be used as dusting powders during surgical operations. A mixture of sulphadiazine (previously dried at 110°C. for 3 hours) and potassium benzylpenicillin (1650 U/mg.) may be heated at 140°C. for 3 hours without appreciable loss of penicillin, although 160°C. produces complete inactivation in 1 hour. On the other hand, with the sodium salt of benzylpenicillin,

destruction was rapid even at 140°C. Penicillin cream, of the same formula as that of the British Pharmacopœia, infected by the addition of earth containing *B. subtilis*, was sterile after heating for 20 minutes at 120°C. G. M.

GALENICAL PHARMACY

Resorcinol, Stabilisation of, for Topical Preparations. A. Halpern and H. A. Getz. (*J. Amer. pharm., Ass., Pract. Pharm. Ed.*, 1950, **11,** 24.) Varying proportions of ascorbic acid, sodium formaldehyde sulphoxylate, sodium metabisulphite and α-tocopherol were added to ointment bases, lotions and emulsions containing 5 per cent. of resorcinol, and the preparations suitably exposed to light and air until discoloration occurred. Ascorbic acid, 1·4 per cent., delayed discoloration in ointment bases for about 140 hours, α-tocopherol, 1·0 per cent., for about 130 hours, sodium metabisulphite, 1·0 per cent., for about 30 to 40 hours, and sodium formaldehyde sulphoxylate, 1·0 per cent., for 30 to 60 hours. In the fluid preparations, the order of efficiency was ascorbic acid, sodium metabisulphite, sodium formaldehyde sulphoxylate, and α-tocopherol, except that when an oily phase was present, α-tocopherol was the best antioxidant. Sodium metabisulphite and sodium formaldehyde sulphoxylate had the disadvantage that they were incompatible with certain of the preparations used. Similar experiments in which the aqueous phase of water-containing preparations was buffered at different *p*H levels confirmed the view that an alkaline *p*H accelerates discoloration.

G. R. K.

NOTES AND FORMULÆ

Antazoline Hydrochloride (Antistine Hydrochloride). (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1950, **142,** 258.) Antazoline hydrochloride is 2-(*N*-phenyl-*N*-benzylammomethyl)-imidazoline hydrochloride, $C_6H_5.N(CH_2.C_6H_5).CH_2.C_3H_5N_2,HCl$. It occurs as white, odourless crystals with a bitter taste, m.pt. 232° to 238°C. with decomposition, sparingly soluble in water and in alcohol and insoluble in ether and in benzene; a 1 per cent. aqueous solution has *p*H 5·6 to 6·6. The base liberated on adding sodium hydroxide to an aqueous solution melts at 114° to 118°C.; with nitric acid antazoline hydrochloride gives a red colour which becomes green on standing; with trinitrophenol it gives a crystalline picrate which melts at 155° to 159°C. When dried at 105°C. for 4 hours, it loses not more than 0·5 per cent. of its weight; sulphated ash is not more than 0·2 per cent. When assayed by precipitation as the picrate it contains 97 to 103 per cent. of antazoline hydrochloride. A 0·001 per cent. solution exhibits an ultraviolet absorption maximum at 2420Å with an extinction coefficient of 520 ± 5. Assay processes are also described for solution and tablets. Antazoline hydrochloride is milder and less irritating to the tissues than other antihistamine substances. G. R. K.

Theophylline Sodium Glycinate. (*New and Nonofficial Remedies; J. Amer. med. Ass.*, 1949, **139,** 1149.) Theophylline sodium glycinate contains slightly more than 2 g.-mol. of glycine to 1 of theophylline sodium, and 49 to 50 per cent. of theophylline. It is a white odourless powder with a bitter taste. It decomposes between 190° and 210°C. and is soluble in water and decomposed by acids. It has the action of theophylline sodium acetate and theophylline with ethylenediamine, but is more stable in air and less irritating to the stomach. It is given by mouth as powder, tablets, elixir and syrup in a dose of 0·3 to 1 g. every 4 to 6 hours, or as suppositories containing

0·78 g. For children, the oral dose is 0·15 to 0·4 g. (over 12 years), or 0·1 to 0·2 g. (6 to 12 years) every 4 to 6 hours. It may be administered as an aerosol with oxygen in bronchial asthma; 2 ml. of a 5 to 10 per cent. solution every 4 hours is usually effective, but severe dyspnœa may require continuous therapy or alternation with inhalation of penicillin or other antibiotic.

G. R. K.

PHARMACOGNOSY

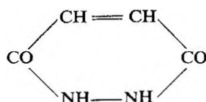
Anthraquinone derivatives, a new Microchemical Outside Indicator. B. V. Christensen and I. A. Abdel-Latif. (*J. Amer. pharm. Ass.*, 1949, **38,** 651.) Potassium hydroxide pellets are used to detect traces of anthraquinone derivatives in any extract of the emodin drugs or in any solvent. A drop of the liquid is placed on the pellet which immediately develops a red colour.

J. W. F.

Aloin, Distribution Chromatography of. A. B. Svendsen and K. B. Jensen. (*Scientia Pharm.* 1949, **17,** 118.) Two samples of commercial barbaloin were examined, one dating from 1898, the other a recent one. A column of kieselguhr, with 30 to 40 per cent. of water, was used; the mobile phase being composed of ether, chloroform, isopropyl alcohol or mixtures of the latter two liquids. The results showed that after prolonged storage (not well protected from light and air) a small amount of anthranol or anthrone is converted into emodin. There is also formed an appreciable quantity of a red-violet substance which does not give either the Bornträger or the Schonteten reaction, but gives a distinct positive reaction for sugar. A similar substance may be obtained by repeated heating of aloin on the water bath.

G. M.

Maleic Hydrazide; A selective Herbicide. H. B. Currier and A. S. Crafts. (*Science,* 1950, **111,** 152.) A solution of the diethanolamine salt of maleic hydrazide,



containing the equivalent of 0·2 per cent. of maleic hydrazide, and in some instances a wetting agent, was sprayed on to 2-weeks-old barley and 5-weeks-old cotton. A few days after treatment the leaves of the barley turned dark green and slowly died back from the tips; in about 6 weeks the barley was dead. In contrast the cotton was completely unaffected and in no way different from control cotton plants. Subsequent tests have shown that various types of plants react quite differently to the compound, and that age is critical, young plants responding to a much greater extent. Cotton treated in the cotyledonous stage was severely inhibited whereas plants 16 in. in height showed no apparent response. Young water grass and Johnson grass stopped growing, developed anthocyanin pigmentation and died; older plants showed some response but survived. Results already obtained seem to justify very thorough testing of this compound.

G. R. K.

Senna Leaves (Alexandrian), a new Fluorometric Method of Assay. B. V. Christensen and I. A. Abdel-Latif. (*J. Amer. pharm. Ass.*, 1949, **38,** 652.) An ether extract of senna leaves is made according to the procedure previously described (*J. Amer. pharm. Ass.*, 1949, **38,** 589.) This extract is shaken with ammonia solution and the fluorescence of the ammonia layer

is measured in a " Lumetron " fluorometer, which is described. The fluorescence was found to be inversely proportional to the concentration of senna extract. The method is only comparative as no reference standard for senna leaves has been established. J. W. F.

PHARMACOLOGY AND THERAPEUTICS

Antihistamines, Dermatitis following use of. W. B. Sherman and R. A. Cooke (*J. Allergy*, 1949, **21**, 63). Exacerbations of contact dermatitis of the eyelids, face and neck are reported following the use of pyribenzamine ointment in 2 cases, and of antistin eye-drops in 1 case. In the last patient who showed reactions to many antihistamine drugs there was also a maculopapular rash of the trunk apparently due to oral use of pyribenzamine. In the case of the pyribenzamine ointment there was no reason to suspect the base as a causative agent, and control patch tests with powdered pyribenzamine and antistin eye-drops on 3 normal persons showed no reaction. All 3 patients affected gave past histories of allergic manifestations. The 3 patients illustrated striking variations in sensitivity to different antihistamines; one patient reacted on patch tests to pyribenzamine but not to other drugs of the group; the second reacted to pyribenzamine, thenylene and neo-antergan but not to 6 other antihistamine drugs; and the third reacted to 8 antihistamine drugs but not to thephorin which differs in chemical structure. S. L. W.

Artane Therapy for Parkinsonism. L. J. Doshay and K. Constable. (*J. Amer. med. Ass.*, 1949, **140**, 1317.) Pharmacological tests established that the action of artane mildly resembles that of atropine in the control of sialorrhœa, in cycloplegic effects and cerebral stimulation, but that it is entirely free from the toxic effects of atropine on the cardiac vagus, blood pressure and circulation. Clinical studies in 117 patients have established its usefulness against parkinsonism and its freedom from disturbing side reactions. Though the usual dosage is between 6 and 10 mg. a day, 15 to 20 mg. is easily tolerated, and doses of 30 to 50 mg. have not had deleterious effects. Hypertension, cyanotic induration of the liver or renal disorders do not contraindicate the use of the drug. The investigation was conducted with 47 post-encephalitic, 33 idiopathic and 37 arteriosclerotic patients for periods ranging from 6 months to 2 years. In this series, 76.1 per cent. of the patients were improved and 23.9 per cent. unimproved. A favourable response was observed more frequently among members of the arteriosclerotic and idiopathic groups than among the post-encephalitic. In addition to the physical improvement in rigidity, tremor and oculogyria, it has a cerebral-stimulating action which is particularly effective in combating the depression and inertia. S. L. W.

Boric Acid as a Poison. E. G. Young, R. P. Smith, and O. C. MacIntosh. (*Canad. med. Ass., J.*, 1949, **61**, 447.) The deaths of 6 babies, 6 to 11 days old, are reported as the result of one feeding of milk which had been diluted with a 2·5 per cent. aqueous solution of boric acid in error for sterile water. The deaths occurred in from 19 hours to 5½ days. It was estimated that the amount of boric acid ingested was less than 3 g. The clinical diagnosis of death was intoxication with respiratory failure. Associated symptoms of poisoning which developed progressively in this group of infants were: (1) vomiting and diarrhœa, increasing in intensity

after the first day, (2) excoriations of the skin on the second and subsequent days, (3) convulsive movements, especially of the facial muscles and the extremities, (4) cyanosis and collapse as terminal picture. The authors emphasise that solutions of boric acid or borax should be labelled "poison."

S. L. W.

Cation Exchange Resin for Treatment of Œdema. L. Irwin, E. Y. Berger, B. Rosenberg and R. Jackenthal. (*J. clin. Invest.*, 1949, **28,** 1403.) A study was made of the electrolyte interchange between the diet and a cation exchange resin, liquonex CRW, a sulphonated polystyrene. The resin was given orally in gelatine capsules in doses sufficient to neutralise from 100 to 250 molar equivalents of base per day, in three equal doses. The general effect was to immobilise sodium in the gut, thus causing a decrease in the urinary sodium and an increase in the fæcal sodium. With a dosage of resin sufficient to neutralise 150 molar equivalents of sodium (6 g. salt), the daily urinary excretion was reduced to 50 molar equivalents, while a dosage of resin equivalent to 250 molar equivalents reduced the urinary excretion to 8 molar equivalents. Administration of the resin enabled a patient with congestive heart failure to take a relatively normal diet without absorbing an excessive amount of sodium. In two œdematous patients there was a diuresis of existing depots of fluid and no reaccumulation. Calcium and potassium are preferentially taken up by the resin and a deficiency of either or both may theoretically occur although not noticed in this study. The liberation of acid in the gut may create an acidosis. The principal disadvantage of the treatment is the large number of capsules, 20 to 25, to be taken with each meal. H. T. B.

Chloramphenicol, Observations on. J. D. Gray. (*Lancet,* 1950, **258,** 150.) Chloramphenicol, given by mouth, is effective against a wide variety of organisms. Bacteria do not acquire a resistance to the drug, and a "chloramphenicolase" has not yet been encountered; the substance seems to be inactivated only when it is exerting its antibiotic effect. In patients being treated with chloramphenicol, the mucous surfaces of the upper respiratory tract become completely sterile, and the sterile condition is maintained for 2 or 3 days after the last dose is given. This may be of value in surgery, especially if sterilisation is found to extend into the lungs. An urticarial reaction to a single dose is reported; this may have been due to massive bacterial death. Large doses of chloramphenicol hasten the onset of fatigue in skeletal muscles and also give rise to a rapid fatigue of accommodation of the eyes on reading, with recovery after a short rest. Owing to the intensely bitter taste of this substance, chloramphenicol may have to be administered to young children by stomach tube; rectal administration is not successful. Preliminary trials suggest that chloramphenicol may be effective in the treatment of whooping-cough. G. B.

Chloramphenicol in Paratyphoid B Fever. M. Curtin. (*Brit. med. J.*, 1949, **2,** 1504.) In view of previous favourable clinical reports, chloramphenicol was tried in 6 patients in whom a diagnosis of paratyphoid B fever was confirmed by culture of *Salmonella paratyphi B.* All had been ill for some days before treatment was instituted. The drug was always given four-hourly but dosage and duration of treatment were varied. Usually an initial dose of 1 or 2 g. was followed by 0·5 g. for 1 to 4 days after which dosage was reduced to 0·25 g. for a further 4 to 5 days. Prompt improvement occurred in all cases, usually within 24 hours, and was followed by clinical recovery. The temperature returned to normal in a maximum of 3 to 4 days. Recovery was more rapid when the treatment was instituted in the early days of the

infection. In 4 cases a convalescent carrier state developed persisting for 6 to 9 weeks. Three apparently healthy fæcal carriers were treated with 0·5 g. four-hourly for 4 days and then 0·25 g. four-hourly for 3 days. This dosage had no effect on the carrier state. H. T. B.

Curare Preparations; Assay by the Rabbit Head-drop method. N. K. Dutta and F. C. MacIntosh. (*Analyst*, 1949, **74**, 588.) A solution of the drug is infused into the ear veins of a rabbit until the animal is so weakened that it can no longer hold up its head. The volume of solution required to produce this end-point is noted. The unknown preparation is compared with the standard in a two-day cross-over test. The apparatus employed by the authors (which is described, with a diagram) enables 4 rabbits to be treated simultaneously. The error of a cross-over test on 8 animals is usually $\pm$ 10 per cent. (limits for $P = 0{\cdot}95$). S. L. W.

Decamethonium Bromide in Anaesthesia. D. A. Holaday, A. M. Harvey and D. Grob. (*New Engl. J. Med.*, 1949, **241,** 816.) Bis-trimethylammonium decane dibromide (C10) was employed as a curarising agent during 172 anæsthesias. It was found capable of producing adequate abdominal relaxation but was not without some interference with respiration, observable respiratory depression being encountered in 55 per cent. of the patients. Its effect was of considerably briefer duration than that of *d*-tubocurarine, necessitating more frequent injections. On the other hand, a standard dosage range may be used regardless of the anæsthetic agent employed, since no potentiating effect is observed as when *d*-tubocurarine is administered to patients under ether anæsthesia. The most satisfactory method of administration was to give 1 to 2 mg. of the dibromide, in a solution containing 1 mg./ml., intravenously at a rate not exceeding 1 mg./minute. Doses of 0·5 to 0·1 mg. are then injected every 5 or 10 minutes for as long as curarisation is required, with a maximum total dose of 10 mg. S. L. W.

Deoxycortone and Ascorbic Acid, Effect of, on Formaldehyde-induced Arthritis in Normal and Adrenalectomised Rats. G. Brownlee. (*Lancet*, 1950, **258,** 157.) Experimental arthritis can be induced in rats by the injection of formaldehyde. Normal and adrenalectomised rats can be protected against this arthritis by parenteral administration of deoxycortone (1 mg.) and ascorbic acid (20 mg.) daily. Parenteral administration of ascorbic acid only gives some degree of protection to normal rats but not to adrenalectomised rats. Deoxycortone, administered alone, aggravates the arthritis in normal rats and has little effect on adrenalectomised rats. A possible explanation is that both deoxycortone and ascorbic acid are components of an essential enzyme system, or alternatively that the deoxycortone has to be changed chemically by the ascorbic acid before it is effective. The aggravating effect of deoxycortone by itself seems to suggest that unaltered deoxycortone is not anti-arthritic, but that it can compete as a building unit with the anti-arthritic sterols, a mechanism analogous to the antagonism of sulphanilamide and *p*-aminobenzoic acid. G. B.

Diamino-diphenyl sulphone, Oral Treatment of Leprosy with. J. Lowe. (*Lancet*, 1950, **258,** 145.) Diamino-diphenyl sulphone (D.A.D.P.S.) is almost completely absorbed from the gut, and only slowly eliminated by the kidney; a high concentration can be maintained in the blood by administration of small oral doses, daily or twice weekly. Toxic effects, previously reported,

are due to excessively high dosage and are not produced by a starting dose of 100 mg. daily, gradually increased during 5 weeks, to 300 mg. This drug is at least as effective against lepromatous and tuberculoid forms of leprosy as promin, diasone or sulphetrone. The activity of these drugs may be attributed to diamino-diphenyl sulphone formed by their hydrolysis. Absorption and hydrolysis of these more complicated sulphones is incomplete, and the cost of treatment is reduced to about 1/20th when diamino-diphenyl sulphone is used. The drug is well tolerated by patients with tuberculosis of the lungs, and may possibly be of value in this condition. G. B.

Digitoxin, Quantitative Detection of Minute Concentrations of. R. Bine and M. Friedman. (*Proc. Soc. exp. Biol. N.Y.*, 1949, **69**, 487.) Studies were made of various concentrations of digitoxin upon the duck heart immersed in (1) Tyrode's solution, (2) rat serum, and (3) human serum. The method of quantitative determination employed was the same as that previously described by these authors (*Proc. Soc. exp. Biol. N.Y.*, 1947, **64**, 162; *Amer. J. med. Sci.*, 1948, **216**, 534). It was found that the embryonic duck heart was not only extraordinarily sensitive to minute quantities of digitoxin but also offered a means whereby the concentration of digitoxin could be assessed in a quantitative fashion. The embryonic heart preparation was able to detect the presence of 0·005μg. of digitoxin in 1 ml. of Tyrode's solution, and with increasing concentrations of the drug there was a progressive reduction in the time taken for the occurrence of the digitalis effect. In rat serum the action of digitoxin was much less effective, only quantities of 0·2μg. or more per ml. being detected. Human serum was even more inhibitory, only quantities of 0·6μg. or more of digitoxin per ml. being detected. S. L. W.

Dimercaprol and Parathyroid Extract; Effects on Distribution of Lead in Rabbits. K. R. Adam, M. Ginsburg and M. Weatherall. (*Brit. J. Pharmacol.*, 1949, **4**, 351.) The distribution of lead in the tissues of rabbits 13 and 21 days after the intravenous injection of lead acetate (2·07 g. Pb/kg.) was studied by use of the isotope Pb^{210} (radium D). Some of the rabbits were treated with dimercaprol, or parathyroid extract, or both, for some days during the week before they were killed. Parathyroid extract was employed for its reputed effect in mobilising lead from bones, since at this phase in the distribution of a single intravenous dose of lead most of the lead remaining in the body would be found in the bones and it would be unlikely that dimercaprol would have much effect on lead so deposited. Apart from a transient increase in the urinary excretion of lead after dimercaprol none of the treatments caused any substantial change in the distribution or the excretion of lead. About 50 per cent. of the lead was excreted in 21 days. The bones contained about 25 per cent. of the dose 21 days after injection, the bone marrow and liver being the only other tissues consistently containing more than 1 per cent. The authors conclude that dimercaprol and parathyroid extract have no useful effect in rabbits subacutely poisoned with lead. S. L. W.

Fat Emulsion for Intravenous Feeding. S. R. Lerner, I. L. Charkoff and C. Entenman. (*Proc. Soc. exp. Biol., N.Y.*, **70**, 388.) A stable olive oil emulsion was prepared as follows. Two parts of olive oil and one part of glycerol monostearate were heated, with stirring, to give a clear solution. This was diluted with glucose (5 per cent.) to yield a final concentration of 10 per cent. of fat. By high pressure homogenisation, an

emulsion with most of the fat particles of diameter 1μ or less, and *p*H 7·0, was obtained, which could be sterilised by autoclaving at 5 lbs. for 30 minutes. The emulsion is stable for several months. It is well tolerated by dogs when injected intravenously at rates of 1 ml. per minute per kg. and is rapidly removed from the bloodstream. Although dogs that had received as many as 31 injections were not free of tissue responses, the reactions were quite mild. E. N. I.

Gantrisin: Studies on Solubility, Absorption and Excretion. F. A. Svec, P. S. Rhoads and J. H. Rohr (*Arch. intern. Med.*, 1950, **85,** 83). This compound, 3:4-dimethyl-5-sulphanilamido-isoxazole (formerly known as NU445) shows promise as a sulphonamide, particularly in the treatment of coliform infections of the urinary tract. Toxic manifestations have been similar to those produced with other sulphonamides except for the absence of renal complications. The solubility of gantrisin in urine rapidly increases from 60 mg./100 ml. at *p*H 5·4 to 327 mg./100 ml. at *p*H 6·14, whereas the solubility of sulphadiazine at *p*H 6·3 is only 12·9 mg./100 ml. and approaches that of gantrisin only after the *p*H of the urine is raised to 7·5 or 8. Administration of 1 g. 4-hourly orally or intramuscularly, or 2 g. 8-hourly intravenously, maintains adequate average blood levels. Up to 12 g. daily has been given without untoward effect, and the intramuscular or intravenous administration of a 40 per cent. solution of the ethanolamine or diethanolamine salts of the drug produces no reaction. From 88 to 90 per cent. of the drug is excreted in the urine within 48 hours after administration of a single dose by any of the three routes, the most rapid excretion occurring during the first 8 hours. From 28 to 35 per cent. of the drug appears in the urine in the conjugated form, regardless of the route of administration, and a similar degree of acetylation occurs in the blood. Levels of the drug in the spinal fluid are about one-third those in the blood. S. L. W.

Leptazol and Strychnine for Testing Anticonvulsant Drugs. H. L. Williams and C. C. Pfeiffer. (*Proc. Soc. exp. biol., N.Y.*, 1949, **70,** 254). A method of evaluation of anticonvulsant drugs depends upon the determination of the degree of protection conferred against leptazol or strychnine. The anticonvulsant is administered intraperitoneally and the mouse placed in a cone-shaped holder of plexi-glass, the tail being drawn out through a slit in the holder. Leptazol (0·5 per cent.) or strychnine sulphate (0·01 per cent.) is injected at the rate of 0·05 ml. every 10 seconds. The degree of protection is judged by comparison of the time of the first twitch, pseudo-convulsion, persistent convulsion and death, and of the type of seizure and percentage mortality. By use of this method in which the effect of graded dosage of convulsant is observed in each animal, a statistical analysis of results is possible when a smaller number of animals is used than is the case when a test involving a fixed subcutaneous dose of convulsant is used. G. B.

Methadone and its Isomers, Side effects of. J. E. Denton and H. E. Beecher. (*J. Amer. med. Ass.*, 1949, **141,** 1148.) The incidence of side effects following the injection of *dl*-methadone, *l*-methadone, *dl-iso*-methadone, *l-iso*methadone and morphine was observed in two groups of healthy young men, as it was found impossible to distinguish between the side effects of these drugs and the after effects of anæsthesia and surgery in post-operative patients. The drugs were administered subcutaneously as solutions of the hydrochlorides, and isotonic sodium chloride solution was

given as a control. Records of the nature, incidence and duration of signs and symptoms, respiratory rate, pulse rate and blood pressure were made before, and for 5 hours following an injection. *dl*-Methadone, *l*-methadone and *dl-iso*methadone, in comparable analgesic doses, are as toxic as morphine in respect to the occurrence and duration of side effects. *l-iso*Methadone produces less nausea than morphine. All four forms of methadone slow the pulse rate and depress the respiratory rate to the same degree as morphine. None of the drugs tested alters systolic or diastolic blood pressure.

G. R. B.

Procaine-Penicillin G, Prophylaxis of Tetanus with. M. Novak, M. Goldin and W. I. Taylor. (*Proc. Soc. exp. Biol., N.Y.*, **70,** 573.) The possible prophylactic value of penicillin in tetanus was determined by injecting mice with an LD100 infective dose of *Clostridium tetani* spores, detoxified by heating at 80°C. for 30 minutes, followed at varying intervals by similar injections of penicillin G procaine in oil containing 2 per cent. of aluminium monostearate. A statistical analysis of data on mortality and time of death of the mice receiving penicillin prophylaxis and untreated controls showed a significant decrease in mortality and prolongation of life in the treated mice. The lowest mortalities resulted when doses of 150 and 300 units of penicillin were given immediately after the injection of the spores and when 150 units were given after 3 and 6 hours' delay. The series having low mortalities were also observed to have the greatest time lapse before symptoms and deaths. The results also show that penicillin injected into the necrotic areas is more effective than the same unitage injected at a different site.

E. N. I.

Phenadoxone, Pharmacology of. B. Basil, N. D. Edge and G. F. Somers (*Brit. J. Pharmacol.*, 1950, **5,** 125). Phenadoxone (heptalgin, *dl*-6-morpholino-4:4-diphenyl-heptan-3-one hydrochloride, CB11) is a colourless, odourless crystalline solid. It is freely soluble in water at *p*H 4; above *p*H 4 the free base begins to come out of solution, precipitation being complete in weakly alkaline solutions. It can be autoclaved without decomposition in acid solution at *p*H 2·5, but at *p*H 3·5 precipitation may occur unless the solution is suitably buffered. Phenadoxone is the most active of some 40 amino-ketones and amino-esters related to amidone examined for their analgesic activities. It has been shown to be more active on rats than morphine, amidone, pethidine or Hoechst 10581 (the hexane analogue). In spite of this its acute toxicity to mice is lower than that of amidone and its therapeutic index is therefore correspondingy higher. Its pharmacological properties closely resemble those of amidone. At therapeutic dose levels undesirable effects such as cardiac depression and vasomotor disturbances are absent, but it has a strong respiratory depressant action when given in high doses and should be used with special caution intravenously. It has a weak spasmolytic action on smooth muscle and has some surface analgesic action. No irritant action was observed after injection by the subcutaneous, intramuscular or intravenous route. There was no evidence of tolerance developing in rats dosed daily for 3 weeks, though it has been reported that dogs dosed twice daily developed analgesic tolerance in about a month.

S. L. W.

Procaine Penicillin, with Aluminium Monostearate. E. J. Wayne, J. Colquhoun and J. Burke. (*Brit. med. J.*, 1949, **2,** 1319.) Serum penicillin levels were estimated at frequent intervals for the first 24

hours and then 12 hourly for 2 days in 10 adults who received single doses of 300,000 units of procaine penicillin in arachis oil with 2 per cent. of aluminium stearate, and in 33 adults who received 600,000 units. The minimum bacteriostatic level was taken as 0·06 μ/ml., a level above this figure was maintained for 24 hours in all but one of the 10 cases receiving doses of 300,000 units, and in every case receiving the higher dosage. In the latter, the average level remained above 0·06 μ/ml. for 72 hours. Preparations with three different sizes of particle were given to 15 patients in doses of 600,000 units daily for 4 days. Constant serum levels were obtained with one preparation in which 95 per cent. of the particles were below 5 μ in diameter and with another preparation in which the majority of crystals were below 5 μ in size but which contained some rather large crystals. With the third preparation, containing particles 5 to 20 μ in size, the levels were not so constant. Detectable quantities were present in the blood 72 hours after injections ceased. Serum pencillin levels were estimated in one patient who received caronamide before and after a single dose of 600,000 units. During caronamide administration, significantly higher blood levels were noted 36, 48 and 96 hours after penicillin had been given. Serum penicillin levels were estimated by dilution in phenol-red-glucose-serum medium inoculated with *Streptococcus pyogenes* and comparison with similarly inoculated dilutions of standard penicillin in the same medium. G. R. B.

Steroid Metabolism, Abnormality in, Associated with Rheumatoid Arthritis. I. F. Sommerville, J. J. R. Duthie, G. F. Marrian and R. J. G. Sinclair. (*Lancet,* 1950, **258,** 116.) The authors have discovered an abnormality in the metabolism of progesterone in rheumatoid arthritis, a clinical condition which responds to treatment with 11-dehydro-17-hydroxycorticosterone (cortisone), a closely related steroid. Two successive daily doses of 60 mg. of progesterone in arachis oil were injected intramuscularly into a group of men and post-menopausal women suffering from rheumatoid arthritis, and into a similar control group. Daily urine specimens were collected from each subject 2 days before injection, the 2 days when the hormone was being administered and for the following 4 days. Pregnanediol determinations were made on the samples and the results clearly showed that rheumatoid arthritics of both sexes excrete in the urine as pregnanediol an abnormally high proportion of the administered progesterone. Further work is needed to prove whether this abnormality is specific to rheumatoid arthritis and related conditions, but some support is given to the hypothesis that rheumatoid arthritis may be associated with an analogous abnormality in the metabolism of the adrenocortical steroid hormones. E. N. I.

Tetraethylthiuram disulphide and Apomorphine, Action of, in Alcoholism. R. Lecoq, P. Chauchard and H. Mazoué. (*C.R. Acad. Sci. Paris,* 1949, **229,** 1261.) Tetraethylthiuram disulphide, like alcohol, has a diphasic effect on chronaxia, which first decreases, then finally increases. When both are administered, either together or in succession, the phase of diminution disappears and the phase of augmentation is amplified and prolonged. Thus the compound has the effect of exaggerating the action of the alcohol, being in this respect quite different from substances such as vitamin B which minimise the chronaxial effects of alcohol. The latter effect is produced even after preliminary treatment with tetraethylthiuram disulphide, showing that this compound produces an accumulation of intermediate products of the catabolism of alcohol. Apomorphine acts similarly. It is this effect which causes

conditioned reflexes resulting in distaste for alcohol, but, on account of it, the use of apomorphine and tetraethylthiuram disulphide is not entirely free from danger. G. M.

Theophylline Ethylenediamine and Dihydroxypropyltheophylline, Comparative Toxicity of. K. B. Jensen. (*Arch. Pharm. Chemi,* 1949, **56,** 741.) In view of the low solubility of theophylline, and of the objection to the use of more soluble compounds such as theophylline ethylenediamine, it has been proposed to use a soluble derivative in the form of dihydroxypropyltheophylline. The toxicity tests previously recorded with the latter compound must, however, be considered as only preliminary. The two compounds have now been compared by injection into the peritoneal cavity of mice, using 15 mice at each dose level. The results show that theophylline ethylenediamine is about 5 times as toxic as dihydroxypropyltheophylline, the values for LD50 being respectively 1450 and 261 mg./kg. G. M.

Thyroid Preparations, Acetonitrile Test for Control of. P. Laland and K. F. Støa. (*Acta pharmacol.,* 1949, **5,** 1.) The test is still valuable as a biological control, supplementary to chemical estimations. For routine tests the present procedure is to use 3 groups of male mice, each mouse weighing 18 to 20 g. and fed on an ordinary diet. The first group is used to determine the LD50 of a 10 per cent. solution of acetonitrile, and the second and third the LD50 of the same solution after administration of thyroxine and thyroid respectively on the previous day. The acetonitrile solution is given by intravenous injection into the tail, the thyroxine by subcutaneous injection and the thyroid by stomach tube as a 4 per cent. suspension in water. There are in each group sufficient mice to allow 10 to receive each selected dose of acetonitrile solution, and not less than 3 doses are used. Results are given for 8 different thyroid samples together with the total iodine content of each sample. The relation between biological activity and total iodine is somewhat variable and the factors to which the variations may be due are discussed. G. R. K.

***d*-Tubocurarine Chloride; Statistical Examination of Assay on Isolated Rat-Diaphragm.** G. A. Mogey, J. W. Trevan and P. A. Young. (*Analyst,* 1949, **74,** 577.) A Bülbring preparation of the rat diaphragm and phrenic nerve is supended in 100 ml. of oxygenated Ringer-Locke solution at 37°C., and the nerve stimulated by approximately rectangular pulses of direct current, not exceeding 1 m.sec. in duration, passing down the nerve. The assay starts with doses chosen to keep the responses between 20 and 80 per cent. paralysis, each dose being allowed to act for 5 minutes. Three washes with Ringer-Locke solution are used between each pair of doses, taking about 5 minutes altogether. The 4 doses (2 of Standard and 2 of test) are assigned at random to the elements of a 4 × 4 Latin square to determine the order of testing; the analysis of variance determines whether an order effect is significant and can be eliminated. The accuracy obtainable by this method is very high for a biological assay. When the test gives fiducial limits ($P = 0{\cdot}95$) with little more than 1 per cent. either side of the mean, as it frequently does, it is of interest to examine the following possible elements of variance: (1) errors of measurement of dose, (2) errors of measurement of volume of Ringer-Locke solution, (3) inherent variability of response of endplate, and (4) possible errors due to variation in temperature, etc. Then, when the fiducial limits are just over 1·0 per cent., the standard deviation will

be 0·5 per cent. and the variance 0·25 on a percentage scale. This is the mean derived from 16 observations and the variance will be 1·0 for a single observation. Divided equally between the first 3 sources of variability the variance for each will be 0·3 and the standard variation 0·548 per cent. This corresponds to filling the bath to a mark with 100 ml. of Ringer-Locke solution with a standard deviation of 0·548 ml., and measuring 1 ml. to 0·0548 ml. with a "tuberculin" syringe of 4 cm. length for each ml., setting the piston with a standard deviation of about 0·25 mm. S. L. W.

BACTERIOLOGY AND CLINICAL TESTS

Chemicals, Bacterial Contamination of. A. T. Dalsgaad and N. Kjaer. (*Arch. Pharm. Chemi*, 1950, **57**, 57.) Tests were carried out on samples of 30 chemicals, used for injection solutions and eye-drops, with the object of comparing the official test for resistant spores with the results obtained with agar plates and by a filtration method. The greatest degree of contamination was found with glucose, sodium chloride, sodium bicarbonate, magnesium sulphate and sodium sulphate (exsiccated). It was concluded that, in general, the contamination was small, and the proportion of resistant germs especially so. Tests on samples infected by the addition of earth showed that it is necessary to use a fairly large volume of solution for the official boiling test in order that the results may be of practical value, and it is to be supposed that the content of resistant germs (Gram-positive rods) would be too small to be shown by the test. In suitable cases the use of agar plates is of advantage. The filtration method is very delicate, and can only be carried out by using aseptic precautions, since the organisms to be expected may also be present in air. G. M.

Cobalt: Effect on Bacterial Growth. A. L. Schade. (*J. Bact.*, 1949, **58**, 811.) The growth of a wide variety of bacteria of all types, aerobic or anaerobic, Gram-positive or Gram-negative, can be completely inhibited by the addition of cobalt to the culture medium, the proportion needed being from 1 to 100 p.p.m. The factors concerned in determining the effect of cobalt were investigated in a strain of *Proteus vulgaris* isolated from a patient with cystitis. The nature of the medium is of primary importance, the amount of cobalt necessary to prevent growth in a meat extract peptone broth being 100 times that needed in a synthetic medium containing glucose and ammonium sulphate. Of 17 amino acids tested only histidine and cysteine can overcome the inhibitory action of cobalt on growth, the amount required being equivalent to a molar ratio, histidine to cobalt, of at least 2 to 1. Temperature and *p*H have no effect on the activity of cobalt. The concentration of cobalt needed to show an initial effect is the same for *P. vulgaris* as for *Staphylococcus aureus*, but under anaerobic conditions the cobalt concentration needed to inhibit growth completely is 2-3 times that needed under aerobic conditions. Cells of *P. vulgaris* differ in sensitivity to cobalt at different stages of their life cycle. Viability during the resting stage is little affected by 4 hours' exposure to cobalt, while cells in the lag phase are of intermediate sensitivity. The viability after 4 hours' exposure to cobalt in the resting, lag and logarithmic phases of growth is decreased respectively to 75, 40 and 2 per cent. of the initial value. The variations in sensitivity as determined by viability at different stages of the life cycle are closely paralleled by the respiration sensitivity of *P. vulgaris* to cobalt. H. T. B.

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BOOK REVIEW

LE CURARE. Journées Thérapeutiques de Paris, 1948. G. Doin et Cie., Paris 1949.

It would have astonished both the discoverers of curare and early workers on it to read in a volume such as this the diversity of purposes for which curare has come to be used. But one pioneer, perhaps, might even be disappointed that we still understand so little of some of the problems he raised. An account by L. Binet of a few unedited notes by Claude Bernard reveals his versatility. In them, he distinguishes contractility from nervous irritability; he records variations of sensitivity to curare among different muscles, different species of animal, at different temperatures; and he speculates on the action of curare on ferments, its relation to the circulation, and the modification of its absorption by mucus.. Scanty though these notes are, they make a fitting introduction to the whole symposium.

The remaining articles (nine in number) provide a useful summary of our present knowledge, without delving far below the surface. They are written for the clinician, rather than for the physiologist or pharmacologist. Thus M. Polonovski, in a chemical article, prudently avoids the quicksands, in which so many have floundered, of speculation about the chemical structure necessary for curare-like activity. He rightly draws attention, however, as others have done, to three significant points: (a) the intensification of activity by introducing a second quaternary group; (b) the existence of compounds of tertiary nitrogen, whose curare-like action is diminished by quaternisation; (c) the extent to which the existence of so many choline derivatives possessing curare-like action corroborates "*s'il en était besoin, l'hypothèse féconde de Dale et Feldberg.*"

J. Reuse, discussing the pharmacology and physiology of curare, divides his paper in the conventional way according to the actions of the drug on the various systems of the body. He also adopts a simple definition of curarisation as " a reversible interruption of transmission of excitation from the motor nerve of a striated muscle." Such division and such definition are sufficient for many purposes; but the lucidity which they confer on the discussion is to some extent spurious. For the definition, as it stands, includes the effect of procaine, Ca^{++} lack, and Wedenski inhibition, and excludes the effects of curare itself at the ganglionic synapse. Similarly the division of the description of the actions of curare according to the systems of the body involves the dispersal of one group of actions (say that due to histamine release, or to ganglion blokade) over several independent sections. It will soon be essential in such discussions, if it is not already, to bring out explicitly and individually those functions of curare which are well-defined. The clinician will not be the last to appreciate classification of this sort. Apart, however, from such general considerations, this section is useful, particularly in setting some of the continental literature in relation to that of this country and the U.S.A.

J. Cheymol and E. Corteggiani contribute a comprehensive and valuable section on assay. There are a few omissions: the method suggested by Feldberg and Lin using the peritaltic reflex of the intestine; the perfusion method of Laidlaw; and recent methods using the righting reflex or the fall from a rotating drum of mice and rats. But it is important that they choose,

as the test of most interest in assessing a curare-like compound for human therapeutic use, the rabbit head-drop test. The priority, in this context, of experiments on the whole animal over tests *in vitro* deserves emphasis.

The remaining sections concern the use of curare in surgery, convulsion therapy, neurology, obstretrics, gynæcology, and vascular disease, which are outside the province of the reviewer. The techniques used are based on those elaborated by Griffiths, Bennett, Gray, Cullen and other pioneers. There are a few minor errors, including one by which Scott M. Smith, the subject of the well-known and courageous experiment in complete curarisation becomes, by binary fission, two American anæsthetists Scott and Smith. It is clear from such accounts as these that the difficulties of sustained curarisation (e.g. for spastic nervous disorder, or for tetanus) are far from being conquered. This is not simply a question of paralytic side-effects; for instance, an outstanding problem is the state of shock into which patients with tetanus treated by curare appear to fall. It seems probable that curare will preserve its baffling but fascinating role in pharmacology for many years to come. W. D. M. PATON.

ABSTRACTS (*continued from page* 468)

Iodine and Available Chlorine. Determination of Bactericidal Efficiency by Semi-micro Methods. L. Gershenfeld and J. A. Palisti. (*Amer. J. Pharm.*, 1949, **121,** 337.) Two procedures were used, namely that of Klarmann and Wright (*Amer. J. Pharm.*, 1948, **120,** 146) and a modification of the U.S. Food and Drug Administration method. The organisms used in both instances were 24-hour cultures of *Salmonella* (*E.*) *typhosa* and *Staphylococcus aureus,* and the materials tested were tincture of iodine U.S.P., and sodium hypochlorite solution containing 2 per cent. of available chlorine. In the first method, 0·05 ml. of the culture was mixed with 0·5 ml. of different dilutions of the disinfectant. After being maintained at 20°C. for 10 minutes, 2 ml. of N/10 sodium thiosulphate was added, followed by 10 ml. of culture medium. The tubes were then incubated for 48 hours at 37°C. The phenol controls were treated similarly, except that the sodium thiosulphate was omitted. In the modified F.D.A. method, 0·5 ml. of culture was added to 5 ml. of diluted disinfectant at 20°. After 10 minutes, 2 ml. of N/10 sodium thiosulphate was added and one 4 mm. loopful of the mixture was transferred to 10 ml. of culture medium in a tube which was then incubated at 37°C. for 48 hours. With the Klarmann and Wright method, 1:4,000 of available chlorine killed *S. typhosa* and 1:5,000 killed *Staph. aureus* in 10 minutes, the corresponding concentrations of iodine being 1:8,000 and 1:6,000 respectively. With the modified F.D.A. method the corresponding concentrations were: available chlorine, 1:16,000 and 1:12,000; iodine, 1:12,000 and 1:13,000. The phenol coefficients were, by the Klarmann and Wright method, available chlorine, 44 against *S. typhosa* and 62 against *Staph. aureus*; iodine, 89 against *S. typhosa* and 75 against *Staph. aureus*; by the modified F.D.A. method the corresponding figures were: available chlorine, 177 and 171; iodine, 133 and 185. H. T. B.

Penicillin and Tubercle Bacillus: Tubercle Penicillinase. C. N. Iland and S. Baines. (*J. Path. Bact.*, 1949, **61,** 329.) While several authors have reported inhibition by penicillin of the growth of *Mycobacterium tuberculosis,* Ungar and Muggleton (*J. Path. Bact.*, 1946, **58,** 501) claimed that penicillin

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LETTERS TO THE EDITOR

Intravenous Injection of Dextrose, 5 per cent.

SIR,—It is common knowledge that on heating solutions of dextrose in an autoclave they become markedly acid in reaction. This phenomenon was noted by Hudson and Tarlowski[1] who suggested that such solutions should either be buffered before autoclaving or be sterilised by filtration. The fact that large quantities of dextrose solution are used and that all the commercial preparations we have examined have had a low *p*H suggest that the acidity is not widely regarded as a very serious matter. The *p*H is usually in the region of 4·2 to 4·5. Recent experience (especially with infants) of venous thrombosis following prolonged intravenous administration of 5 per cent. dextrose solution has caused us to re-examine the matter. So far as we are aware no explanation of the phenomenon has been put forward. The following notes give the gist of our findings, which are incomplete. (1) *Addition of Phosphate buffer.* The addition of sufficient sodium phosphate buffer to prevent the *p*H falling below 6 during autoclaving brought about discolouration of the solution to a degree which rendered it unfit for use. (2) *Saturation with nitrogen.* Saturation of the solution with nitrogen and displacement of the air above the solution with nitrogen gave solutions of slightly higher *p*H than the controls, viz. controls of 4·2, nitrogenated solutions 4·5. This was consistent over a number of experiments. (3) *Addition of Sodium Ascorbate.* 20 mg. of ascorbic acid as sodium ascorbate solution (*p*H 7) was added to each 100 ml. of dextrose solution. This was then nitrogenated. After autoclaving the *p*H was 5·5. 100 mg. of ascorbic acid in a similar experiment gave a *p*H of 6·0. (4) *Irgalon* (*ethylenediamine tetracetic acid*). As the solution salt (*p*H 7), in a concentration of 10 mg./100 ml. of dextrose solution this substance gave *p*H 5·2 and 100 mg./100 ml. gave *p*H 6·0. As with the ascorbic acid solution, the solutions were nitrogen saturated. Irgalon is used as a sequestering agent for trace metals and as an anti-oxidant for fats owing to its property of sequestering peroxide oxygen. (5) *Hydrazine Hydrate.* Hydrazine hydrate 10 mg. was added to each 100 ml. of nitrogenated dextrose solution. After autoclaving *p*H 6·0 was given. It appears to us that the above findings suggest a dual oxidation taking place in the dextrose solutions, viz. (a) an " external " oxidation using dissolved atmospheric oxygen: this is to some extent prevented by saturation with nitrogen and (b) an " internal " oxidation of the dextrose molecule. This appears to be prevented or retarded by the addition of reducing substances as shown. The processes appear to be addition, since non-nitrogenated solutions treated with reducing agents give lower *p*H readings than the nitrogenated ones. In view of the serious difficulty of prolonged intravenous administration of dextrose to infants, we investigated the effect of the " stabilised " solutions on red cells, expecting to find them less damaging than the unadjusted, acid reacting fluids. Both the ascorbic acid and the irgalon-treated samples brought about a marked *in vitro* auto-agglutination and hæmolysis at 37 per cent. Slight agglutination was also found in the unadjusted control but hæmolysis was not noticeable. The hæmolysis may be due to fact that our solutions were approaching the critical *p*H for hæmolysis noted by Hendrie[2]. There was, however, no apparent correlation between the degree of hæmolysis and the degree of agglutination. The latter phenomenon could well be associated with that of venous thrombosis and since in infants especially the volume of injected fluid is high in relation to blood volume and moreover,

since little or no dilution with blood takes place for some distance along the vein it seems possible that such a connection exists. We believe the matter to be worthy of fuller investigation. We are particularly impressed by the fact that plain dextrose solution is not free from the agglutination property.

GEOFFREY BRIAN,
Queen Elizabeth Hospital for Children,
London, E.2.

HERBERT S. GRAINGER,
Westminster Hospital,
London, S.W.!.

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REFERENCES

1. Hudson, T. A., and Tarlowski, L., *Pharm* J. 158, 1947, 451.
2. Hendrie, E. B., *Edin. med. J.,* 1948, 40, 427.

ABSTRACTS *(continued from page 470)*

stimulated the growth of human tubercle bacilli. The possibility therefore arose that Ungar and Muggleton's results were due to retarded growth because of lack of oxygen in the controls as compared with the test cultures in which the containers were frequently opened for the assay and replacement of penicillin. To test this possibility, 3 sets of cultures were set up, the bottles containing the controls and those containing the test cultures being opened at the same time. One set of bottles was a control; no penicillin was used in them and the bottles were not opened. A second set were identical with the first but they were opened for the same periods as the test set. The test cultures contained 5 1.U. {m!. of penicillin, the antibiotic level being maintained by assay at 48-hour intervals and adjustment by addition of more penicillin as found necessary. The results showed that the greater growth in set 3 than in set 1 could be due to the greater oxygen supply. The penicillin disappears completely in a few days and by assaying at 2-hourly intervals it was fo-und that it was destroyed by the culture at a rate of about 5 units per m!. *in* 2 hours. A 2-months' culture, *in* which a considerable amount of lysis had occurred, centrifuged until free from cells, destroyed 50 units of penicillin per m!. per hour at 37°C.; the crude culture fluid destroyed the antibiotic at the same rate. Tubercle bacilli therefore produce a penicillinase and further experiments showed that the enzyme loses its activity after 1 hour at 60°C., that it is adsorbed by a bacterial filter, that it is destroyed by shaking and that it is most active at *pH* 6'0. The lower activity of tubercle penicillinase as compared with other varieties may be due to the presence of iron in the medium used, this metal having been previously reported to inhibit penicillinase. Since young cultures show little penicillinase production it is probable that the enzyme is intracellular in the living organism and liberated only on its death and lysis. H. T. B.

Phenylmercuric Acetate, as Preservative in Antigens. 1. Williams and G. Piness. *(J. Allergy,* 1949, 21, 45.) On the basis of laboratory and clinical studies covering a period of 3 years, the authors recommend a 1:25,000 dilution of phenylmercuric acetate as the most satisfactory preservative for use in antigens. The bacteriostatic and fungistatic efficiency in this dilution was demonstrated by in *vitro* tests employing cultures of *Staphylococcus aureus, Staph. albus, Bacterium coli, Bacillus subtilis* and *Aspergillus.* Skin tests conducted on a number of patients, using a 1: 25,000 dilution in buffered saline solution, showed that intracutaneous injections gave rise to no pain or irritation. Comparative skin tests also showed that phenylmercuric acetate in this dilution neither destroys nor inhibits antigenic potency. S. L. W.