REVIEW ARTICLE

ANALGESICS—A GENERAL SURVEY

By A. H. Beckett, B.Sc., Ph.D., Ph.C., F.R.I.C.

Senior Lecturer in Pharmaceutical Chemistry, The Chelsea School of Pharmacy, Manresa Road, London, S.W.3

Synthetic analgesics were reviewed comprehensively in 1948 by Bergel and Morrison,[1] especially from the chemical point of view. Since that time, other reviews[2,3,4,5,6,7,8,9,10] have appeared dealing with various aspects of the problem of producing compounds with the analgesic activity of morphine but without its undesirable side-effects. The research in this important field of medicinal chemistry continues with little sign of reduction in its volume, although its emphasis changes from time to time as fresh clues to analgesic activity are unearthed, or new theories for activity proposed. The present article will survey the whole field of analgesics briefly, and emphasise especially:—1. The chemical aspect of the work since 1948. 2. The absorption, distribution, metabolism and excretion of analgesics. 3. The theories relating to the mode of action, and the attempts to relate chemical structure and analgesic activity. Compounds of weak activity such as aspirin, phenacetin, phenazone, and related substances, called by Fourneau[11] "antalgics," will not be included.

Modifications of the Morphine Molecule

The structure for morphine (I) put forward by Gulland and Robinson[12] has recently been proved conclusively to be correct by the synthesis of tetrahydrodesoxycodeine[13] and of morphine[14] itself. Morphine has been modified chemically in many ways in attempts to reduce its undesirable side effects, the chief of which are its great liability to produce addiction and its depressant effect upon the respiratory centre. Summaries of the results of this work have been presented elsewhere[1,3,11,15] and only the more important or newer compounds derived from morphine-type alkaloids are described here. Table I shows the structure of many of these compounds and the approximate analgesic activities.

6-*Acetylmorphine* (II). This substance, although 4 times as active as morphine, exhibits a 4-fold increase in the unwanted side effects.

Diacetylmorphine (*Diamorphine*; *Heroin*). (C-OH in position 3 and 6 replaced by C-$OCOCH_3$). Although possessing a higher analgesic activity than morphine, it is more toxic and possesses a greater liability to habitation, and its manufacture has been prohibited in many countries.

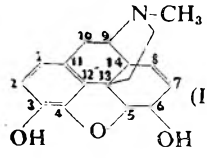
Dihydromorphinone (*Dilaudid*) (IV). Unfortunately an increased toxicity accompanies its increased analgesic action. However, it is said to be less habit forming, and to have less emetic action than morphine.

Methyldihydromorphinone (*Metopon*) (V). This compound was prepared by Small *et al.*[16] in the course of a fundamental study of the reaction of thebaine with organomagnesium halides. Its structure has not yet

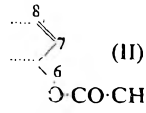
TABLE I

MORPHINE AND ITS DERIVATIVES

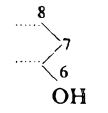
The relative analgesic activities are given as numbers in parenthesis


(I)

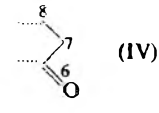
Morphine (100)


(II)

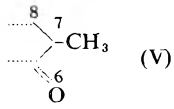
6-Acetylmorphine (400)


(III)

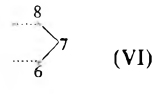
Dihydromorphine (200)


(IV)

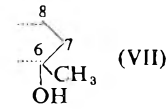
Dihydro-morphinone (500) (Dilaudid)


(V)

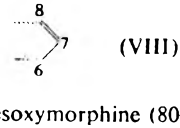
Methyldihydromorphinone (1000) (Metopon)


(VI)

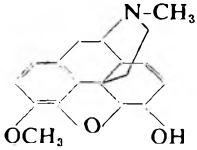
Dihydrodesoxymorphine-D (900) (Desomorphine)


(VII)

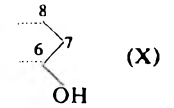
6-Methyldihydromorphine (100)


(VIII)

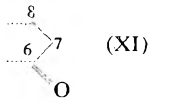
Δ^{7}—Desoxymorphine (800)


(IX)

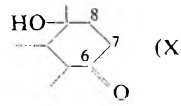
Codeine (10)


(X)

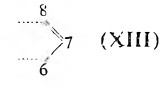
Dihydrocodeine (15)


(XI)

Dihydrocodeinone (50) (Dicodid)


(XII)

Dihydrohydroxycodeinone (50) (Eucodal)


(XIII)

Δ^{7}—Desoxycodeine (5)

been rigidly established. In America, metopon has been studied clinically in cases of inoperable cancer[17,18] and the results indicate that it is a more powerful analgesic than morphine, with apparently fewer undesirable side effects. Metopon can be administered orally, but unfortunately it is difficult and expensive to make.

Dihydrodesoxymorphine-D (*Desomorphine*) (VI). Although about 10 times as active as morphine as an analgesic, and only possessing about 3 times its toxicity, this compound has only a short duration of action.

6-*Methyldihydromorphine* (VII). This compound was synthesised recently by Small and Rapoport[19] by the action of methyl-lithium upon dihydromorphine. It possesses about the same analgesic action as morphine, but the duration of the action is almost doubled, and its addictive tendencies seem to be less than that of morphine.[17] However, it has been classified as an addiction-producing drug.[20]

Δ^7-*Desoxymorphine* (VIII). Preliminary pharmacological tests[21] indicate that it is about 8 times as active as morphine and that the onset of the effect is very rapid but the duration of the effect is short.

Codeine (IX) *and phenolic ethers of morphine.* The blocking of the phenolic hydroxyl group of morphine produced a reduction in analgesic activity and toxicity. Codeine, ethylmorphine (dionine) and benzylmorphine (peronine) have been used in medicine. Codeine, unlike morphine, does not have a depressant action on the respiratory centre, and because it has also little action on the intestine and is not such a powerful drug of addiction as morphine, it is of value as a mild analgesic and cough sedative. Ethylmorphine and benzylmorphine are more active than codeine but also more toxic.

Dihydrocodeinone (*Dicodid*) (XI). This substance has been used in Germany and America principally for cough relief, although apparently there is more danger of addiction than with codeine.

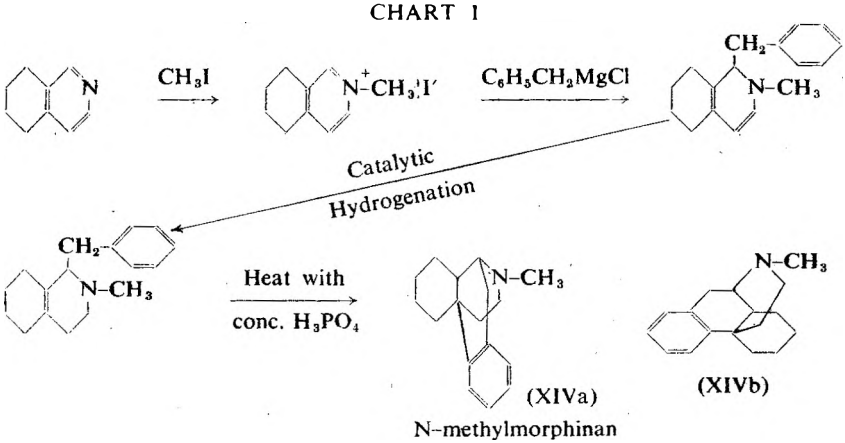
Dihydro-hydroxycodeinone (*Eucodal*) (XII). Results indicate that it has a lower toxicity than dicodid but a higher addiction liability.

Δ^7-*Desoxycodeine* (XIII). This substance was prepared in 1951 by Karrer and Widmark[22] and by Rapoport and Bonner[23] independently. Its analgesic action is about half that of codeine.[22]

The Morphinans and Synthesis of the Morphine Structure

The synthetic approaches to the morphine structure have been reviewed recently by Stern.[24] Grewe's fundamental studies in this field led to the preparation of *N*-methylmorphinan from 5:6:7:8-tetrahydro*iso*quinoline as follows[25,26,27] (Chart I).

CHART I

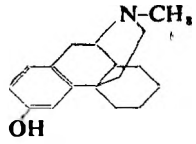


N-methylmorphinan

N-Methylmorphinan is usually written as (XIVb) which illustrates graphically its relationship to morphine. Pharmacological animal tests showed that this substance possessed morphine-like properties.[26] The steric identity of *N*-methylmorphinan with morphine was shown by Grewe *et al.*[28] in the synthesis of tetrahydro-desoxycodeine (XVI) by

cyclisation of compound (XV), the *lævo* isomer being identical with *l*-tetrahydrodesoxycodeine,

prepared from codeine. This was the first total synthesis of a compound in the morphine series. The same workers also prepared 3-hydroxy-N-methylmorphinan (XVII) by a similar cyclisation procedure.



(XVII) (HBr salt is Dromoran)

Schnider and Grussner[29] had also prepared this compound by a number of methods, and reported that it had an intensive and long-lasting analgesic effect on oral as well as parenteral administration and that its ethers and acyl derivatives were also active. Later pharmacological and clinical reports on the hydrobromide of (XVII) (called *Dromoran*)[30,31,32,33,34,35] demonstrated that it was about 4 times as potent an analgesic as morphine, with a greater duration of effect, and less frequent or severe side reactions.

The optically active isomers of dromoran have been prepared[36] and the *l*-isomer has approximately the same toxicity but a higher analgesic action than the racemic compound, while the *d*-isomer is less toxic and inactive.[37] *l*-Dromoran is also a greater respiratory depressant than the *d*-isomer, and *d*-, *l*- and *dl*-methyl ethers of dromoran exhibit parallel analgesic characteristics, although they are less potent and more toxic than the parent compounds.[38] Schnider *et al*[29,36,39] have prepared other derivatives related to dromoran and state that the replacement of the *N*-methyl group by *N*-ethyl, *N*-allyl, or *N*-benzyl reduces activity, and the same effect is produced by the introduction of an hydroxyl group at C_2. The 2 (or 4)-hydroxy derivative of *N*-methylmorphinan is inactive. Recent patents[40] cover the synthesis of many derivatives belonging to the morphinans. In Grewe's synthesis of *N*-methylmorphinan,[27] small quantities of two by-products were obtained. Gates *et al.*[41,42,43] have synthesised a morphinan-type structure by a totally different synthetic approach, and have shown it to be identical with one of Grewe's by-products. It has been called *N*-methyl*iso*morphinan,[43] and is stated to show considerable analgesic activity in animal tests. The successful synthesis of racemic β-Δ^6-desoxydihydrocodeine methyl ether[44] confirms that the steric configuration of these *iso*morphinan compounds is epimeric at

C_{14} to that of the morphinans and morphine itself. A prediction in Stern's recent review[24] that "within the next few years the synthesis of morphine, and with it a most interesting and difficult chapter of alkaloid chemistry, will be brought to a successful conclusion" has been justified by the recent preliminary communication by Gates and Tschudi[45] of the total synthesis of morphine.

Partial Structures of Morphine and Miscellaneous Substances

While the previously described approach to the synthesis of a new drug *via* the route of "operation upon the alkaloid" was in progress, the alternative route of synthesis of fragments of the parent molecule, to emphasise certain structural features, was not ignored. Hundreds of compounds have been prepared as partial structures of morphine, and Table II gives some of these basic structures. The formulae are drawn to indicate their relationship to morphine.

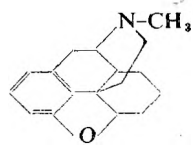
Phenanthrenes (XVIII), *dibenzofurans* (XIX) *and carbazoles* (XX). Under the auspices of the Drug Addiction Committee of the National Research Council of America, systematic work on synthetic analgesics was commenced in 1929, and although many derivatives of these compounds were prepared, none had a greater analgesic activity than codeine. This work has been summarised.[15]

Aralkylamines. In 1943, Dodds *et al*[46,47] reported that diphenylethylamines (XXII) relieved pain, and that α:β-diphenylethanolamine produced definite analgesic effects in cancer patients, but later[48] it was stated that it would only relieve the particular pain associated with pressure upon nerve, and was inactive in animals compared with pethidine or morphine. A similar line of approach has been investigated by other workers[49,50,51,52,53,54,55,56,57] but compounds have not been produced which possess significant analgesic activity. Kulz[58] claimed analgesic activity in phenolic bisphenylethylamines (XXI) and Lee *et al.*[59] prepared substances in which one of the phenyl groups was replaced by the *cyclo*hexyl group, and methyl-2-*p*-hydroxy-phenylethyl-2'-*cyclo*hexylethylamine was stated to have 1/7th the activity of morphine. Ullyot and co-workers[60,61,62] prepared a series of aminophthalidylalkanes, and 1-amino-1-phthalidylpropane was shown to possess considerable activity.[63,64] Because of reports of analgesic activity found for sympathomimetic amines, and the implied significance of adrenaline in analgesia, Fellows and Ullyot[5] undertook a systematic investigation of aralkylamines, and although some of these showed analgesic activity, three of the most promising ones, when subjected to clinical trials, were found to have only a low order of potency. For a more comprehensive treatment of the aralkylamines see the review by Fellows and Ullyot.[5] Recently the preparation of the four isomers of α:β-diphenyl-β-hydroxyethylamine has been reported[65] but the pharmacological data was not given. Burckhalter and Johnson[66] have prepared a series of di- and tri-phenylpropylamines and state that α-(benzhydrylmethyl)-benzylamine exhibits activity approaching that of morphine. Little activity was

TABLE II

PARTIAL STRUCTURES RELATED TO MORPHINE

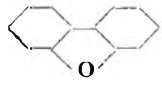
Basic structures



Morphine

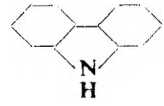
(XVIII)

Phenanthrene



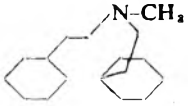
(XIX)

Dibenzofuran



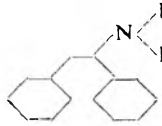
(XX)

Carbazole



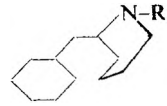
(XXI)

Bisphenylethylamines



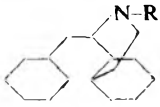
(XXII)

Diphenylethylamines



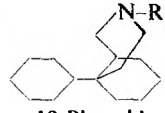
(XXIII)

2-Benzylpiperidines



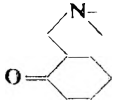
(XXIV)

Benzyl*iso*quinolines



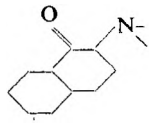
(XXV)

10-Phenyldecahydro-quinoline



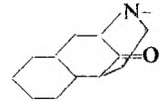
(XXVI)

Aminoalkyl-*cyclo*hexanones



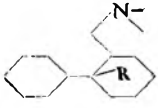
(XXVII)

2-Amino-1-tetralones



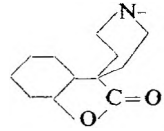
(XXVIII)

1-Aminoalkyl-2-tetralones



(XXIX)

Phenylaminoalkyl-*cyclo*hexanes



(XXX)

*iso*Coumaranones

obtained in a number of α-methylbenzylamines prepared by McCoubrey.[67,68]

2-*Benzylpiperidines* (XXIII), *benzyl*isoquinolines (XXIV), 10-*phenyldecahydroquinolines* (XXV) *and related substances.* Many compounds of this type have been prepared[59,69,77] but little analgesic activity was obtained. (See Suter[5] pp. 443 to 451). Smith *et al.*[78] prepared α-aminophenacylpyridines and quinolines and some of the derivatives were equal to codeine in analgesic activity. The work was continued by the preparation of 2- and 5-phenacylpyrimidines.[79] The benzyl*iso*quinolines prepared by Shapiro[80,81] were devoid of analgesic action.

*Aminoalkyl*cyclo*hexanones* (XXVI), *tetralones* (XXVII) *and hydrindones.* Lee *et al.*[59] found that 2-aminomethyl-1-tetralone and derivatives possessed analgesic properties, the most active having about 1/7th the activity of morphine. Reduction of the compounds had but little effect, while acetylation of these alcohols reduced the activity. The corresponding *cyclo*hexanone derivatives were without significant activity. Scheuing and Walach[82] had previously claimed that 2-alkylamino-1-tetralones had analgesic properties. Barltrop[73] prepared hydrindones and aminoalkyl derivatives of 2-tetralone, and *cyclo*hexanones containing phenyl groups and basic groups have also been prepared,[83,84] but either no analgesic properties have been reported or else they are of a low order.

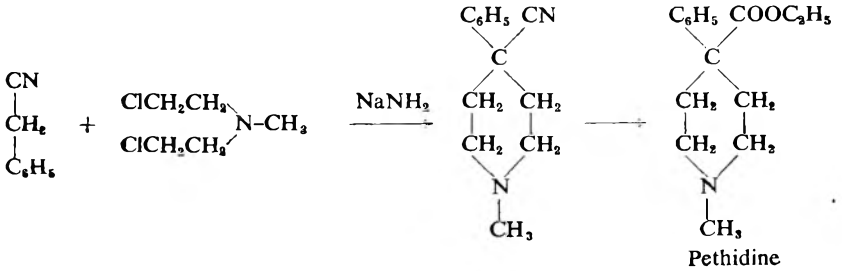
*Phenylaminoalkyl*cyclo*hexanes* (XXIX) *and related compounds.* Compounds prepared by Lee *et al.*[59] and by Goldschmidt *et al.*[85,86] showed little analgesic activity.

iso*Coumaranones* (XXX) *and related substances where the oxygen containing ring system of morphine is considered.* The *iso*coumarones and lactones prepared by Bergel *et al.*[87] were shown to be inactive by Macdonald *et al.*[88] Bovet and Simon[89] found that diethylaminomethylbenzodioxan and related compounds showed some analgesic properties. A study of condensed cyclic aryl ethers[90,91] and sulphides[92] carrying alkylamine side chains has been carried out, but little activity resulted. Compounds emphasising the ether ring of morphine prepared by other workers[93,94,95,96,97,98] possessed little activity.

From the above brief account of synthetic fragments of the morphine molecule, it is apparent that morphine has been mentally dissected in almost every conceivable way, in the hope that analgesically active compounds would result. Despite the thoroughness and comprehensive character of this attack, it has not yielded compounds with significant activity comparable with morphine. However, fortuitous events are not unknown in the field of scientific endeavour, and the fact is emphasised in the search for analgesics. The discovery by Eisleb and Schaumann[99] in 1938, that pethidine had analgesic activity was the necessary clue to guide the search into more profitable channels, and pethidine was prepared by these workers in a search for spasmolytic agents, regarding atropine as the parent structure.

Pethidine and Related Compounds

Pethidine was synthesised originally as follows:[100]



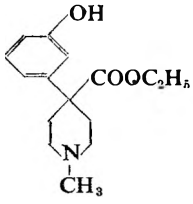
Pethidine

but because this route involved the use of the dangerous vesicant methylbis-2-chloroethylamine, other synthetic routes were devised (see reviews[1,2] for routes and references). Although pethidine is not so powerful an analgesic as morphine (about 1/5th in animal tests and 1/8th in humans) it rarely depresses the respiratory centre and has less powerful addictive properties than morphine (see Yonkman[101] for a review of the pharmacology). Since the discovery of pethidine, much work has been performed in preparing modifications of the molecule in attempts to increase the activity and lower the incidence of side effects (for reviews see references[1,5,102]). These modifications include moving, removing, and substituting the phenyl group, substituting and breaking open the piperidine ring, replacing the *N*-methyl group by other alkyl groups, and replacing the ethyl ester by other ester groups, hydrogen, ketonic and reversed ester groupings.

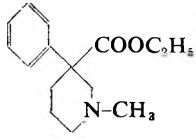
Only the most important compounds are considered here, the structures of which are given in Table III.

TABLE III

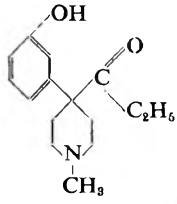
PETHIDINE-TYPE COMPOUNDS



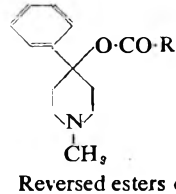
Hydroxypethidine (XXXI)



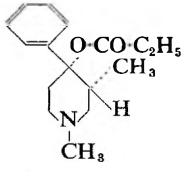
β-Pethidine (XXXII)



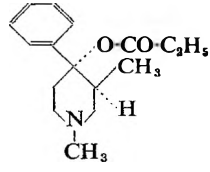
Ketobemidone (XXXIII)



Reversed esters of pethidine (XXXIV)



Alphaprodine (XXXVa)



Betaprodine (XXXVb)

Hydroxypethidine (XXXI) (bemidone). This substance has about the same analgesic activity as pethidine[88] and is reported to have given promising clinical results when used as a general anæsthetic by intravenous injection.[103]

β-Pethidine (XXXII). Bergel *et al.*[104] prepared this compound and it has been studied pharmacologically[88] and clinically.[105] It has only a low toxicity, but it is a less potent and shorter-acting analgesic than pethidine.

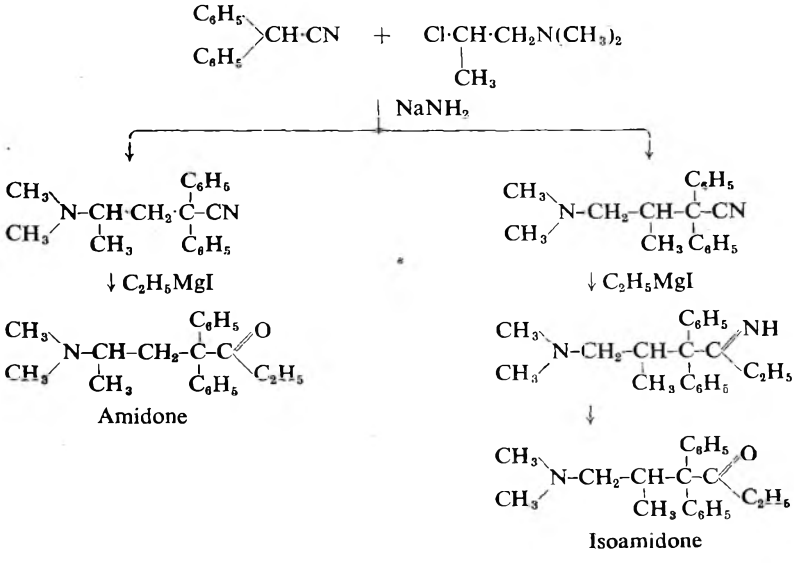
Ketobemidone (XXXIII). The change from the ester group of hydroxypethidine to the ethyl ketone was reported to give a compound with 20 times the activity of pethidine,[103] and although clinical trials by

Kirchoff[106] showed that it had excellent analgesic properties, it has proved to be a powerful drug of addiction,[107] comparable with heroin in this respect.

Reversed ester of pethidine (XXXIV). In 1943, Jensen *et al.*[108] reported that these compounds were more active than pethidine, especially the propionoxy derivative (R = C_2H_5), which was stated to have 5 times the activity of pethidine. Independently, workers in the Roche laboratories (for references see review[1]) prepared many compounds of this type. Ziering and Lee[109] obtained compound (XXXV) in its *cis*-(XXXVa) and *trans*-(XXXVb) modifications (configurations assigned only provisionally) and resolved the *trans*-form into its optical enantiomorphs. Randall and Lehmann[110] obtained the following pharmacological results on rats, morphine being taken as 100, Nu 1196 (*cis*-form racemate) 97, Nu 1779 (*trans*-form racemate) 550, Nu 1831 (*l*-form *trans*-) 350, Nu 1832 (*d*-form *trans*-) 790, but in man the difference in action between the *cis*- and *trans*-racemates is not so pronounced.[111] The World Health Organisation has recognised Alphaprodine and Betaprodine as international non-proprietory names, for the *cis*- and *trans*-racemate respectively. Gross *et al.*[112] investigated Nu 1196 and Nu 1779 in man, and Houde *et al.*[18] reported that Nu 1196 had a weaker analgesic action than morphine and showed side effects in 10 per cent. of the patients. Evidence that these substances show addiction properties has been obtained by Isbell.[107]

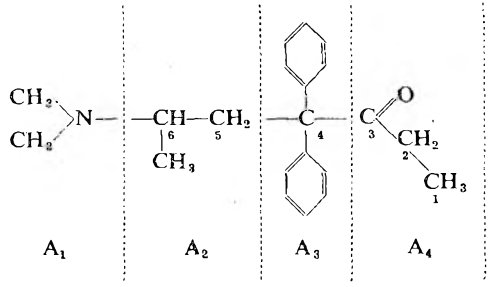
Amidone and Related Substances

The synthesis of amidone proceeds as follows and results in two products, amidone and isoamidone.



For further references to synthetic routes and the mechanism of the above sodamide condensation, see the review by Bergel.[1] Early reports published after the war,[103,113] gave the information that amidone was 5 to 10 times as active as pethidine. The literature on the pharmacology of amidone and related compounds is too extensive to be dealt with here (for detailed accounts see references[114,115,116,119]).

Since 1945, many workers have investigated modifications of the amidone structure, and the results of these modifications are discussed briefly below.



AMIDONE

Modification of the Basic Group (A_1). Various basic groups have been tried[103,117,118,119,120,121,122] and, in general, a decrease in activity is produced except in the case of the morpholino (O N–) or piperidino (N–) analogues. The morpholino compound[117,118] (CB. 11, heptalgin, *phenadoxone*) was reported by Wilson and Hunter[123] and Hewer *et al.*[124] to be at least as potent as amidone in human subjects, but Winter and Flataker[125] found that in rats it was shorter-acting than amidone. Its detailed pharmacological actions have been described by Basil *et al.*[126] Isbell and Fraser[127] state that its addiction liability is quite low, but "the drug is a relatively ineffective and short-acting analgesic." The piperidino analogues of amidone and isoamidone have received a favourable report by Ofner *et al.*,[128] and Prescott *et al.*[129] have found that the isoamidone analogue has less respiratory depressant effect than morphine, amidone or pethidine in man. Removal of the basic group results in a complete loss of analgesic activity[119] while quaternisation of the group markedly decreases activity.[115,130]

Modifications in A_2. The effects of length, branching and position of branching in the carbon atoms joining the tertiary nitrogen atom and quaternary carbon atom have been investigated. A methyl group on C_6, as in amidone, is advantageous,[103,117,131] and when the methyl group is on C_5, as in isoamidone, some reduction of activity occurs[118,119,112] (exception is the piperidyl analogue). Isoamidone has been studied in some detail[133,134] and is reported to have less respiratory depressant activity than amidone. Lengthening or shortening the chain results in reduction or complete loss of activity.[117,120]

The presence of a methyl group on C_6 (amidone) or C_5 (isoamidone)

introduces an asymmetric carbon atom in the molecule, and many of the compounds have been resolved (amidone,[120,135,136,137,138] isoamidone,[137] sulphone analogue of amidone[139]) into their optical enantiomorphs, and pharmacological studies have shown that one enantiomorph is always much more active than the other.[116,119,139] This point will be considered in more detail later.

Modifications of A_3. The phenyl groups have been substituted,[103,117,119] one or both groups replaced by *cyclo*hexyl,[119] alkyl,[119,140] thiazole,[141] benzyl,[120] pyridyl[142] or thienyl[143,144] groups, but a reduction or complete loss of activity occurs. The migration of one phenyl group to the neighbouring carbon atom (C_5) resulted in loss of activity.[119,120] The replacement of the whole group by the fluorenyl group[120,145] led to reduced activity.

Modifications of A_4—*the ketonic portion.* 1. Other ketones were less effective.[103,120,121] 2. Replacement by

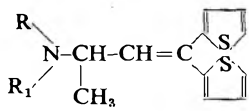
$$\text{-COOH, -COOR, -CH}_2\text{OH, -CHO, -O·CO·R, -CH = CH·CH}_3\text{,}$$
$$\text{-CH}_2\text{O·COR, -CONH}_2\text{, H, -OH, -CH}\begin{matrix}\text{Cl}\\ \text{C}_2\text{H}_5\end{matrix}$$

led to a reduction or complete loss of activity.[115,118,119,120,121,146,147,148] 3. Reduction of the ketonic group of amidone, and related compounds, to a secondary alcohol[118,149,150,151] led, in general, to a reductionof toxicity and also of activity which can be restored by acetylation. The platinum oxide hydrogenation or the lithium aluminium hydride reduction of *dl*-amidone (or *dl*-isoamidone) only gave one of the two possible racemic alcohols in each case. Pohland *et al.*[153] obtained the optically active forms of the same isomer (designated the α-isomer) by hydrogenation of *d*- and *l*-amidone. The name methadol has been approved for α-*dl*-methadol (one of the secondary alcohols from *dl*-amidone), and methadyl acetate for the acetyl ester of α-*dl*-methadol. By the use of sodium/propanol reduction of *d*-, *l*-, and *dl*-amidone Eddy *et al.*[152] have obtained α-*dl*- and β-*dl*-methadol and the four corresponding optical isomers (α-*d*-; α-*l*-; β-*d*-; β-*l*-), and have converted them to the acetyl esters. Both α- and β-*dl*-methadols were less effective than *dl*-amidone, the parent compound, but the acetyl esters were similar to *dl*-amidone in toxicity, but had greater analgesic effect. The corresponding compounds (α-*d*-, β-*l*-) obtained from *l*-amidone showed similar results to the above. Rather remarkably, α-*l*-methadol, and α-*l*- and β-*d*-acetylmethadols, derived from the only weakly analgesic *d*-amidone, showed very high analgesic activity both orally and subcutaneously, and are now being tried clinically. Chen[119] has reported that in rats, by subcutaneous injection, α-*d*-acetylmethadol is about 5 times as active as the α-*l*-isomer and twice as active as *dl*-amidone, but the α-*l*-isomer shows a long duration of action (see also Sherrod *et al.*[154]). The analgesic effects in man[155] and the addiction potentialities[156] of the α-acetylmethadol isomers have been reported. It is possible that some of the reduction products, and their esters, derived from amidone-type compounds may prove to be of great importance.

4. Compounds obtained by replacements of the ketonic group by ketimine $-C\begin{matrix}NH\\ C_2H_5\end{matrix}$ and acyl ketimine $-C\begin{matrix}NCOR\\ C_2H_5\end{matrix}$ groups, have been described by various workers.[157,158,159] Cheney *et al.*[159] stated that the order of decreasing toxicity and increasing therapeutic index was ketone: ketimine: acetylketimine in both the amidone and isoamidone series.

5. The replacement of $-C\begin{matrix}O\\ C_2H_5\end{matrix}$ by the sulphone ($-SO_2-R$) group[160] leads to active compounds, especially when $R = C_2H_5$, and this substance was claimed to have the analgesic activity of amidone but only half its toxicity. Resolution into the optical isomers was accomplished[139] and the *l*-form was 20 times as active as the *d*-form, and has been claimed to be one of the most powerful analgesics.[161]

Miscellaneous compounds related to amidone. The ketonic group of amidone has been incorporated into one of the reduced phenyl groups,[162] the arrangement of the two phenyl groups, ketone and basic side chain about the quaternary carbon atom has been altered,[84] basic and ketonic groups have been introduced into the fluorene molecule,[145,163] but little analgesic activity has been obtained. However, Adamson[164,165] has reported high analgesic activity in compounds of type (XXXVI).

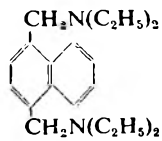


(XXXVI)

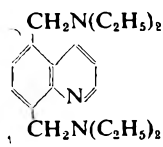
Despite the absence of a ketonic group and a quaternary carbon atom, the compounds are stated to be as active as morphine in the rat with undesirable side-effects (in the dog) at a minimum. Two of these compounds have been resolved and the analgesic activity has been shown to be present mainly in the *d*-isomer.[166] Reduction of the double bond reduces the analgesic activity.[166] Some of the compounds have been tested in man, and they appear to be more active than pethidine but less active than amidone.[167]

Other Series of Miscellaneous Compounds

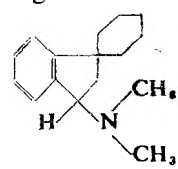
Compound (XXXVII) has been claimed by Badger *et al.*[75] in a



(XXXVII)



(XXXVIII)



(XXXIX)

preliminary communication to be as active as pethidine, and further details of compounds of this type, which are so completely different from previous analgesics, are awaited with interest. Martin and Hanslick[168] have described salts of the closely related compound

(XXXVIII) as analgesic agents. Horning and Rutenberg,[169] stressing the importance in analgesics of a quaternary C atom attached to an aromatic ring with an amino *N* in β-relationship to it, have prepared a series of oxindoles, but the pharmacological results were not recorded. A similar approach has been made by Schwartzman[170,171] in preparing *spirocyclo*hexyl quinolines and indanes (XXXIX) and activity has been obtained in some of the compounds. *Spiro*piperidino-*iso*quinolines have been described by Kägi and Miescher.[172] Close *et al.*[173] prepared benzoxazolones but they were found to possess much less analgesic activity than pethidine. The bispidines prepared by Kyi and Wilson[174] and the dihydroglyoxaline derivatives prepared by Wilson[175] were shown to be devoid of analgesic activity by Marshall *et al.*[176]

The Absorption, Distribution, Fate and Excretion of Analgesics

The study of the distribution and fate of analgesics is one approach to the problem of elucidating the mechanism of action of these drugs which is already of importance. It may well yield more important results in the future, now that techniques have been perfected for detecting and separating small quantities of drugs in tissues and body fluids. The analgesics which have received attention up to the present have been morphine, codeine, pethidine, and amidone. The absorption and fate of morphine and codeine was reviewed comprehensively in 1941 by Krueger *et al.*,[177] but since that time important publications have appeared as the more powerful analytical methods have been applied.

Morphine. The information which had been accumulated up to 1940 indicated that an animal which absorbs a dose of morphine begins to excrete the unchanged drug in urine and fæces at once, but only about 15 to 35 per cent. could be accounted for. It was presumed that the rest was destroyed or eliminated promptly by unknown chemical processes. However, in 1941 Gross and Thompson[178] showed that previous workers had only measured "free" morphine and that in dogs both "free" and "combined" morphine was present in the urine, and they were able to account for 80 to 85 per cent. of a given dose by this route of excretion. Oberst[179] showed that in man a large percentage of morphine was excreted in the urine, chiefly as the "combined" form. It had previously been suggested[180] that morphine was possibly conjugated with glucuronic acid, but Gross and Thompson[178] failed to find any evidence of morphine glucuronate in urine. These workers also showed that a smaller percentage of a given dose of morphine appears in the urine of the tolerant than of the non-tolerant dog. Later work of Bernheim and Bernheim[181,182] established that the liver is the only organ which conjugates morphine. Zauder[183] has shown that, in rats, 80 per cent. of the administered dose is excreted as free and combined morphine, and that in tolerant rats less of the conjugated form appears in the urine. He further showed that liver slices conjugated morphine, but, unlike Abord and Koon[184] he could not demonstrate any oxidation of morphine by these slices, and concluded that the mechanism responsible for the destruction of morphine is extrahepatic.

Recently, radioactive morphine (morphine -N-$C^{14}H_3$) has been prepared[185] and its tissue distribution, metabolic fate and excretion studied in rats.[186] All the radioactivity disappeared from the animal within 48 hours, about 2/3rds *via* the urine and 1/3rd *via* the gastrointestinal tract, and the results indicated that a greater part was excreted by the liver into the bile. A small percentage of radioactivity was excreted *via* the respiratory route indicating an *N*-demethylation process. The central nervous system contained a negligible amount of radioactivity.

Thus, from the evidence at present available, it appears that about 70 to 80 per cent of the morphine is excreted in the urine in free or conjugated form, and a small percentage is excreted in the fæces. The conjugation occurs in the liver and excretion *via* the bile occurs. A small percentage is *N*-demethylated, but the mechanism by which the rest of the morphine (probably less than 10 per cent.) is destroyed is not yet known. A fairly rapid removal of morphine from the body occurs and only very small traces of morphine (or metabolic product) ever reach the central nervous system.

Codeine. By the use of C^{14}-methoxy-labelled codeine synthesised by Chang *et al.*,[187] Latham and Elliot[188] showed that there was a general distribution of codeine (or metabolite) in rats, and that no "site of action" could be inferred as might be evidenced by excessive concentration in any tissue. Only a small percentage reached the brain or central nervous system. A consideration of the results of the radio-active tracer work, and the publications in which other techniques have been applied, indicate that a number of different mechanisms and routes of excretion come into operation after codeine has been administered:—1. Conjugation to a more water-soluble form excreted, along with unchanged codeine, in the urine. Oberst[189] showed that about 11 per cent. of free base and 32 per cent. of conjugated base was excreted in humans. Latham and Elliot[188] showed similar results in rats. 2. Formation of codeine -X, a biologically inactive metabolite excreted *via* the bile into the intestinal tract from which it is apparently later reabsorbed.[188,190] 3. Demethylation to form morphine has been shown to occur using rat liver slices[182,191,192] and *in vivo* in rats.[192] When radio-active codeine (C^{14} methoxy) was used, radio-active carbon dioxide was found in the expired air.[188] Under *in vitro* conditions approximately 1/3rd of the metabolised codeine appears as morphine, both in free and combined forms.[193] The site of demethylation is the liver.[181,192,193] 4. Conjugation of the liberated morphine[191] and excretion in the urine in this form.[193]

Pethidine. The study of the metabolism of pethidine using the N-$C^{14}H_3$ labelled material has shown that only very small amounts of pethidine (or metabolite) reach the brain or cerebrospinal fluid.[194,195] After oral administration, over 90 per cent is absorbed from the gut within 4 hours.[194] If a subcutaneous injection is used, radio-activity is present at the site after 12 hours, which indicates slow absorption from a subcutaneous depot.[195] In human subjects it has been shown that there is no pethidine in the milk of lactating mothers within 1 to 6 hours after injection, and very little in the urine of new-born infants whose mothers had received

injections of the drug.[194] Plotnikoff[196] has reported that, after a subcutaneous injection of radio-active pethidine in rats, 50 per cent. of the radio-activity could be recovered in the urine and 4 per cent. in the fæces. This radio-activity is a measure of both unchanged pethidine and some of the metabolic products because the combined results of other workers[197,198,199] indicate that less than 10 per cent. of a given dose is excreted as pethidine in the urine. The liver has been shown to be the main organ for metabolising pethidine by hydrolysis in *vitro* and in *vivo*, the enzyme responsible being an unknown esterase.[194,200,201] When radio-active pethidine ($N\text{-}C^{14}H_3$) was used, radio-activity was found in the expired air,[196] indicating *N*-demethylation. A recent publication by Plotnikoff *et al.*[195] confirmed that one metabolic route involves hydrolysis of the ester group and another pathway involves *N*-demethylation, because pethidine, hydrolysed pethidine and nor-pethidine were identified in human and rat urine. However, the sum total of these substances did not account for all the radio-activity in the urine. They also showed that the liver was probably responsible for the demethylation because $C^{14}O_2$ was evolved from rat liver slices in the presence of pethidine ($N\text{-}C^{14}H_3$).

Amidone. As in the case of the analgesics already mentioned, only very small concentrations of amidone reach the brain and central nervous system.[202,203,204] The drug is fairly rapidly mobilised from the site of a subcutaneous injection,[203,205] is carried by the blood plasma,[206] and 10 minutes after an injection radio-activity is found in the bile.[207] When radio-active amidone was used, high radio-activity was found in the adrenals[203] and this fact may be significant because of the reports which implicate adrenaline as a mediator of the analgesic effects of certain drugs. Many publications have appeared dealing with the metabolism and excretion of amidone. Elliot *et al.*[203] showed that C^{14} labelled amidone ($-C(=O)C^{14}H_2{\cdot}CH_3$) may be recovered as radio-active material to the extent of 80 per cent. in the fæces and 20 per cent. in the urine, and a later publication[207] indicated that biliary excretion was chiefly responsible for the radio-activity appearing in the gastro-intestinal tract. The whole of the radio-activity did not represent unchanged amidone because other workers[202,208] found that about 10 per cent. was excreted in urine and 10 to 20 per cent. in fæces, and it was suggested that the methods appeared to measure degradation products in addition to amidone itself. Subsequent work by Way *et al.*[209] showed that amidone was excreted unchanged in about 8 to 10 per cent. in the urine and 8 to 10 per cent. in the fæces. These results agree well with those obtained by Richards *et al.*[204] using a different method. Way *et al.*[209,210] partially separated and characterised a basic amino metabolic product from the bile of rats and dogs, and also indicated that there is possibly another metabolic product in the fæces. Richards *et al.*[204] considered that one possible metabolic pathway could involve the introduction of hydroxyl groups into the phenyl rings of amidone. The liver has been shown[204,211,212]

to be the chief organ for metabolising amidone *in vitro* and *in vivo*, and it has been recently reported[213] that liver slices from tolerant rats appeared to metabolise the drug less rapidly than slices from normal rats. The tissue distribution and excretion of the optical isomers of amidone has been studied.[214,215] Rat liver slices have the same effect upon both isomers.[214] Although the two isomers differ so markedly in pharmacological activity, the distribution in the various tissues follows the same pattern as that of racemic amidone, and *l*-amidone is not localised to any higher degree than the *d*-isomer in the brain.

Hypothesis Concerning the Mode of Action of Analgesics

This section of the work on analgesics will only be considered very briefly because the theories are highly speculative, and the search for clues to the explanation of activity in the investigation of effects upon enzyme systems or biochemical processes has not proved very fruitful (Krueger *et al.*[177]). The effect of analgesics upon the enzyme cholinesterase has received some attention. Bernheim and Bernheim[216] showed that morphine strongly inhibited cholinesterase in *vitro* and work in this direction has been performed using both morphine and other analgesics.[217,218,219,220] Thus, the effect of this inhibition would be to block the hydrolysis of acetylcholine, and this leads to speculation as to whether this may be connected with the action of analgesics on the central nervous system. On the other hand, Burn[221] has suggested that analgesics may be substances which antagonise acetylcholine in parts of the central nervous system, and has presented evidence in support of this hypothesis. Analgesics have been shown to inhibit the oxygen uptake of brain tissue.[222,223,224,225,226] However, in general, the concentrations of analgesics which have been required to produce a significant inhibition have been far in excess of the concentrations which have been shown to reach the brain in the intact animal, and so the results are of little practical significance. Other workers[227,228] have shown that the oxidation of glucose, succinate, ascorbate, lactate, etc., by brain tissue is inhibited by analgesics. Because of this inhibition of oxidation processes, Wang and Bain[229] have investigated the sensitivity to morphine of the various steps in the cytochrome system.

Pero[230] has advanced the hypothesis that pain is a cholinergic, and analgesia is an adrenergic phenomenon (stimulation of secretion of adrenaline at the synapses), and numerous reports (see Ivy *et al.*[231]) state that adrenaline and sympathomimetric amines have analgesic action. However, it has been shown[232,233] that an injection of morphine results in the liberation of adrenaline from the adrenals—a cholinergic phenomenon! Furthermore, when the adrenals are removed, the analgesic response to a given dose of morphine is below normal.[234,235] In connection with this possible implication of adrenaline as a mediator of the analgesic effect of drugs, the relatively high concentration of radio-activity in the adrenals after the subcutaneous injection of radioactive amidone may be of significance.[203] The effect of certain analgesics and adrenal cortical hormone on the brain of normal and hypophy-

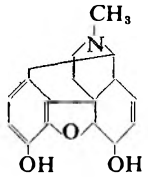
sectomised rats, as measured by the thiobarbituric acid reagent, has been investigated by Zauder.[236] His results seem to indicate that these analgesics, *via* the mechanism of adrenaline release, induce a release of adrenocorticotrophic hormone from the hypophysis with a consequent stimulation of the adrenal cortex, but the recent report by Irwin and Shideman[237] does not support these results.

Morphine and other opium derivatives produce hyperglycæmia[177] and the newer analgesics have also been shown to do the same.[238,241] The effect is possibly due to the stimulation of a supraspinal centre with the subsequent release of adrenaline and then mobilisation of liver glycogen.[177,242] It is of interest that *l*-amidone produces a much greater hyperglycæmia in dogs than does the *d*-isomer,[242] whereas the two isomers affect *in vitro* tissue respiration to the same extent.[227] Pfeiffer *et al.*[243] have suggested that analgesics specifically block certain metabolites such as amino-acids which are essential for the central nervous system, but experimental evidence is lacking. Schueler *et al.*[244] state that the activity of analgesics may be traced to effects involving the autonomic nervous system.

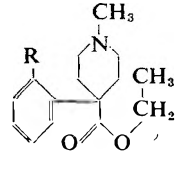
It is therefore apparent that a clear picture of the mode of action of analgesics is, as yet, a distant goal, despite the multitudinous array of facts which have been collected.

Chemical Constitution and Analgesic Action

Many of the workers engaged in the search for synthetic analgesics have tried to explain any analgesic activity in their compounds in terms of a relationship with the morphine molecule. This is not unexpected when the basic plan underlying much of the research was the synthesis of partial fragments of the morphine structure. The publications of Fourneau[11] and Small *et al.*[15] illustrate this approach. After the discovery of the analgesic activity of pethidine and related compounds, these structures were related to the structure of morphine,[88,245,246] and this relationship has been illustrated as follows:—



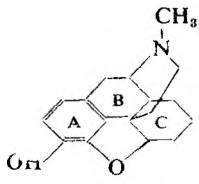
Morphine



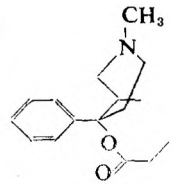
Pethidine series

Macdonald *et al.*[88] stated that their results seemed to indicate that "the shape or fit of the molecule as a whole is more important in determining its analgesic value than any precise duplication of any one fraction of the morphine structure." Ziering and Lee[109] suggested that the *trans*-isomer (XLI) was more closely related to dihydrodesoxymorphine (XL) than the *cis*-isomer (XLII) (see Randall and Lehmann[110] also) and that this accounted for the *trans*-isomer being more active than the *cis*-. The propionoxy chain and the methyl group in the piperidine ring are together

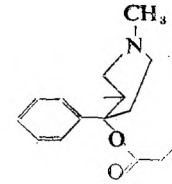
supposed to simulate ring C of dihydrodesoxymorphine, and the carbonyl oxygen atom of the propionoxy group is said to occupy spatially the exact position of the ether oxygen in (XL).



(XL)



XLI (*trans*-)



(XLII) (*cis*-)

According to Bergel and Morrison[1] (excluding diphenylpropylamine derivatives) "those substances which contain almost unimpaired certain elements of morphine, such as the phenyl and piperidine rings and an appropriate chain or ring on the quaternary carbon, are true morphine-like analgesics." After previously emphasising the steric arrangement and compactness of the morphine molecule, they state, concerning the diphenylpropylamine derivatives such as amidone, "when an atomic model is made, the spatial compactness of amidone and its close similarity to morphinan and the phenylpiperidines becomes evident." However, as Adamson and Green[164] have pointed out, it is difficult to discern any structural similarity between analgesically active dithienylbutenylamines and morphine. Furthermore, Eddy[3] and Bochmühl[120] state that they fail to see any direct relationship between the structures of amidone and morphine. Even if amidone and other analgesics show some similarity to morphine, dealing in terms of the relationship alone does not carry us much further forward towards a statement as to the simplest pharmacodynamic group required for analgesic activity. However, the approach does emphasise the necessity of stereochemical considerations in the treatment of the problem. It has been repeatedly emphasised in many publications (e.g.,[84,169,170,247]) that morphine, pethidine and amidone possess in common a tertiary nitrogen group and a quaternary carbon atom separated by a $-CH_2 \cdot CH_2-$ linkage. Eddy,[3] in a recent review, has stated that a tertiary nitrogen seems to be essential for analgesic action and a $-CH_2 \cdot CH_2-$ link joining tertiary nitrogen and quaternary carbon seems to be desirable and perhaps optimal for analgesic action. A different approach to the problem has been made by Schueler *et al*,[244] who suggested that the presence of both sympathomimetic and parasympathomimetic moieties, connected (in general) by the same nitrogen atom, was necessary for analgesic action. A subsequent publication[248] indicated that this was not likely to be a fruitful approach.

One important factor which undoubtedly emerges from any consideration of chemical structure and analgesic activity is the importance of the stereochemical configuration. *N*-methylmorphinan, for instance, is just a collection of aromatic and hydroaromatic rings, joined together in a certain way, and possessing a basic centre, and yet this compound is analgesically active. However, the fact that *N*-methyl*iso*morphinan also

possesses activity suggests that more than one spatial arrangement may be permissible for activity. The importance of spatial configuration is also seen in the difference of activity between the diastereoisomers of 1-amino-1-phthalidyl propane,[60] and the diphenylethanolamines[52] and the *cis*- and *trans*-isomers of the pethidine type compounds.[109] The clearest examples are provided by the analgesics containing one asymmetric carbon atom, where in all cases in which the optical enantiomorphs have been prepared, one enantiomorph is always very much more active than the other (e.g., amidone, amidone-type esters and sulphones, isoamidone, β-pethidine, dithienylbutenylamines). The distribution within the body of the *d*- and *l*-isomers of amidone is the same,[215] and although these measurements of tissue distribution are on a macroscopical level, and specific agents act on a molecular level, the possibility of a stereochemical fit upon a certain receptor surface of one isomer, and not the other, does receive some support. Thus, before analgesic action can be mediated directly or indirectly, it is possible that the stereochemical configuration of the drug must be complementary to that of a certain tissue surface or enzyme system.

It appears probable from a consideration of the diverse types of compounds which have an analgesic activity equal to, or greater than, that of pethidine, that the minimum requirement for activity may be a hydrophobic group (or collection of groups) containing a basic centre with an overall optimum spatial arrangement. Once this essential minimum is present, functional groups can increase or decrease the effect because of affecting the distribution, the metabolism, or the fit at a particular receptor surface.

References

1. Bergel and Morrison, *Quart. Revs*, 1948, **2**, 349.
2. Weill and Weiss, *Bulletin on Narcotics*, 1951, **3**, 12.
3. Eddy, *J. Amer. pharm. Ass. (Sci. Ed.)*, 1950, **39**, 245.
4. Wolff, *Bull. World Hlth. Org.*, 1949, **2**, 193.
5. Fellows and Ullyot, *Medicinal Chemistry*, Suter, 1951, Vol. I, 390; Lee, *ibid.*, 438.
6. Macdonald, *J. Pharm. Pharmacol.*, 1949, **1**, 569.
7. Robson and Nissim, *Chem. products*, 1949, **12**, 284.
8. Wikler, *Pharmacological Revs.*, 1950, **2**, 435.
9. Isbell and Fraser, *ibid.*, 1950, **2**, 355.
10. Wolff, *J. Pharm. Pharmacol.*, 1951, **3**, 1.
11. Fourneau, *Chim. et Industr.*, 1938, **39**, 1043.
12. Gulland and Robinson, *Mem. Proc. Manchester Lit. Phil. Soc.*, 1924–5, **69**, 79.
13. Grewe, Mondon and Nolte, *Liebigs Ann.*, 1949, **564**, 161.
14. Gates and Tschudi, *J. Amer. chem. Soc.*, 1952, **74**, 1109.
15. Small, Eddy, Mosettig and Himmelsbach, *Suppl. No.* 138 *to Public Health Reports, U.S. Treasury Dept.*, 1938.
16. Small, Fitch and Smith, *J. Amer. chem. Soc.*, 1936, **58**, 1457.
17. Eddy, *Ann. N.Y. Acad. Sci.*, 1948, **51**, 51.
18. Houde, Rasmussen and La Due, *Ann. N.Y. Acad. Sci.*, 1948, **51**, 161.
19. Small and Rapoport, *J. org. Chem.*, 1947, **12**, 248.
20. W.H.O. *Technical Report Service* No. 57.
21. Rapoport and Bonner, *J. Amer. chem. Soc.*, 1951, **73**, 5485.
22. Karrer and Widmark, *Helv. chim. Acta.*, 1951, **34**, 34.
23. Rapoport and Bonner, *J. Amer. chem. Soc.*, 1951, **73**, 2872.
24. Stern, *Quart. Revs.*, 1951, **5**, 405.
25. Grewe, *Naturwiss*, 1946, **33**, 333.
26. Grewe, *Z. angew Chem.*, 1947, **59**, 194.

27. Grewe and Mondon, *Ber. dtsch. chem. Ges.*, 1948, **81**, 279.
28. Grewe, Mondon and Nolte, *Liebigs Ann.*, 1949, **564**, 161.
29. Schnider and Grussner, *Helv. chim. Acta.*, 1949, **32**, 821.
30. Randall and Lehmann, *J. Pharmacol.*, 1950, **99**, 163.
31. Junkerman, Heen and Pohle, *J. Lab. clin. Med.*, 1949, **34**, 1615.
32. Zager, Sawtelle, Gross, Nagfy and Tidrick, *ibid.*, 1949, **34**, 1530.
33. Denton and Beecher, *J. Amer. med. Ass.*, 1949, **141**, 1051.
34. Keutmann and Foldes, *The New Eng. J. of Med.*, 1951, **244**, 286.
35. Slomka, Keasling and Gross, *J. Pharmacol.*, 1951, **101**, 33.
36. Schnider and Grussner, *Helv. chim. Acta.*, 1951, **34**, 2211.
37. Fromherz, *Arch. int. pharmacodyn.*, 1951, **85**, 387.
38. Benson, Stifko and Randall, *Fed. Proc.*, 1952, **11**. 322.
39. Schnider and Hellerbach, *Helv. chim. Acta.*, 1950, **33**, 1437.
40. U.S. 2,524,855 (1950), U.S. 2,524,856 (1950), Ger. 802,571 (1951), Brit. 624,391 (1949).
41. Gates and Newhill, *J. Amer. chem. Soc.*, 1948, **70**, 2261.
42. *Idem.*, *Experimentia*, 1949, **5**, 285.
43. Gates, Woodward, Newhill and Künzli, *J. Amer. chem. Soc.*, 1950, **72**, 1141.
44. Gates and Tschudi, *ibid.*, 1950, **72**, 4839.
45. *Idem.*, *ibid.*, 1952, **74**, 1109.
46. Dodds, Lawson and Williams, *Nature, Lond.*, 1943, **151**, 614.
47. *Idem.*, *Proc. Royal Soc.* (*London*), 1944, B.**132**, 119.
48. Dodds, Lawson, Simpson and Williams, *J. Physiol.*, 1945, **104**. 47.
49. Goodson, Wiegand and Splitter, *J. Amer. chem. Soc.*, 1946, **68**, 2174.
50. Moffett and Hoehn, *ibid.*, 1947, **69**, 1792.
51. McPhee and Erickson, *ibid.*, 1946, **68**, 624.
52. Holck, Kimura and Kimura, *J. Amer. pharm. Ass.* (*Sci. Ed.*), 1950, **39**, 354.
53. Mercier, Lespagnol and Mercier, *C. R. Soc. Biol. Paris*, 1947, **141**, 820.
54. Wawzonck and Smolin, *J. org. Chem.*, 1951, **16**, 746.
55. Goodson and Moffett, *J. Amer. chem. Soc.*, 1949, **71**, 3219.
56. Lespagnol, Mercier, Bertrand, Mercier, *Ann. pharm. franc.*, 1950, **8**, 241.
57. Lespagnol and Bertrand, *C.R. Acad. Sci., Paris*, 1950, **230**, 658.
58. Kulz, U.S.P. 2,276,618, 2,276,619 (1942).
59. Lee, Ziering, Berger and Heineman, *Jub. Vol. Emil Barell*, 1946, 264.
60. Ullyot, Stehle, Zirkle, Shriner and Wolf, *J. org. Chem.*, 1945, **10**, 429.
61. Ullyot, Taylor and Dawson, *J. Amer. chem. Soc.*, 1948, **70**, 542.
62. Wilson, Zirkle, Anderson, Stehle and Ullyot, *J. org. Chem.*, 1951, **16**, 792.
63. Fellows and Cunningham, *Fed. Proc.*, 1945, **4**, 118.
64. Fellows, *ibid.*, 1946, **5**, 178.
65. Weijlard, Pfister, Swanezy, Robinson and Tishler, *J. Amer. chem. Soc.*, 1951, **73**, 1216.
66. Burckhalter and Johnson, *ibid.*, 1951, **73**, 4830.
67. McCoubrey, *J. chem. Soc.*, 1951, 2931.
68. McCoubrey and (in part) Iyengar, *ibid.*, 1951, 3430.
69. Lee, Ziering, Heinemann and Berger, *J. org. Chem.*, 1947, **12**, 885.
70. Bockelheide and Schilling, *J. Amer. chem. Soc.*, 1950, **72**, 712.
71. Anker, Cook and Heilbron, *J. chem. Soc.*, 1945, 917.
72. Külz and Hornung, B.PP. 512,560; 513,512.
73. Barltrop, *J. chem. Soc.*, 1946, 958.
74. Bockelheide, *J. Amer. chem. Soc.*, 1947, **69**, 790.
75. Badger, Cook, Donald, Graham and Walker, *Nature, Lond.*, 1948, **162**. 21.
76. Badger, Cook and Walker, *J. chem. Soc.*, 1948, 2011.
77. Badger, Cook and Donald, *ibid.*, 1951, 1392.
78. Smith, Stewart, Roth and Northey, *J. Amer. chem. Soc.*, 1948, **70**, 3997.
79. Roth and Smith, *ibid.*, 1949, **71**, 616.
80. Shapiro, *J. org. Chem.*, 1950, **15**, 1027.
81. Shapiro, *ibid.*, 1951, **16**, 1247.
82. Scheuing and Walach, U.S.P. 2,369,611, 2,352,020 (1945).
83. Burger and Bennet, *J. Amer. chem. Soc.*, 1950, **72**, 5414.
84. Zaugg, Freifelder and Horrom, *J. org. Chem.*, 1950, **15**, 1191.
85. Goldschmidt and Veer, *Rec. Trav. chim. Pays-Bas*, 1948, **67**, 489.
86. Goldschmidt, Modderman and Overbeck, *ibid.*, 1950, **69**, 1109.
87. Bergel, Howarth and Morrison, *J. chem. Soc.*, 1944, 261.
88. Macdonald, Woolfe, Bergel, Morrison and Rinderknecht, *Brit. J. Pharmacol.*, 1946, **1**, 4.
89. Bovet and Simon, *Anesthesia et Analgesia*, 1935, **1**, 21.

90. Mosettig and Robinson, *J. Amer. chem. Soc.*, 1935, **57**, 2186.
91. Kirkpatrick and Parker, *ibid.*, 1935, **57**, 1123.
92. Burger and Bryant, *ibid.*, 1941, **63**, 1054.
93. Harnest and Burger, *ibid.*, 1943, **65**, 370.
94. *Idem.*, *ibid.*, 1943, **65**, 2382.
95. Henecka, *Medicine in its Chemical Aspects*, 1938, **3**, 280.
96. Burger and Deinet, *J. Amer. chem. Soc.*, 1945, **67**, 566.
97. Fellows and Ullyot, *Medicinal Chemistry*, Suter, 1951 Vol. I, pp. 432–433.
98. Spielman, *J. Amer. chem. Soc.*, 1944, **66**, 1244.
99. Eisleb and Schaumann, *Dtsch. med. Wschr.*, 1939, **65**, 967.
100. Eisleb, *Ber. dtsch. chem. Ges.*, 1941, **74**, 1433.
101. Yonkman, *Ann. N.Y. Acad. Sci.*, 1948, **51**, 59.
102. Foster and Carman, *J. Pharmacol.*, 1947, **91**, 195.
103. Report No. P.B. 981 U.S.A. Office of the Publ. Board, Dept. of Commerce.
104. Bergel, Hindley, Morrison and Rinderknecht, *J. chem. Soc.*, 1944, 269.
105. Glazebrook and Branwood, *Lancet*, 1945, **249**, 528.
106. Kirchoff, *Fed. Proc.*, 1948, **7**, 234.
107. Isbell, *J. Pharmacol.*, 1949, **97**, 182.
108. Jensen, Lindquist, Rekling and Wolffbrandt, *Dansk. Tidskr. Farm.*, 1943, **17**, 173.
109. Ziering and Lee, *J. org. Chem.*, 1947, **12**, 911.
110. Randall and Lehmann, *J. Pharmacol.*, 1948, **93**, 314.
111. Gross, Holland and Schueler, *J. Applied Physiol.*, 1948, **1**, 298.
112. Gross, Brotman, Nagyfy, Sawtelle and Zager, *Fed. Proc.*, 1949, **8**, 297.
113. B.I.O.S. Final Report No. 116, Item No. 24, p. 51 (1945).
114. Finnegan, Haag, Larson and Dreyfuss, *J. Amer. med. Ass.*, 1948, **92**, 269.
115. Eddy, Touchberry and Lieberman, *J. Pharmacol.*, 1950, **98**, 121.
116. Denton and Beecher, *J. Amer. med. Ass.*, 1949, **141**, 1146.
117. Dupré, Elks, Hems, Speyer and Evans, *J. chem. Soc.*, 1949, 500.
118. Speeter, Byrd, Cheney and Binkley, *J. Amer. chem. Soc.*, 1949, **71**, 57.
119. Chen, *Ann. N.Y. Acad. Sci.*, 1948, **51**, 83.
120. Bockmühl and Ehrhart, *Ann.*, 1948, **561**, 52.
121. Walton, Ofner and Thorp, *J. chem. Soc.*, 1949, 648.
122. Morrison and Rinderknecht, *ibid.*, 1950, 1478.
123. Wilson and Hunter, *Brit. med. J.*, 1948, **2**, 553.
124. Hewer, Keele, Keele and Nathan, *Lancet*, 1949, **256**, 431.
125. Winter and Flataker, *J. Pharmacol.*, 1950, **98**, 305.
126. Basil, Edge and Somers, *Brit. J. Pharmacol.*, 1950, **5**, 125.
127. Isbell and Fraser, *Pharmacological Reviews*, 1950, 2, 355.
128. Ofner, Thorp and Walton, *Nature, Lond.*, 1949, **163**, 479.
129. Prescott, Ransom, Thorp and Wilson, *Lancet*, 1949, **256**, 340.
130. Jensen, Lauridsen, and Christensen, *Acta chem. Scand.*, 1948, **2**, 381.
131. Attenburrow, Elks, Hems and Speyer, *J. chem. Soc.*, 1949, 510.
132. Ofner, Thorp and Walton, *Nature, Lond.*, 1949, **163**, 479.
133. Jenney and Pfeiffer, *Fed. Proc.*, 1948, **7**, 231.
134. Luduena, Miller, Ananenko and Frick, *Fed. Proc.*, 1948, **7**, 241.
135. Thorp, Walton and Ofner, *Nature, Lond.*, 1947, **160**, 605.
136. Brode and Hill, *J. org. Chem.*, 1948, **13**, 191.
137. Larson, Tullar, Elpern and Buck, *J. Amer. chem. Soc.*, 1948, **70**, 4194.
138. Howe and Sletzinger, *ibid.*, 1949, **71**, 2935.
139. Tullar, Wetterau and Archer, *ibid.*, 1948, **70**, 3959.
140. Burckhalter and Johnson, *ibid.*, 1951, **73**, 4832.
141. Brown, Cook and Heilbron, *J. chem. Soc.*, 1949, S 106.
142. Mosher and Tessieri, *J. Amer. chem. Soc.*, 1949, **73**, 4925.
143. Brown, Cook and Heilbron, *J. chem. Soc.*, 1949, S 111.
144. Leonard and Ehrenthal, *J. Amer. chem. Soc.*, 1951, **73**, 2261.
145. Badger, Cook and Schwarz, *J. chem. Soc.*, 1952, 117.
146. Beckett and Linnell, *J. Pharm. Pharmacol.*, 1950, **2**, 427.
147. Morrison and Rinderknecht, *J. chem. Soc.*, 1950, 1510.
148. Yandik and Larsen, *J. Amer. chem. Soc.*, 1951, **73**, 3534.
149. May and Mosettig, *J. org. Chem.*, 1948, **13**, 459.
150. *Idem.*, *ibid.*, 1948, **13**, 663.
151. Beckett and Linnell, *J. Pharm. Pharmacol.*, 1950, **2**, 418.
152. Eddy, May and Mosettig, *J. org. Chem.*, 1952, **17**, 321.
153. Pohland, Marshall and Carney, *J. Amer. chem. Soc.*, 1949, **71**, 460.
154. Sherrod, Kaiser, Santos-Martinez and Pfeiffer, *Fed. Proc.*, 1948, **7**, 255.

155. Beecher and Keats, *ibid.*, 1952, **11**, 321.
156. Fraser and Isbell, *J. Pharmacol.*, 1951, **101**, 12.
157. Easton, Gardner, Evanick and Stevens, *J. Amer. chem. Soc.*, 1948, **70**, 76.
158. Schultz, Robb and Sprague, *ibid.*, 1947, **69**, 2454.
159. Cheney, Smith and Binkley, *ibid.*, 1949, **71**, 53.
160. Klenk, Suter and Archer, *ibid.*, 1948, **70**, 3846.
161. Lewis, *J. Pharmacol.*, 1949, **96**, 31.
162. Brown, Cook and Heilbron, *J. chem. Soc.*, 1949, S 113.
163. Ginsberg and Baizer, *J. Amer. chem. Soc.*, 1949, **71**, 1500.
164. Adamson and Green, *Nature, Lond.*, 1950, **165**, 122.
165. Adamson, *J. chem. Soc.*, 1950, 885.
166. Adamson, Duffin and Green, *Nature, Lond.*, 1951, **167**, 153.
167. Keele, *Analyst*, 1952, **77**, 111.
168. Martin and Hanslick, U.S.P. 2,507,313 (1950).
169. Horning and Rutenberg, *J. Amer. chem. Soc.*, 1950, **72**, 3534.
170. Schwartzman, *J. org. Chem.*, 1950, **15**, 517.
171. Schwartzman and Woods, *ibid.*, 1952, **17**, 492.
172. Kagi and Miescher, *Helv., chim. Acta.*, 1949, **32**, 2489.
173. Close, Tiffany and Spielman, *J. Amer. chem. Soc.*, 1949, **71**, 1265.
174. Kyi and Wilson, *J. chem. Soc.*, 1951, 1706.
175. Wilson, *ibid.*, 1950, 2173.
176. Marshall, Ahmed and Weston, *Brit. J. Pharmacol.*, 1952, **7**, 85.
177. Krueger, Eddy and Sumwalt, *The Pharmacology of the Opium Alkaloids, Suppl. No.* 165 *to the Public Health Reports.*
178. Gross and Thompson, *J. Pharmacol.*, 1940, **68**, 413.
179. Oberst, *ibid.*, 1940, **69**, 240.
180. Endo, *Jap. J. med. Sci. IV Pharmacol.*, 1938, **11**, 140.
181. Bernheim and Bernheim, *J. Pharmacol.*, 1944, **81**, 374.
182. *Idem., ibid.*, 1945, **83**, 35.
183. Zauder, *ibid.*, 1952, **104**, 11.
184. Abord and Koon, *Fed. Proc.*, 1949, **8**, 270.
185. Rapoport, Lovell and Tolbert, *J. Amer. chem. Soc.*, 1951, **73**, 5900.
186. March and Elliot, *Fed. Proc.*, 1952, **11**, 373.
187. Chang, Oneto, Sah, Tolbert and Rapoport, *J. org. Chem.*, 1950, **15**, 634.
188. Latham and Elliot, *J. Pharmacol.*, 1951, **101**, 259.
189. Oberst, *ibid.*, 1941, **73**, 401.
190. Latham and Elliot, *ibid.*, 1951, **101**, 22.
191. Adler and Shaw, *ibid.*, 1952, **104**, 1.
192. Adler and Latham, *Proc. Soc. exp. Biol., N.Y.*, 1950, **73**, 401.
193. Adler and Shaw, *J. Pharmacol.*, 1951, **103**, 337.
194. Way, Gimble, McElway, Ross, Sung and Ellsworth, *ibid.*, 1949, **96**, 477.
195. Plotnikoff, Elliot and Way, *ibid.*, 1952, **104**, 377.
196. Plotnikoff, *ibid.*, 1951, **101**, 31.
197. Oberst, *ibid.*, 1943, **79**, 10.
198. Lehman and Aitkin, *J. Lab. clin. Med.*, 1943, **28**, 787.
199. Lief, Burns, Papper, Berger, Wollack and Brodie, *Fed. Proc.*, 1952, **11**, 369.
200. Bernheim and Bernheim, *J. Pharmacol.*, 1945, **85**, 74.
201. Way, Swanson and Gimble, *ibid.*, 1947, **91**, 178.
202. Way, Sung and McKelway, *ibid.*, 1949, **97**, 222.
203. Elliot, Chang, Abdou and Anderson, *ibid.*, 1949, **95**, 494.
204. Richards, Boxer and Smith, *ibid.*, 1950, **98**, 380.
205. Eisenbrandt, Abdou and Adler, *Fed. Proc.*, 1949, **8**, 288.
206. Adler and Eisenbrandt, *Proc. Soc. exp. Biol., N.Y.*, 1949, **72**, 347.
207. Eisenbrandt, Adler, Elliot and Abdou, *J. Pharmacol.*, 1950, **98**, 200.
208. Cronheim and Ware, *ibid.*, 1948, **92**, 98.
209. Way, Signorotti, March and Peng, *ibid.*, 1951, **101**, 249.
210. Way, Signorotti and March, *ibid.*, 1950, **98**, 34.
211. Sung and Way, *ibid.*, 1950, **98**, 72.
212. Bonneycastle and Delia, *Proc. Soc. exp. Biol., N.Y.*, 1950, **74**, 489.
213. Sung and Way, *J. Pharmacol.*, 1951, **101**, 34.
214. *Idem., Fed. Proc.*, 1952, **11**, 394.
215. Way and Sung, *ibid.*, 1952, **11**, 401.
216. Bernheim and Bernheim, *J. Pharmacol.*, 1936, **57**, 427.
217. Eadie, *J. biol. Chem.*, 1941, **138**, 597.
218. Wright and Sabine, *J. Pharmacol.*, 1943, **78**, 375.
219. Greig and Howell, *Proc. Soc. exp. Biol., N.Y.*, 1948, **65**, 352.

220. Eadie, Bernheim and Fitzgerald, *J. Pharmacol.*, 1948, **94**, 19.
221. Burn, *Chemistry and Industry*, 1948, 790.
222. Quastel and Wheatley, *Biochem. J.*, 1932, **26**, 725.
223. Gross and Pierce, *J. Pharmacol.*, 1935, **53**, 156.
224. Seevers and Shideman, *ibid.*, 1940, **71**, 373.
225. Elliot, Warrens and James, *ibid.*, 1947, **91**, 98.
226. Elliot, *Fed. Proc.*, 1947, **6**, 327.
227. Watts, *Arch. Biochem.*, 1950, **25**, 201.
228. Grieg and Howell, *ibid.*, 1949, **19**, 441.
229. Wang and Bain, *J. Pharmacol.*, 1951, **103**, 365.
230. Pero, *Giorn. ital. anestesia ē analgesia*, 1939, **5**, 128.
231. Ivy, Goetzl, Harris and Burrill, *Quart. Bull. Northwestern Univ. Med. School*, 1944, **18**, 298.
232. Bodo, Co Tui and Benaglia, *J. Pharmacol.*, 1937, **61**, 48.
233. Puharich and Goetzl, *Permanent Found. Med. Bull.*, 1947, **5**, 19.
234. Friend and Harris, *J. Pharmacol.*, 1948, **93**, 161.
235. Gross, Holland, Carter and Christensen, *Anesthesiology*, 1948, **9**, 459.
236. Zauder, *J. Pharmacol.*, 1951, **101**, 40.
237. Irwin and Shideman, *Fed. Proc.*, 1952, **11**, 359.
238. Wilkler, Frank and Eisenman, *ibid.*, 1947, **6**, 384.
239. Finnegan, Haag, Larson and Dreyfuss, *J. Pharmacol.*, 1948, **92**, 269.
240. Kimura, Keith and Foster, *Fed. Proc.*, 1952, **11**, 363.
241. Kimura, and de Boer, *J. Pharmacol.*, 1951, **101**, 20.
242. Watts, *ibid.*, 1951, **102**, 269.
243. Pfeiffer, Santos-Martinez and Sherrod, *Fed. Proc.*, 1948, **7**, 248.
244. Schueler, Gross and Holland, *J. Amer. pharm. Ass.* (*Sci. Ed.*), 1949, **38**, 74.
245. Schaumann, *Arch. exptl. Path. Pharmakol.*, 1940, **196**, 109.
246. Morrison and Rinderknecht, *Jubilee Vol. Emil. Barell*, 1946, 253.
247. Horning and Schock, *J. Amer. chem. Soc.*, 1948, **70**, 2941.
248. Slomka and Schueler, *J. Amer. pharm. Ass.* (*Sci. Ed.*), 1951, **40**, 47.

RESEARCH PAPERS

THE CHEMISTRY OF ANTI-PERNICIOUS ANÆMIA FACTORS

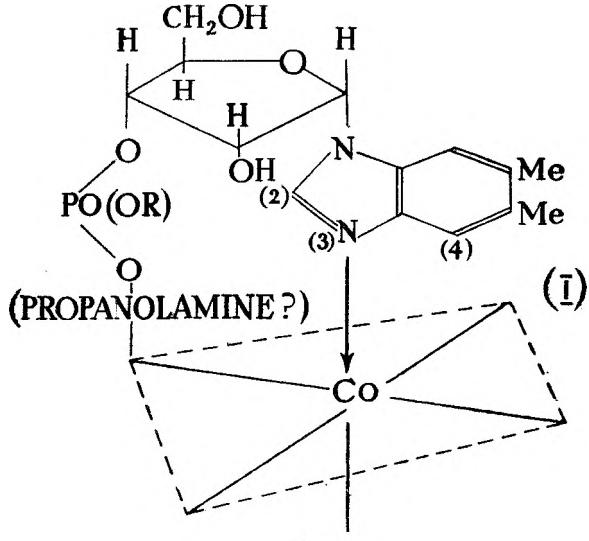
PART IX. THE PREPARATION AND SPECTROGRAPHIC PROPERTIES OF SOME BENZIMINAZOLE-COBALT CO-ORDINATION COMPOUNDS

BY M. T. DAVIES, P. MAMALIS, V. PETROW, B. STURGEON, G. H. BEAVEN, E. R. HOLIDAY and E. A. JOHNSON

From The Research Laboratories, The British Drug Houses Ltd., London, N.1, and the Medical Research Council Spectrographic Unit, London Hospital, E.1

Received May 19, 1952

THE evidence upon which Beaven, Holiday, Johnson, Ellis, and Petrow (Part VI)[1] based their conclusion that the phosphoryl 1α-D-ribofuranosyl-5:6-dimethylbenziminazolyl residue present in the B_{12} molecule was co-ordinately linked to cobalt, as shown in (I), was largely spectroscopic in character. In the main it rested on the recognition of two anomalies



I

in the contribution of the benziminazole chromophore to the spectrum of the parent vitamin, and the association of these anomalies with co-ordination of the type specified.

Two spectroscopic features were held to distinguish the co-ordinated glycosylbenziminazole structure from an unattached phosphoryl-1α-D-ribofuranosyl-5:6-dimethylbenziminazole: (i) the absence of a short wave shift in passing from *p*H 12 to *p*H 2, and (ii) the absence of a well-resolved fine structure band at $\lambda = 2885$Å, characteristic of glycosyl-5:6-dimethylbenziminazoles, but only evident in the cyanocobalamin spectrum as a slight inflection.

Their validity as criteria for co-ordination, however, was open to criticism, as the results were not based upon data derived from authentic benziminazolocobalt co-ordination compounds, *per se*, the preparation of which had not at the time been described in the literature. Additional studies were therefore initiated in order to provide the remaining links in the chain of evidence leading to partial structure (I).

The π-electron density at N^3 of a 1-substituted benziminazole must clearly play an important part in determining the stability of the resulting cobalt co-ordination complex.[2] At the same time the electron density must also bear some relation to the *p*Ka of the base. The first part of our study was therefore concerned with the relationship between chemical constitution and basicity in the benziminazole and glycosylbenziminazole series (Davies, Mamalis, Petrow and Sturgeon, Part VIII)[3]. The present communication takes the investigation a stage further by describing the preparation of some authentic benziminazolocobalt co-ordination compounds, and by showing that such materials fulfil the spectral criteria (i) and (ii) laid down in Part VI as evidence for co-ordination.

Our first experiments were directed to the preparation of quadrico-ordinate cobaltous complexes of the type $[CoCl_2 \cdot py_2]$[4,5,6] (where py = pyridine), which are generally prepared by simple admixture of the components in solvent media. Extension of this preparative method to 1-alkylbenziminazoles proved unsuccessful in those cases in which the *p*Ka of the base was less than 3·22 in ethanol (50 per cent.) (1-methyl-5:6-dichlorobenziminazole, Part VIII[3]). Glycosylbenziminazoles, though relatively basic compounds (*p*Ka 3·92 to 4·70 in water), likewise failed to react, a result probably due to the operation of unknown factors associated with the sugar group. 1-Alkylbenziminazoles of *p*Ka $\not<$ 3·88 in ethanol (50 per cent.), in contrast, passed readily into the required dibenziminazolo-cobaltous chloride complexes $[CoCl_2 \cdot Bzm_2]$ (II) (see Table IV), which were obtained as crystalline blue solids of striking appearance.

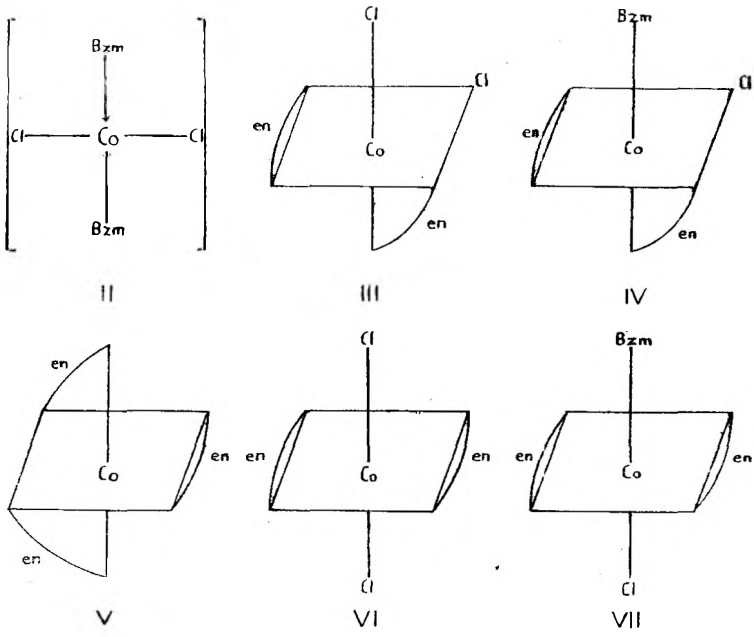
Attempts to dissolve these compounds in water led to complete hydrolysis as shown by (*a*) the colour change from deep blue to pink, (*b*) the identification of free benziminazole in the solution by spectroscopic methods (*vide infra*), (*c*) conductimetric measurements, the molar conductance at infinite dilution being that of cobaltous chloride itself, and (*d*) solvent extraction, when over 90 per cent. of the ligand could be recovered. Concentration of such solutions led to reversal of the hydrolytic reaction through a mass-action effect

$$Co^{++} \cdot aq. + 2Bzm + 2Cl^- \rightleftharpoons [CoCl_2 \cdot Bzm_2] + aq.$$

(where Bzm = 1-substituted benziminazole)

with separation of the complex in its crystalline form. In this state the benziminazole molecules were very firmly combined, not being removed by heating *in vacuo* at 150° to 160° C. We had thus to seek for organic solvents in which the compounds could be dissolved without dissociation in order to obtain solutions suitable for spectroscopic study.

Success was ultimately achieved by employing dry acetone and dry *n*-butanol, wherein the compounds dissolved to give solutions retaining



II—VII

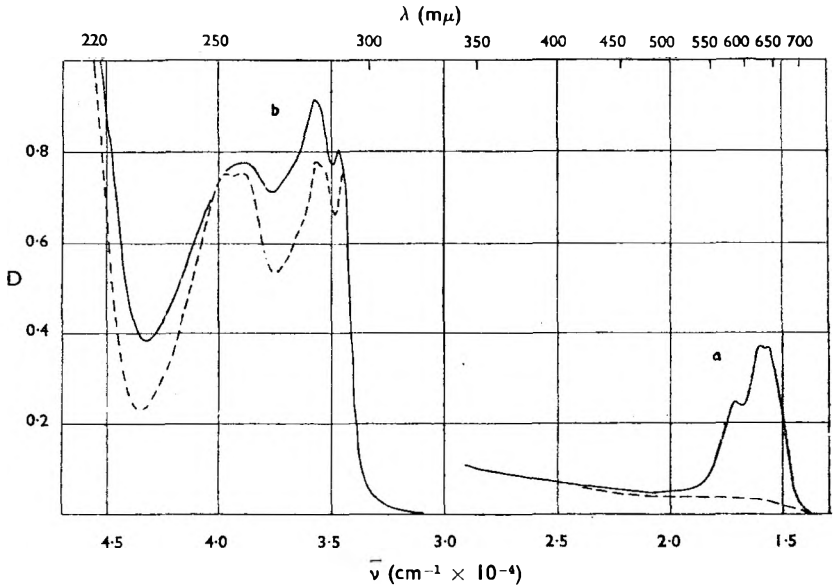


FIG. 1. Di-[1 : 5 : 6-trimethylbenziminazolo]-cobaltous chloride

Solvent .. *n*-Butanol
Concentration Saturated at 20 C° (*ca.* 1 g./litre, 0·002 M)
Cell length .. (*a*) 4·75 mm., (*b*) 0·475 mm.
—— .. Dry *n*-butanol
— — .. After adding 5 per cent. vol. of water.

unchanged the blue colours characteristic of the undissociated salts. Spectroscopic study of such solutions showed unmistakably that the authentic dibenziminazolocobaltous chloride co-ordination compounds (II) satisfied the spectral criterion (ii) (above) laid down by Beaven *et al*[1] as evidence for co-ordination, and thus provided the first direct evidence for its validity. The results obtained are illustrated in Figure 1 by reference to di-(1 : 5 : 6-trimethylbenziminazolo)-cobaltous chloride in dry *n*-butanol solution. Inspection of the curve shows that the absorption of the benziminazole chromophore is anomalous with reduced resolution of the fine structure and with both the general form and location of the absorption bands intermediate between those of a free benziminazole in acid (cation) and alkaline (free base) solution. Addition of 5 per cent. volume of water increases the fine-structure resolution, and the spectrum alters towards that of the free base, though the full resolution of the fine structure is not regained. Solution in water, in contrast, leads to complete destruction of the complex, as shown in Figure 2, with reversion of the spectrum to that of the free benziminazole (*cf.* (*b*), p. 449).

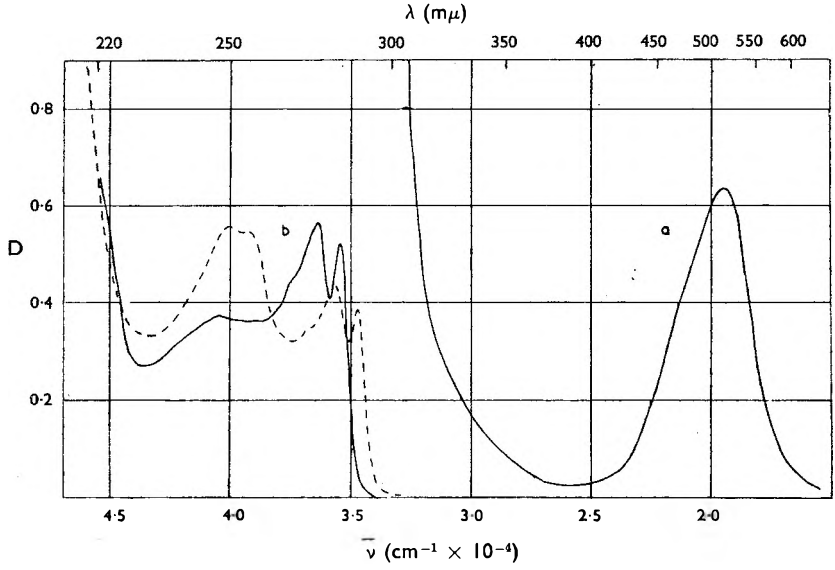


FIG. 2. Di-[1 : 5-dimethylbenziminazolo]-cobaltous chloride

Solvent .. Water
Concentration (*a*) Saturated at 20° C. *ca.* 0·1 M
(*b*) *ca.* 0·0001 M
Cell length .. (*a*) 2 cm. (*b*) 1 cm.
—— .. *p*H 2
— — — .. *p*H 10.

The absorption spectra in the visible region of the blue dibenziminazolocobaltous chloride complexes (II) differ markedly from that of the pink hydrated cobaltous ion, but resemble that of the blue $[CoCl_4]^{--}$ ion, which

has been studied by Macwalter and Barratt,[7] Gròh and Schmidt,[8] and others (see Macwalter and Barratt for references).

Compound	$\lambda_{max.}$(mμ)				Solvent
$[CoCl_2.(1\text{-MeBzm})_2]$	(518)	(592)	617	637	acetone
$[CoCl_2.(1:5:6\text{-TriMeBzm})_2]$	(526)	(592)	625	637	acetone
	(*ca.* 530)	585	621	637	*n*-butanol
Co^{++}		510			water*
$[CoCl_4]^{--}$			625	680	ethanol/HCl†
			585	678	acetone†

* See *Tables Annuelles de Constantes et Donnes Numeriques*, No. 39, vol. 13, section 33, p. 12 (Hermann, Paris, 1943).
† This work.

The molar extinction coefficient ($\epsilon_{max.}$) of the 637 mμ. band (Fig. 1) of (II) is *ca.* 200, compared with *ca.* 5 for the 510 mμ. band of hydrated Co^{++}, and with *ca.* 300 for the 678 mμ. band of $[CoCl_4]^{--}$ (in acetone). The latter figure is in good agreement with the values (630, 609) found by Macwalter and Barratt[7] and by Gròh and Schmidt,[8] respectively, as in neutral acetone only one-half of the cobalt is in the form of the complex ion:—

$$2CoCl_2 \rightarrow [CoCl_4]^{--} + Co^{++}$$

The intense 678 mμ. band present in the spectrum of the $[CoCl_4]^{--}$ ion is not evident in the spectra of the dibenziminazolocobaltous chloride complexes (II). This observation provides further proof that the latter exist in acetone and *n*-butanol solutions in the undissociated forms.

It was, of course, not possible to confirm spectral criterion (i) (above) with the dibenziminazolocobaltous chloride complexes (II), owing to their marked instability to traces of moisture (see also Experimental part). We therefore turned our attention to the preparation of co-ordinated complexes derived from cobaltic cobalt, as it was thought that such compounds might show greater stability in aqueous solution (*cf.* Pauling[9]). Meisenheimer and Kiderlen,[10] Ablov,[11,12] and Bailar and Clapp[13] had previously shown that *trans*-bisethylenediamino-dichlorocobaltic chloride $[Co.en_2Cl_2]Cl$ (VI; en = ethylenediamine) reacts readily with arylamines and with heterocyclic bases to give compounds of the type $[Co.en_2.Base.Cl]Cl_2$. We now find that by heating (VI) with 1-methylbenziminazole in aqueous ethanolic solution at 100° C. for 3 to 4 hours, or preferably by allowing the reaction to occur at room temperature for 1 to 2 days, a rose-red compound of empirical constitution $[CoCl_3.en_2.1\text{-MeBzm}]$, is readily obtained in excellent yield.

Conductance measurements show that the molar conductance of this complex at infinite dilution corresponds to a salt of the type $[Co\ complex]^{++}Cl_2$. Conductance titrations with silver nitrate likewise show that two-thirds of the chlorine content is ionic. There is little doubt, on this evidence alone, that the compound has the desired constitution of a [bis-ethylenediamino-1-methylbenziminazolochlorocobaltic] dichloride (IV, *cis*-form; (VII), *trans*-form). It thus represents the first example of an authentic benziminazolocobaltic complex to be prepared in the laboratory. In addition, its physico-chemical characteristics

make it a suitable model compound for spectroscopic study. At the same time it is impossible to define rigidly its stereochemical configuration. The balance of evidence derived from chemical (p. 454) and spectroscopic studies (p. 457), however, appears to indicate that the *cis*-structure (IV) and not the *trans*-structure (VII) is the preferred form. (IV) has, therefore, been adopted for usage in the present communication.

Extension of the reaction to other 1-substituted benziminazoles has given the results listed in Table I, alongside the basicity of the ligands.

Three types of behaviour are evident:—

(*a*) 1:5:6-Trimethyl-, 1:5-dimethyl-, 1-ethyl-, and 1:2-hydroxyethylbenziminazole react readily to give the co-ordination compound (IV) in yields greater than 60 per cent.

(*b*) 1-*iso*Propyl-, 1-*n*-propyl-, 1-allyl-, and 1:6-dimethyl-5-chlorobenziminazole give mixtures consisting largely of *cis*[bis-ethylenediaminodichlorocobaltic] chloride (III), admixed with small quantities (< 5 per cent.) of the co-ordination compound, which is separated by fractional crystallisation from aqueous acetone. 1-*n*-Butyl-, and 1-methyl-5-chlorobenziminazole also appear to react in this way, as distinctly red fractions are obtained. Purification of the co-ordination compounds, however, could not be effected.

(*c*) 1:2:5:-Trimethyl-, 1α-D-arabopyranosyl-5:6-dimethyl-, and 1β-D-glucopyranosylbenziminazole afford only the *cis*-compound (III), no evidence for complex formation being obtained.

TABLE I

Benziminazole Bzm	*p*Ka Ethanol (50 per cent.)	Reaction product(s)
1:5:6-Trimethyl-	5·45	[Co.en$_2$.BzmCl]Cl$_2$ yield < 60 per cent.
1:5-Dimethyl-	5·22	
1-Ethyl-	4·88	
1-Methyl-	4·88	
1:2-Hydroxyethyl-	4·82	
1-*iso*Propyl-	4·97	(III) plus [Co.en$_2$.BzmCl]Cl$_2$ in less than 5 per cent. yield
1-*n*-Propyl-	4·83	
1-Allyl-	4·58	
1:6-Dimethyl-5-chloro-	4·1†	(III) plus possibly some [Co.en$_2$.BzmCl]Cl$_2$
1-*n*-Butyl-	4·75	
1-Methyl-5-chloro-	3·88	
1:2:5-Trimethyl	6·07	(III)
1α-D-Arabo-5:6-dimethyl-	4·2*	
1β-D-Glucopyranosyl-	3·69	

* Estimated from value measured in aqueous solution.
† Calculated from figures given in Table V, Part VIII[3].

$$[Co.en_2.Cl_2]^+ \underset{trans\text{-}}{\text{(VI)}} \longrightarrow \{Co.en_2.Cl\}^{++}\ Cl^- \underset{\text{intermediate cation}}{\text{(VIII)}}$$

$$\{Co.en_2.Cl\}^{++} \xrightarrow[(k)]{+\ Bzm} [Co.en_2.BzmCl]^{++}\ \text{(IV)}$$

$$\{Co.en_2.Cl\}^{++} \xrightarrow[(k')]{+\ Cl^-} [Co.en_2.Cl_2]^+ \underset{cis\text{-}}{\text{(III)}} + \text{possibly trace of (VI)}$$

The explanation for this multiplicity of behaviour lies in the dual nature of the changes which can occur in mixtures of (VI) and a benziminazole in aqueous ethanolic solution. In addition to formation of (IV), we now find that (VI) undergoes spontaneous conversion into (III), a change previously thought to occur through participation of the co-present ligand.[10,13]

Both these competing reactions may be visualised as proceeding through initial formation of the intermediate cation (VIII) from the *trans*-salt (VI) by an S_N1 type of mechanism. Addition of benziminazole or chloride ion then takes place, the relative proportions of the two products formed being governed by the specific reaction rates k and k'. Thus benziminazoles in category (*a*) react quickly with formation of complex (IV) in excellent yield. Category (*b*) benziminazoles, in contrast, show slow reaction with concomitant production, in major amount, of the auto-transformation product (III), whilst category (*c*) compounds form either a special instance of such behaviour, or do not react at all.

Inspection of Table I shows that, apart from the exceptions mentioned below, the yields of $[Co.en_2.Bzm{\cdot}Cl]Cl_2$ run parallel to the basicity of the ligands. The relationship is not quantitatively proportional, however, as there is a definite break in the yield at about pK 4·8. The benziminazoles thus resemble the arylamines, in which compounds stronger than aniline (pKa 4·62 in 30 per cent. ethanol) give co-ordination compounds in high yield, whereas weaker bases appear to give solely the *cis*-compound (III) (Ablov[11]). At the same time the marked influence of basicity on complex formation may sometimes be overshadowed by the effect of other factors which cannot be strictly defined at present. Thus the basic 1-*iso*propylbenziminazole gives only a minute yield of complex with (IV), whilst the strongly basic 1:2:5-trimethylbenziminazole fails to give evidence of reaction. The reason for this anomalous behaviour is not clear, but in the case of the latter compound may well be related to steric factors associated with the 2-substituent.

In spite of the general similarity between the reactions of benziminazoles and arylamines with (VI), a point of difference is nevertheless observed in the behaviour of the former series with the *cis*-salt (III). Bailar and Clapp[13] had previously reported that arylamines react with both (III) and (VI) to give similar products, but that "it cannot be stated with certainty that the compounds from the two series are identical." Benziminazoles, in contrast, fail to react with (III) under the conditions studied. This observation, however, does not provide data for a decision on the stereochemistry of the [bis-ethylenediamino-1-methylbenziminazolo-chlorocobaltic]dichloride complexes (p. 453), as their formation is thought to occur through the intermediate cation (VIII), from which both *cis*-(IV) and *trans*-(VII) types can be formed. It is known, moreover, that aqueous ammonia converts both (III) and (VI) into the *cis*-chloroammine $[Co.en_2.(NH_3)Cl_2,]$ whilst methyl- and ethylamine react with *dextro*-(III) to give derivatives which are optically active (though readily racemised) and thus unambiguously formulated as *cis*-complexes analogous to (IV).[13] It seems reasonable to assume, on this evidence, that the *cis*-

TABLE II

ABSORPTION SPECTRA OF (BIS-ETHYLENEDIAMINO-BENZIMINAZOLOCHLOROCOBALTIC)DICHLORIDE COMPLEXES IN WATER (*p*H *ca.* 6)

Benziminazole substituents	$\lambda_{max.}$	$\epsilon_{max.}$	$\lambda_{min.}$	$\epsilon_{min.}$	$\lambda_{max.}$	$\epsilon_{max.}$	$\lambda_{max.}$	$\epsilon_{max.}$		$\lambda_{max.}$	$\epsilon_{max.}$	$\lambda_{max.}$	$\epsilon_{max.}$
1-Methyl	513	70	429	32	(370)	(110)	—	—	Modified benziminazole absorption with poorly resolved fine structure in 260-290 mμ region: see Table III for example.	—	—	233	19,400
1:5-Dimethyl	510	88	430	29	—	—	—	—		—	—	—	—
1-Ethyl	521	84	433	31	(385)	(120)	—	—		—	—	—	—
1-2′-Hydroxyethyl ..	513	85	427	30	(370)	(120)	—	—		—	—	—	—
1-*iso*-Propyl	505	50*	425	24*	—	—	—	—		—	—	—	—
1-Allyl	515	97	425	38	(370)	(130)	—	—		—	—	235	19,700
Mean values	513	85	428	32	(375)	(120)	—	—		—	—	234	19,500
cis-[Co·en$_2$·Cl$_2$]Cl† ..	535	60	405	24	380	70	(303)	(815)	—	—	—	244	15,500
trans-[Co·en$_2$Cl$_2$]Cl ..	621	34	524	8	(445)	(28)	(400)	(36)	—	304	1100	241	15,500
									λmin. ϵmin.				
[Co·en$_3$]Cl$_3$	464	83	383	12	337	70	—	—	286 5	—	—	—	—

* Not corrected for unknown water of crystallisation: excluded from mean. † Also λ infl. 603 mμ, ϵ infl. 44.
Wavelenths in mμ: values in parentheses denote unresolved inflections.

TABLE IV

DIBENZIMINAZOLO-COBALTOUS CHLORIDE COMPLEXES [$CoCl_2$·Bzm_2]

Substituted Benziminazole	*p*Ka Ethanol (50 per cent.)	Product	Analysis: Found per cent. C	H	N	Cl	Required per cent. C	H	N	Cl	Description
1-Methyl-	4·88	[$CoCl_2$·($C_8H_8N_2$)$_2$]	48·6	4·0	—	18·6	48·8	4·1	—	18·0	Deep blue prisms
1:5-Dimethyl-	5·22	[$CoCl_2$·($C_9H_{10}N_2$)$_2$]	51·6	4·6	13·1	—	51·2	4·8	13·3	—	Deep blue prisms
1:5:6-Trimethyl- ..	5·45	[$CoCl_2$·($C_{10}H_{12}N_2$)$_2$]	53·4	4·8	12·3	16·6	53·4	5·4	12·4	15·8	Dark blue prisms
1:2:5-Trimethyl- ..	6·07	[$CoCl_2$·($C_{10}H_{12}N_2$)$_2$]	53·0	5·3	12·5	15·3	53·4	5·4	12·4	15·8	Turquoise prisms
1-*n*-Butyl-	4·75	[$CoCl_2$·($C_{11}H_{14}N_2$)$_2$]	—	—	11·7	14·9	—	—	11·7	14·9	Dark blue prisms
1-Methyl-5-chloro- ..	3·88	[$CoCl_2$·($C_8H_7N_2Cl$)$_2$]	—	—	12·2	30·2	—	—	12·1	30·6	Dark blue prisms
1-Methyl-6-chloro- ..	3·88	[$CoCl_2$·($C_8H_7N_2Cl$)$_2$]	—	—	12·0	30·4	—	—	12·1	30·6	Lustrous blue prisms

structure (IV) may well be the preferred form in the present instance. Spectroscopic results, detailed below, point likewise in this direction.

In contrast to the cobaltous complexes (II), the cobaltic salts (IV) proved readily soluble in water to give solutions stable at room temperature over the *p*H range 2 to 10. We were thus able to obtain further experimental evidence in support of criterion (ii) page 448), and, in addition, to establish beyond reasonable doubt the validity of criterion (i). For this purpose 6 of the complexes listed in Table I were examined spectroscopically in aqueous solution. The results obtained are presented in such a way as to separate the absorption due to the "cobalt complex" chromophore (Table II) from that of the co-ordinated benziminazole moiety (Table III):

TABLE III

FINE STRUCTURE BANDS OF [bis-ETHYLENEDIAMINO-1-METHYLBENZIMINAZOLOCHLOROCOBALTIC] DICHLORIDE AND OF 1-METHYLBENZIMINAZOLE

		$\lambda_{max.}$	$\epsilon_{max.}$	$\lambda_{max.}$	$\epsilon_{max.}$	$\lambda_{max.}$	$\epsilon_{max.}$
Complex (IV)	*p*H 2	278·2	7600	270·6	9400	(c. 262·5)	(10500)
	*p*H 10	278·5	7000	271·7	9100	263·9	9250
1-MeBz*	*p*H 2	274·4	6600	267·8	6950	261·0	5400
	*p*H 10	279·8	4600	272·9	5000	265·2	4500

Values in parentheses refer to unresolved inflections.
Wavelengths in mμ.
* See Beaven, Holiday and Johnson.[14]

(*a*) The absorption of the "cobalt complex" chromophore is characterised by a band in the visible region at *ca.* 515 mμ., an inflection in the near ultra-violet at *ca.* 375 mμ., and an intense band at *ca.* 234 mμ. The absorption contribution of the benziminazole moiety is superimposed on the long-wave side of this complex band, which thus makes an appreciable contribution to the apparent densities of the benziminazole bands (*cf.* Table III).

(*b*) The absorption of the benziminazole moiety is characterised by:

(1) The absence of a long-wave shift when the *p*H of the solution is changed from 2 to 10, corresponding for a free benziminazole to complete conversion of the "acid" form (benziminazolinium cation) to the free base.

(2) The low resolution of the fine structure in the long-wave band at 270 to 290 mμ.

(3) The positions of the fine structure bands, which lie between the positions occupied by the corresponding bands of the free benziminazoles in acid and alkaline solution, respectively.

These features, which are illustrated in Figure 3 by reference to the absorption of [bis-ethylenediamino-1-methylbenziminazolochlorocobaltic] dichloride (IV), are clearly in marked agreement with those postulated in criteria (i) and (ii) (p. 448) as evidence for co-ordination. In addition, it may be pointed out that donation of an unshared electron pair from $N^{(3)}$ of the benziminazole to a d^2sp^3 orbital of hexacovalent Co^{+++} is formally analogous to donation to a proton with formation of the

benziminazolinium cation. The observation that the absorption spectrum of a co-ordinated benziminazole tends towards the cationic form may therefore be regarded as further evidence for the concepts formulated in the present work.

Comparison of the absorption spectrum (Fig. 3) of [bis-ethylene-diamino-1-methylbenziminazolochlorocobaltic]dichloride (IV) with those

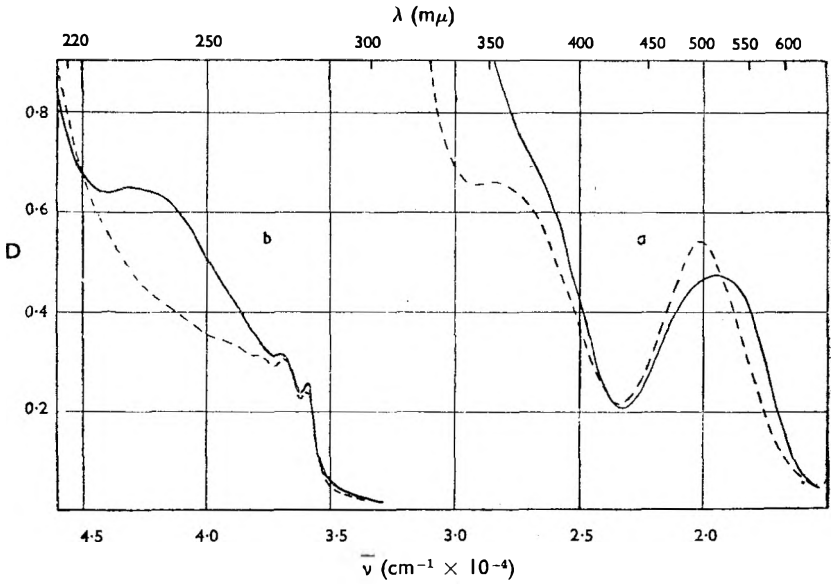


FIG. 3. [Bis-ethylenediamino-1-methylbenziminazolochloro-cobaltic] dichloride

Solvent	.. Water
Concentration	(*a*) 0·00632 M; (*b*) 0·0000335 M
Cell length	.. 1 cm.
—	.. *p*H 2.
— —	.. *p*H 10.

obtained from *cis*-[Co.en$_2$.Cl$_2$]Cl (III) (see Fig. 4) and *trans*-[Co.en$_2$.Cl$_2$]Cl (VI) (see Fig. 5), gives some information regarding the configuration of the benziminazolocobaltic complexes. The spectra of all three compounds show general similarity, although both (III) and (VI) show features at *ca.* 300mμ. which are not present in the benziminazolo-complexes. In (VI) however, the visible band occurs at longer wavelengths (621 mμ.) and is lower in intensity than in (III), and the inflection is similarly altered. It is, therefore, the *cis*-salt (III) which resembles the benziminazole complexes more closely with respect to absorption spectrum. More data on similar pairs of complexes of known configuration would be required, however, before this similarity could be considered as evidence of *cis*-configuration for the benziminazolo-complexes themselves. At the same time it seems reasonable to infer that the spectroscopic results support (IV) rather than the *trans*-structure (VII).

Inter alia, we have examined the absorption spectrum of [Co.en$_3$]Cl$_3$ (V) (see Fig. 6), which is noteworthy for the absence of an intense shortwave

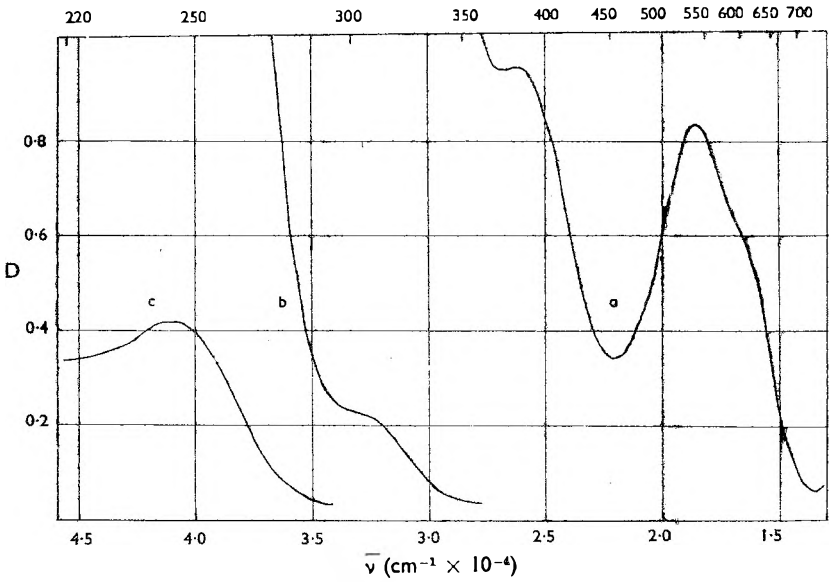


FIG. 4. *Cis*-[bis-ethylenediaminodichlorocobaltic] chloride
Solvent .. Water
Concentration (*a*) 0·0135 M; (*b*) 0·00027 M; (*c*) 0·000027 M
Cell length .. 1 cm.

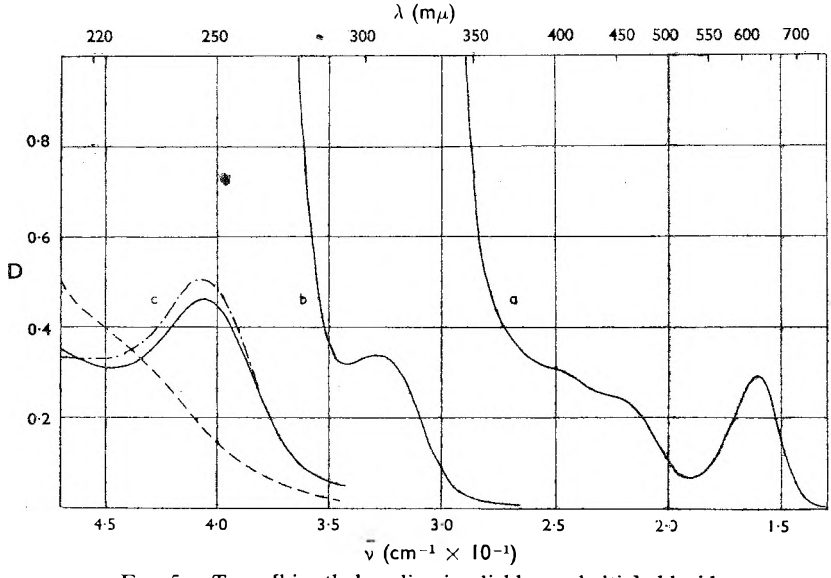


FIG. 5. *Trans*-[bis-ethylenediaminodichlorocobaltic] chloride
Solvent .. Water
Concentration (*a*) 0·0085 M; (*b*) 0·00034 M; (*c*) 0·0000272 M
Cell length .. 1 cm.
·—·—· .. *p*H 2
——— .. *p*H 6
— — .. *p*H 10.

band at wavelengths greater than 220 mμ. (contrast Figures 4 and 5). This feature may prove to be characteristic of complexes of this type, in which case its application in structure determination may be envisaged (*cf.* Tables Annuelles, No. 39, **13**, section 33 (1943) 15.)

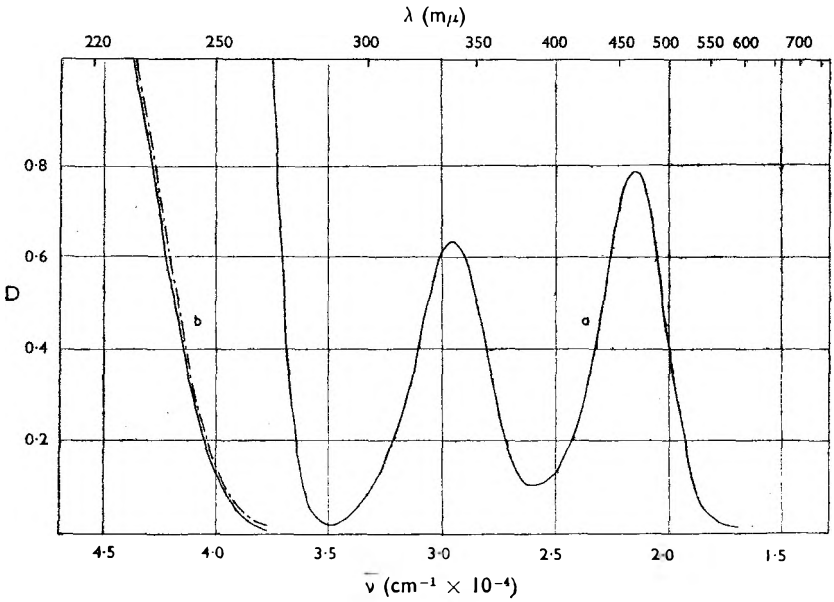


FIG. 6 [Tris-ethylenediaminocobaltic] trichloride

Solvent	.. Water
Concentration	.. (*a*) 0·00925 M; (*b*) 0·0000925 M
Cell length	.. 1 cm.
·—·—·—·	.. *p*H 2
———	.. *p*H 6; *p*H 10 (*b* only).

The above results show clearly that the spectroscopic criteria (i) and (ii) (page 448) may be used to establish the existence of the benziminazole-cobalt co-ordination linkage in simple organo-cobalt co-ordination compounds. Thus they give added support to the existence of such a linkage within the framework of the B_{12} molecule as indicated in partial structure (I).

EXPERIMENTAL

M.pt.s are corrected. Microanalyses are by Drs. Weiler and Strauss, Oxford.

Dibenziminazolocobaltous chloride complexes:—The complexes listed in Table IV were prepared by treating a solution of the benziminazole (1 mole. in warm acetone with a concentrated aqueous solution of cobaltous chloride (0·6 mole.). The products obtained were collected, washed with water and acetone and dried at 100° C.

Di[1-*methylbenziminazolo*] *cobaltous bromide and thiocyanate*:—By carrying out the foregoing preparation in the presence of sodium bromide and ammonium thiocyanate, the corresponding *bromide*, Found: N, 11·6

$[CoBr_2 \cdot (C_8H_8N_2)_2]$ requires N, 11·6, and *thiocyanate*, Found: N, 18·6; S, 14·8 $[Co(SCN)_2 \cdot (C_8H_8N_2)_2]$ requires N, 19·1; S, 14·6 per cent., were obtained. Both complexes crystallised in dark blue prisms of indefinite melting point.

Di[1 : 5-*dimethylbenziminazolo*] *cobaltous nitrate trihydrate* was prepared by adding an aqueous solution of cobalt nitrate (880 mg.) to a warm solution of 1 : 5-dimethylbenziminazole (800 mg.) in acetone (5 ml.). It formed magenta needles. Found: C, 40·8; H, 4·8; N, 16·3. $Co(NO_3)_2 \cdot (C_9H_{10}N_2)_2 \cdot 3H_2O$ requires C, 40·8; H, 5·0; N, 15·9 per cent.

[*Bis* - *ethylenediamino*- 1 - *methylbenziminazolochlorocobaltic*] *dichloride* (*IV*):— (*a*) A mixture of *trans*-(bis-ethylenediaminodichlorocobaltic) chloride (1 g.), 1-methylbenziminazole (1 g.), water (8 ml.), and a few drops of ethanol, was heated at 100° C. in an evaporating basin for 3 hours. The residue was washed with ethanol and then recrystallised twice from water and acetone. The *complex* separated in rose-red prismatic needles, m.pt. 255° C. (decomp.). Found: N, 20·2; Cl, 25·4. $C_{12}H_{24}N_6Cl_3Co$ requires N, 20·1; Cl 25·5 per cent.

(*b*) (VI) (2·9 g.), 1-methylbenziminazole (1·3 g.), water (5 ml.), and ethanol (3 ml.) were shaken together, when the chloride rapidly dissolved and the solution began to assume a red colour. Red prisms began to separate after 3 to 4 hours when ethanol (10 ml.) was added. The complex was collected after standing at 0° C. overnight. Found: N, 19·8 per cent.

[*Bis-ethylenediamino*-1-*ethylbenziminazolochlorocobaltic*] *dichloride* was obtained (90 per cent.) by reacting 1-ethylbenziminazole (1·5 g.) with (VI) (3 g.) by method (*b*). It separated from aqueous acetone in deep red prisms, m.pt. 255° to 260° C. (decomp.) after turning green at 240° C. Found: N, 19·4; Cl, 24·1. $C_{13}H_{26}N_6Cl_3Co$ requires N, 19·5; Cl, 24·7 per cent.

[*Bis* - *ethylenediamino* - 1(2 - *hydroxyethyl*) - *benziminazolo chlorocobaltic*] *dichloride*, prepared (65 per cent.) by method (*b*), formed dark red prisms from aqueous acetone, m.pt. 235° C. (decomp.). Found: N, 19·2; Cl, 23·7. $C_{13}H_{26}ON_6Cl_3Co$ requires N, 18·4; Cl, 23·3 per cent.

[*Bis* - *ethylenediamino* - 1 : 5 - *dimethylbenziminazolochlorocobaltic*] *dichloride* was obtained (72 per cent.) from aqueous acetone in small pink platelets, m.pt. 202° to 204° C. (decomp.). Found: N, 17·4; Cl, 22·2. $C_{13}H_{26}N_6Cl_3Co \cdot 3H_2O$ requires N, 17·4; Cl, 21·9 per cent.

[*Bis* - *ethylenediamino*-1 : 5 : 6 - *trimethylbenziminazochlorocobaltic*] *dichloride* was isolated (80 per cent.) from aqueous acetone in felted pink needles, m.pt. 255° C. (decomp.), after turning green at 230° C. Found: N, 19·0; Cl, 23·6. $C_{14}H_{28}N_6Cl_3Co$ requires N, 18·9; Cl, 23·8 per cent.

[*Bis-ethylenediamino*-1-iso*propylbenziminazolochlorocobaltic*] *dichloride*: —The benziminazole (1·7 g.) was allowed to react with the *trans*-dichloride (VI) (2·9 g.) in cold aqueous ethanol for 1 week. Acetone was then added and the purple crystalline product collected the next day and identified as (III). A second crop, obtained by evaporation of the mother liquor, was red in colour and proved to be a mixture of the desired complex and (III). The latter was removed by repeated crystallisation from aqueous acetone

when [*bis-ethylenediamino*-1-iso*propylbenziminazolochlorocobaltic*] *dichloride* (50 mg.) was obtained as dark red prisms, m.p. 210° to 212° C. (decomp.). Found: N, 17·0. $C_{14}H_{28}N_6Cl_3Co{\cdot}2H_2O$ requires N, 17·4 per cent.

The following complexes were prepared and isolated in essentially the same way:—

[*Bis-ethylenediamino*-1-n-*propylbenziminazolochlorocobaltic*] *dichloride*, rose-red needles, m.p. 215° C. (decomp.). Found: N, 18·9; Cl 23·4. $C_{14}H_{28}N_6Cl_3Co$ requires N, 18·9; Cl, 23·8 per cent.

[*Bis-ethylenediamino*-1-*allylbenziminazolochlorocobaltic*] *dichloride*, purple red crystals, m.p. 208° to 209° C. (decomp.). Found: N, 17·5; Cl, 22·7. $C_{14}H_{26}N_6Cl_3Co,2H_2O$ requires N, 17·5; Cl, 23·3 per cent.

[*Bis-ethylenediamino*-5-*chloro*-1:6-*dimethylbenziminazolochlorocobaltic*] *dichloride*, fine pink needles, m.p. 255° to 256° C., after turning green at 235° C. Found: N, 17·0; Cl, 29·3. $C_{13}H_{25}N_6Cl_4Co,H_2O$ requires N, 17·4; Cl, 29·3 per cent.

Molar Conductivities. Specific conductivities of aqueous solutions were determined employing the bridge and enclosed type cells of 3 ml. capacity manufactured by Mullard Electronic Products, Ltd. Temperature control was effected by immersion of the cells in a thermostatically controlled water-bath maintained at 25 ± 0·05° C., bridge readings being taken after an equilibration period of 20 minutes. The electrodes were replatinised prior to each set of determinations. Potassium chloride, A.R., was employed as a standard in cell-constant determinations, the equivalent conductivity value of the salt being taken from the results of Shedlovsky, Brown and MacInnes.[15] The overall accuracy of the determinations was within ± 3 per cent.

(1) *Complexes of the type* [$CoCl_2{\cdot}2$ Base] (II).

The specific conductivities of solutions of (*a*) dipyridinocobaltous chloride, (*b*) di[1:5-dimethylbenziminazolo]cobaltous chloride, and (*c*) cobaltous chloride, A.R., determined with respect to the bridge standard internal impedance, are given in Table V. On plotting the corresponding values for Λ_M against 100 $M^{\frac{1}{2}}$, linear plots are obtained (Fig. 7) for the

TABLE V

MOLAR CONDUCTIVITIES OF COBALTOUS CHLORIDE COMPLEXES (II) IN AQUEOUS SOLUTION AT 25° C.

Compound	10^4M	$100M^{\frac{1}{2}}$	10^4k	Λ_M
$CoCl_2{\cdot}6H_2O$	44·00	6·63	10·55	240
	22·00	4·69	5·38	244
	4·40	2·10	1·12	213
$[CoCl_2{\cdot}py_2]$	100·00	10·00	21·74	217
	50·0	7·07	11·49	230
	25·0	5·00	5·96	238
$[CoCl_2{\cdot}(1:5\text{-DiMeBzm})_2]$ ..	65·40	8·09	13·97	222
	29·94	5·47	7·06	236
	13·08	3·62	3·17	242
	2·99	1·73	0·76	254

Concentration (M) expressed in moles/l., specific conductivity (k) expressed in mhos.cm^{-1}, molar conductivity (Λ_M) expressed in mhos.cm^2.

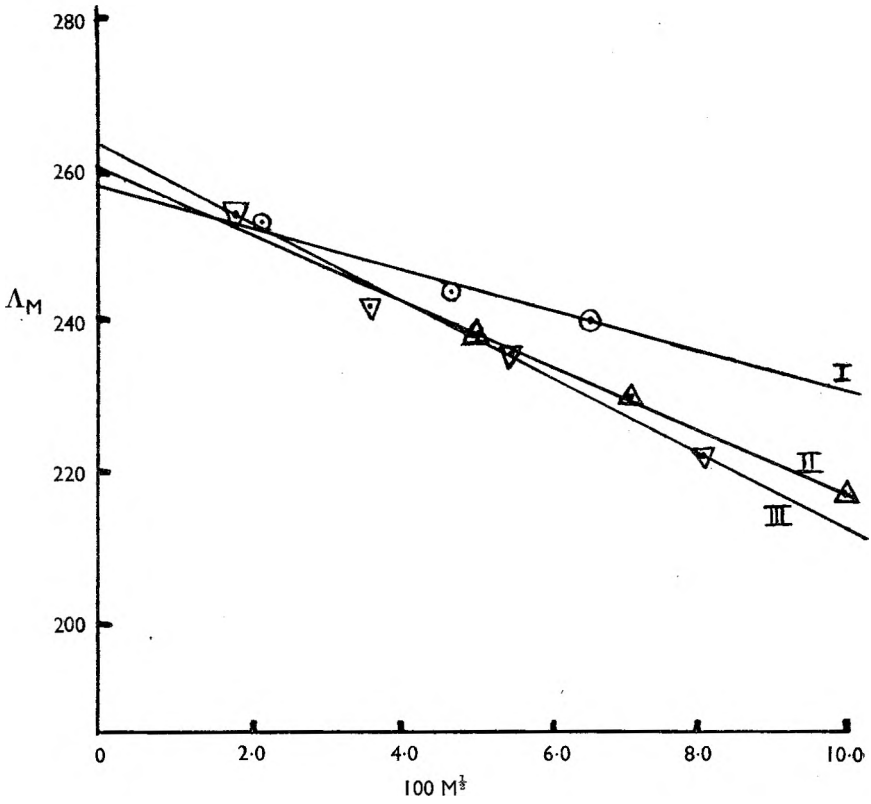


FIG. 7.

(I) ○ $CoCl_2.6H_2O$
(II) △ $[CoCl_2.Py_2]$
(III) ▽ $[CoCl_2.(1\text{-meBzm})_2]$

three substances which co-average on to the same limiting value for Λ_M (where $c = o$). Complete dissociation of (*a*) and (*b*) therefore occurs in dilute solution. Deviations of the molar conductivity values of (*a*) and (*b*) from those of cobaltous chloride at the higher concentrations are possibly due to the presence of undissociated complex.

(2) *Complexes of the type* $[Co.en_2.Bzm.Cl]Cl_2$ (IV).

The results obtained (Table VI) shown graphically in Figure 8, reveal that the complexes are of the type $M^{II}\ Cl_2$.

Estimation of Ionic Chlorine. Ionic chlorine was estimated by titration of the aqueous solutions against 10^{-3} N silver nitrate solution. Equivalence points were determined conductimetrically, the bridge being employed in conjunction with a robust dip-type cell of standard design. Smooth bright platinum foil electrodes were employed.

The results obtained are given in Table VII.

Absorption measurements. Absorption spectra were measured with a twin-beam automatic recording spectrophotometer designed and built

TABLE VI

MOLAR CONDUCTIVITIES OF COMPLEXES $[Co.en_2Bzm.Cl]Cl_2$ (IV) IN AQUEOUS SOLUTION AT 25° C.

Bzm	10^4M	$100\ M^{\frac{1}{2}}$	10^4k	Λ_M
(Pyridine)	123·1	11·09	23·98	194
	24·62	4·96	5·46	222
	12·31	3·51	2·75	224
1-Methylbenziminazole—				
(*a*) run No. 1	20·49	4·53	4·69	229
	10·25	3·20	2·47	241
	5·12	2·26	1·27	247
(*b*) run No. 2	20·11	4·48	4·61	229
	10·06	3·17	2·42	241
	5·03	2·24	1·25	250
1-Ethylbenziminazole—				
(*a*) run No. 1	50·24	7·09	10·37	206
	12·56	3·54	2·80	223
	10·05	3·17	2·28	227
	5·02	2·24	1·18	236
(*b*) run No. 2	50·10	7·08	10·40	208
	12·52	3·54	2·81	225
	10·02	3·17	2·33	232
	5·01	2·24	1·20	240
1:2'-Hydroxyethylbenziminazole—				
(*a*) run No. 1	20·45	4·52	4·44	217
	10·23	3·20	2·35	229
	5·11	2·27	1·21	237
(*b*) run No. 2	12·02	3·47	2·68	222
	6·01	2·45	1·40	233
	3·01	1·74	0·72	238
1:5-Dimethylbenziminazole ($\cdot 3H_2O$)—				
(*a*) run No. 1	18·08	4·25	4·22	233
	9·04	3·01	2·16	239
	4·52	2·13	1·08	240
(*b*) run No. 2	17·83	4·22	4·17	234
	8·92	2·99	2·13	239
	4·46	2·11	1·08	243

TABLE VII

ESTIMATION OF IONIC CHLORINE IN COMPLEXES OF TYPE $[Co{\cdot}en_2{\cdot}BzmCl]Cl_2$ (IV) IN AQUEOUS SOLUTION, AT LABORATORY TEMPERATURE

Bzm	10^4M	$10^3M'$	Ionic chlorine per cent.	Number of chlorine ions per molecule
1-Methylbenziminazole	5·16	4·3	19·1	2·25
1-Ethylbenziminazole	10·61	8·2	17·9	2·18
1:5-Dimethylbenziminazole($\cdot 3H_2O$)	9·10	7·0	17·4	2·38
1:2'-Hydroxyethylbenziminazole	0·84	0·58	19·7	2·50

M = concentration of complex solution, expressed in moles/l.
M' = concentration of complex in the titration vessel, near equivalence point, expressed in moles/l.

by Holiday and Sutton.[16] The spectrograms obtained from it are linear in wave number ($\bar{\nu}$) and optical density (D), as shown in Figures 1 to 6, which are direct tracings of the records after correction for instrumental zero errors. Important features of the spectra listed in Table III were checked manually on a Unicam SP.500 single-beam spectrophotometer.

Observations on (II) in the ultra-violet were made difficult by the sensitivity of the compounds to traces of water in the solvent. Satisfactory results were obtained, however, by using a variable-length cell. It was

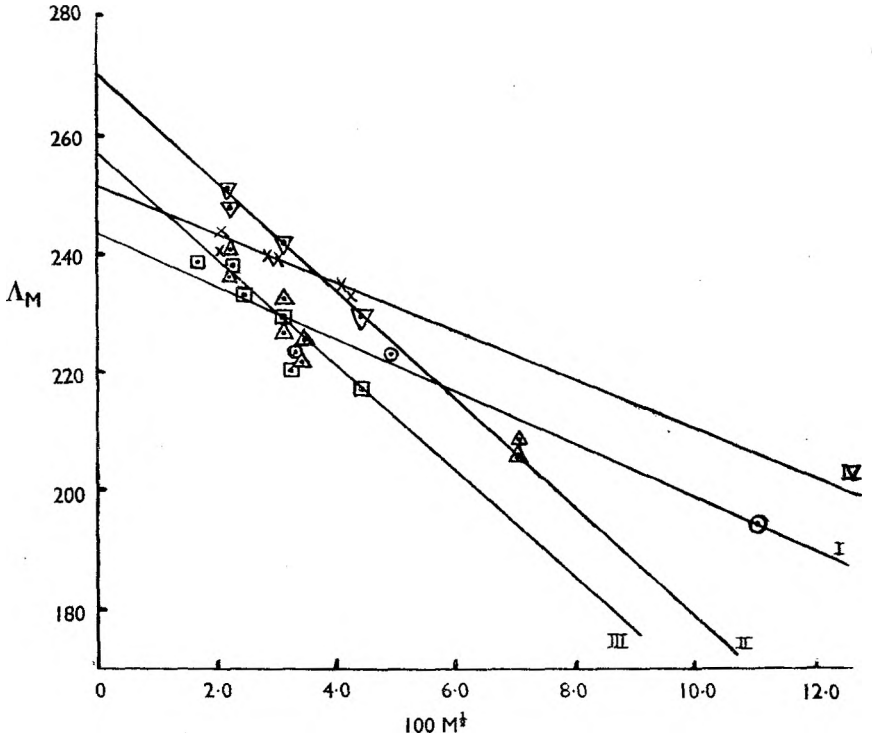


FIG. 8.

○ Pyridine (I)
▽ 1-methylbenziminazole (II)
△ 1-ethylbenziminazole (not graphed)
□ 1 : 2′-hydroxyethylbenziminazole (III)
× 1 : 5-dimethylbenziminazole (IV)

thus possible to determine the absorption spectra of the blue solutions in the visible at long path lengths, and then to reduce the path length for ultra-violet determinations without further dilution of the solutions. By working in this way it was possible to confirm that the blue solutions of (II) had not decomposed before absorption in the ultra-violet had been determined.

Effect of cyanide on the cobaltic complexes (IV). Addition of cyanide to the cobaltic complexes (IV) led to rupture of the benziminazole-cobalt co-ordination linkage with formation of $K_3Co(CN)_6$ (*cf.* Pt VI[1]).

SUMMARY AND CONCLUSIONS

1. The preparation of some authentic benziminazolo-cobaltous and -cobaltic co-ordination compounds has been effected.

2. Their spectroscopic study has proved the validity of the criteria [(i) and (ii), p. 448] employed in Part VI[1] to established the existence of the benziminazole-cobalt co-ordination linkage in vitamin B_{12}.

It is a pleasure to acknowledge the encouragement of the Directors of The British Drug Houses, Ltd., in this work.

REFERENCES

1. Beaven, Holiday, Johnson, Ellis and Petrow, *J. Pharm. Pharmacol.*, 1950, **2**, 944.
2. Fyfe, *Nature, Lond.*, 1952, **169**, 69.
3. Davis, Mamalis, Petrow and Sturgeon, *J. Pharm. Pharmacol.*, 1951, **3**, 420.
4. Reitzenstein, *Liebigs Ann.*, 1894, **282**, 267.
5. Hantzsch and Schlegel, *Z. anorg. Chem.*, 1927, **159**, 273.
6. Cox, Shorter, Wardlaw and Way, *J. chem. Soc.*, 1937, **1556**.
7. Macwalter and Barratt, *J. chem. Soc.*, 1934, 517.
8. Gròh and Schmidt, *Z. anorg. Chem.*, 1925, **146**, 305; 1927, **163**, 321.
9. Pauling, *Nature of the Chemical Bond*, 1945, p. 254.
10. Meisenheimer and Kiderlen, *Annalen*, 1924, **438**, 238.
11. Ablov, *Bull. Soc. chim. Fr.*, 1936 (5), **3**, 2270.
12. Ablov, *ibid.*, 1937 (5), **4**, 1783.
13. Bailar and Clapp, *J. Amer. chem. Soc.*, 1945, **67**, 171.
14. Beaven, Holiday and Johnson, *Spectrochim. Acta*, 1951, **4**, 338.
15. Shedlovsky, Brown and MacInnes, *Trans. Electrochem. Soc.*, 1934, **66**, 165.
16. Holiday and Sutton, to be published. See also Sutton, *Trans. Soc. Instrument Technology*, 1951, **3**, 157; *Photoelectric Spectrometry Group Bull.*, 1950, **2**, 19.

THE ANTAGONISM AND SYNERGISM OF HISTAMINE AND ANTIHISTAMINES IN MICE

By J. L. Ambrus, E. W. Packman, G. V. Rossi, Clara M. Ambrus and J. W. E. Harrisson (with the assistance of S. E. Levin)

From the LaWall Memorial Laboratory of Pharmacology and Biochemistry, Department of Pharmacology, Philadelphia College of Pharmacy and Science

Received February 14, 1952

Bovet and Waltheret[1] and Halpern and Ducrot[2] were the first to report that although antihistamines protect most species against the lethal effect of histamine; a synergistic effect is observed between these substances in mice. This phenomenon was studied in detail by Mayer and Brousseau,[3] using tripelennamine hydrochloride and diphenhydramine hydrochloride. This study was designed to further investigate the above phenomenon.

Besides the aforementioned toxic effect of histamine, we also used its body-temperature decreasing effect as a means of evaluation, since mice, although highly resistant to the toxic action of histamine, are relatively sensitive to its temperature decreasing effect.[4]

Swiss, albino, female mice of 25 $\pm$ 3 g., of our own colony were used. They were maintained on a Rockland complete rat diet and water (*ad lib.*). The animals were kept in air-conditioned quarters at 21° C. and all work was done at this temperature. Body temperature was measured by the thermoelectric method using copper-constantan thermocouples. Readings were made from a potentiometer when the thermoelectric current was counterbalanced as indicated by a galvanometer. A constant temperature water bath at 37 $\pm$ 0·05° was used for the reference junction. Histamine diphosphate U.S.P. and the following antihistamines were used: β-dimethylaminoethyl benzhydryl ether hydrochloride (diphenhydramine hydrochloride; benadryl); *NN*-dimethyl-*N'*-2-pyridyl-*N'*-*p*-methoxybenzyl-ethylenediamine maleate (mepyramine maleate; neoantergan); 2-(*N*-benzylanilinomethyl)-imidazoline (antazoline; antistine); *N*-dimethylamino-ethyl-phenothiazine hydrochloride (3015RP); *NN'*-dimethyl-*N'*-2-pyrimidyl-*N'*-*p*-methoxybenzylethylenediamine hydrochloride (thonzylamine hydrochloride; neohetramine); *NN*-dimethyl-*N'*-2-pyridyl-*N'*-2-thenyl-ethylenediamine hydrochloride (thenylpyramine hydrochloride; histadyl); 2-methyl-9-phenyl-2:3:4:9-tetrahydro-1-pyridindene (phenidamine; thephorin); *N*-methyl-*N'*-4-chlorobenzhydrylpiperazine hydrochloride (chlorcyclizine; di-paralene); 1-phenyl-1:2-pyridyl-3-dimethylaminopropane (prophenpyridamine; trimeton); *N*-ethylpyrolidinyl-phenothiazine hydrochloride (pyrathiazine hydrochloride; pyrrolazote); 2-(10-phenothiazinyl) *iso*propyltrimethylammonium benzenesulphonate (thiazinamium; 3554RP); *NNN'N'*-tetramethyl-*NN'*-bis (β-(10-phenothiazinyl)) ethyl pentamethylene diammonium dibromide (3550RP); diethylaminocarbethoxy-bi*cyclo*hexyl hydrochloride (33536 Merrell). All

preparations were made with normal saline solution and concentrations were calculated as the base.

In determining acute toxicity antihistamines were injected subcutaneously, followed in 15 minutes by the injection of histamine either subcutaneously, intraperitoneally, or intravenously. The animals were then observed for a period of 24 hours.

In the temperature experiments the normal rectal temperature of mice previously fasted for 24 hours was measured three times within 20 minutes prior to the subcutaneous injection of the histamine and antihistamines. The mean of these readings was considered as the normal rectal temperature. The injection of histamine followed the antihistamine injections by 15 minutes. Temperature readings were taken every 10 minutes during the experimental period.

Toxicity. According to preliminary experiments on about 200 mice the intravenous LD100 of histamine in our strain of mice is 277 mg./kg. and the intravenous LD50 is 192 mg./kg., as calculated according to the method of Behrens.[5] We confirmed the results of Mayer and Brousseau[3] and Table 1 extends the available data as observed by us on some other antihistamines. None of the antihistamines protected mice against an intravenous or intraperitoneal LD100 dose of histamine. When an LD50 dose of histamine was given

TABLE I

Antihistamine injected 15 minutes preceding histamine injection	Dose mg./kg. subcutaneously of antihistamine	Histamine intravenously deaths/animals		Histamine intraperitoneally deaths/animals 1500 mg./kg.
		277 mg./kg. (LD100)	192 mg./kg. (LD50)	
Control	0·0	18/18	8/18	14/15
Mepyramine maleate	0·1	10/10	6/10	
" "	0·5	10/10	4/12	
" "	1·0	10/10	9/10	
" "	2·0	11/12	10/10	
" "	5·0	10/10	9/10	
" "	10·0	8/8	8/8	10/10
" "	75·0	6/6	4/4	
Thonzylamine hydrochloride ..	20·0	10/10		
Phenidamine	20·0	10/10		10/10
Prophenpyridamine	20·0	10/10		10/10
Promethazine hydrochloride ..	20·0	10/10		10/10
Thenylpyramine hydrochloride	20·0	10/10		
Antazoline	20·0	10/10		10/10
Pyrathiazine hydrochloride ..	20·0	10/10		
33536 Merrell	20·0	9/10		
3015 RP	20·0	10/10		4/4
3550 RP	20·0	10/10		
Thiazinamium	20·0	10/10		

after a dose of mepyramine maleate larger than 1 mg./kg. a synergistic effect was evidenced. The tests using subcutaneous doses of histamine (Table II) showed wider variation than the intravenous or intraperitoneal tests. However, it seems that definite protection is afforded by 5 mg. of mepyramine maleate/kg.

Effect on body temperature. Figure I shows the composite results of all experiments using different dose levels of histamine as well as mepyramine maleate. This figure is constructed on temperatures recorded 20 minutes after injection of histamine. Each point represents the

TABLE II

Histamine subcutaneously mg./kg.	No pretreatment deaths/animals	Pretreated with 5mg./kg. of mepyramine maleate 15 minutes before histamine deaths/animals
100	4/10	0/10
200	19/30	0/30
300	6/10	0/10
600	7/10	3/10
1000	12/20	3/20
2000	27/30	19/30
LD50	230 mg./kg.	1560 mg./kg.

average of 8 mice. Figure 2 gives the entire course of the reaction following histamine 50 mg./kg. and varied doses of mepyramine maleate. Similar graphs obtained with other dosage levels of which Figure 1 was constructed are not given because of the limitation of space.

From the data of Figure 1 the following conclusions may be drawn: (1) Small doses of histamine (10 and 25 mg./kg.) and small doses of mepyramine maleate (0·5 and 1·0 mg./kg.) act synergistically or additively. Larger doses of histamine and the same small doses of mepyramine maleate are antagonists; (2) by increasing the dose of mepyramine maleate an increased protection occurs against all doses of histamine and maximal protection is obtained with about 5 mg./kg. of mepyramine maleate; (3) upon further increasing the dose of mepyramine maleate (25 mg./kg.) the protection against all doses of histamine decreases.

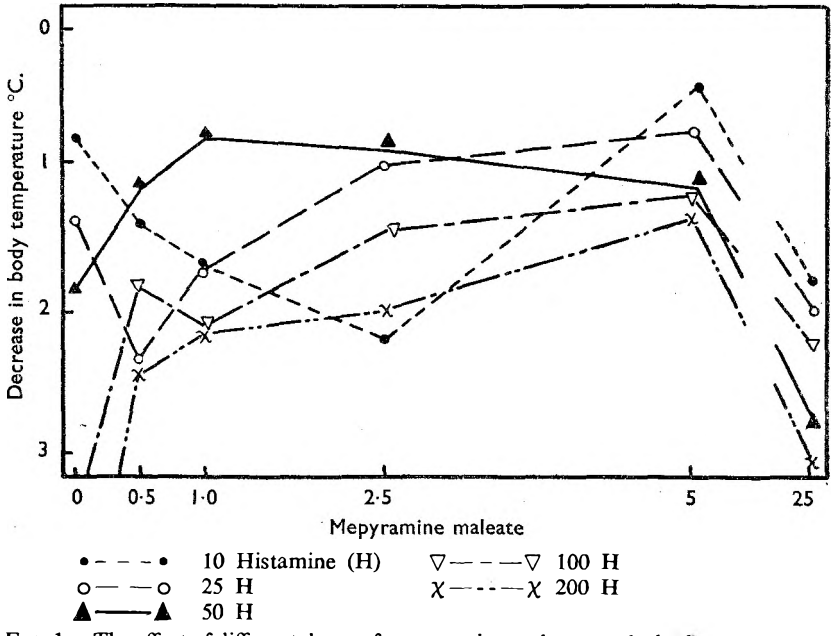


FIG. 1. The effect of different doses of mepyramine maleate on the body temperature-decreasing effect of varied doses of histamine. All doses in mg./kg. of base. Each point is average of 8 mice. Temperature recorded 20 minutes after injection of histamine.

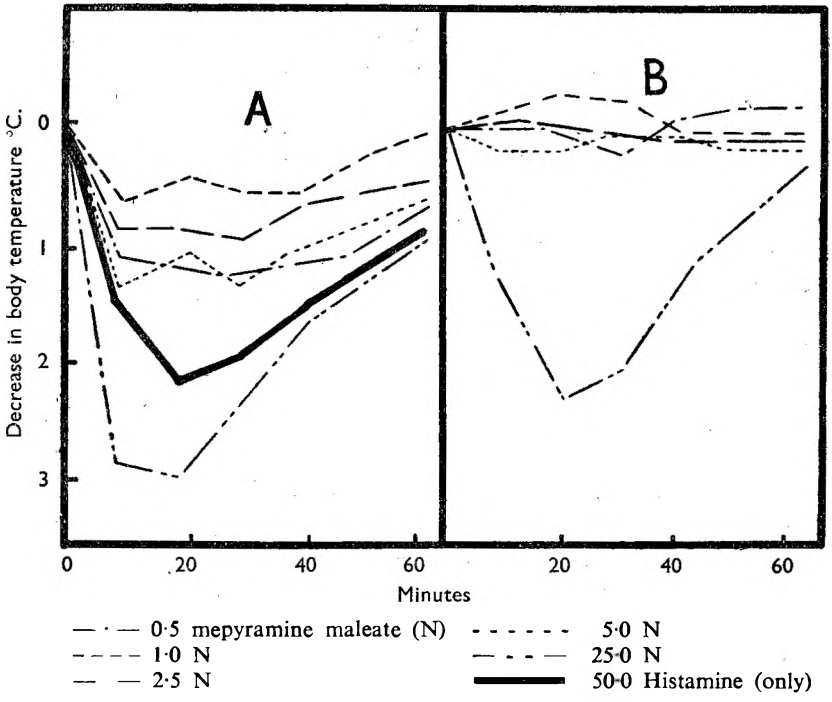


FIG. 2. Effect of histamine and mepyramine maleate on body temperature of mice. All doses in mg./kg. base. Each line is average of 10 mice.

A. 50 mg./kg. Histamine and varied doses of mepyramine maleate.
B. Mepyramine maleate (only).

The body temperature-reducing effect of the different doses of mepyramine maleate alone is set forth in the right portion of Figure 2 wherein it is to be noted that of the doses used only 25 mg./kg. of mepyramine maleate is able to elicit a significant decrease of body temperature.

The observations of other investigators concerning the synergism of histamine and antihistamines in mice from the point of view of intravenous toxicity have been confirmed by our findings. However, a protection was evidenced against toxic doses of histamine given subcutaneously. Loew[6] advanced the hypothesis that because of the great resistance of mice towards histamine, the deciding factor in the death caused by intravenous injections of this substance may be its acidity. As antihistamines are also of acid character, the synergism between these two substances may be interpreted on this basis. It is possible that upon subcutaneous injection, because of the slower absorption and greater efficiency of the neutralizing mechanisms (*a*) acidity plays only a minor role; (*b*) lower levels of histamine are produced against which the protection afforded by the antihistamines is sufficient.

Parfentjev and Goodline[7,8] and Halpern and Roux[9] found that if the sensitivity of mice toward histamine is increased by the injection of

pertussis vaccine, antihistamines exert definite protection against a toxic dose of histamine. Halpern and Wood[10] described that antihistamines protect mice in a similar manner against the lethal effect of histamine, if the histamine sensitivity of these animals is increased by adrenalectomy.

According to our findings, the body temperature-decreasing effect of histamine, an effect to which mice are relatively sensitive, is antagonised by certain doses of antihistamine. Above and below these optimal doses the degree of protection is decreased.

The antagonism or synergism of histamine and antihistamines in mice seems to be related to the high degree of natural resistance of these animals to histamine. Nevertheless there is no difference in principle between the reaction of mice and other species towards these substances.

Summary

1. Mepyramine maleate acts synergistically with toxic doses of histamine upon intravenous injection.

2. 11 other antihistamines failed to protect mice under the same conditions.

3. Of 6 antihistamines tested, none gave protection against toxic doses of intraperitoneally injected histamine.

4. Mepyramine maleate protected mice against subcutaneous toxic doses of histamine.

5. The body temperature-decreasing effect of histamine is antagonised by mepyramine maleate in certain optimal doses, below and above which a synergistic or additive effect, or at least less protection is evidenced.

We are greatly indebted to Dr. Arthur Osol and Dr. W. F. Veraway for their advice and assistance in this study, and to the manufacturers for a generous supply of the drugs used.

References

1. Bovet and Waltheret, *Ann. Pharm. franc.*, 2 (Suppl.), 3.
2. Halpern and Ducrot, *C. R. Soc. Biol. Paris*, 1946, **146**, 361.
3. Mayer and Brousseau, *Proc. Soc. exp. Biol. N.Y.*, 1946, **63**, 187.
4. Harrisson, Rossi, Packman, Ambrus and Ambrus (In the press).
5. Behrens, *Arch. exp. Path. Pharmak.*, 1933, **177**, 379.
6. Loew, *Physiol. Rev.*, 1947, **27**, 542.
7. Parfentjev, *J. Pharmak.*, 1948, **92**, 411.
8. Parfentjev, *Yale J. Biol. Med.*, 1950, **23**, 28.
9. Halpern and Roux, *Semaine Hosp. Paris*, 1950, **26**, 411.
10. Halpern and Wood, *Brit. J. Pharmacol.*, 1950, **5**, 510.

THE STRUCTURE OF THE FLOWER OF *DATURA INNOXIA* MILLER

By T. E. Wallis and S. Rohatgi

From the Museum of the Pharmaceutical Society of Great Britain

Received May 7, 1952

Introduction

The plant, *Datura innoxia*, was first described by Philip Miller[1] in the 8th edition of his *Gardener's Dictionary* in 1768. The plant was confused with other species of *Datura*, particularly *D. metel* Linn., until the matter was clarified by Safford[2] and finally by Timmerman.[3] The confusion, however, still persists and plants of *D. innoxia* Miller, cultivated in Great Britain are frequently erroneously referred to as *D. metel* Linn. Gerlach[4] gives a general morphological description of the plant, including a brief reference to the flower, as follows: "The flowers are white, funnel-shaped, borne in the forks of the branches, about 8 cm. long. The expanded limb of the corolla is 10-angled and the corolla is 10-toothed. The flowers open in the evening for the attraction of night flying moths and emit a powerful fragrance." Since no complete description of the gross morphology is available and the histology of the flower has not hitherto been investigated, a detailed study of the flower and its histology has now been made and the following illustrated description takes the form of a comparison with the flower of *D. stramonium* Linn. described previously.[5]

Material

For the purpose of this work, the following specimens were used:—

(1) Flowers collected at the Ayurvedic Garden, Kanpur, India, during June–July, 1949.
(2) Flowers collected at Dartford during July, 1949, grown from seeds originally obtained from India approximately in 1932.
(3) Flowers collected at the garden of the Institute of Pharmacy, Cairo, grown from seeds received from India.

Gross Morphology

The influorescence of *D. innoxia* is of the same type as that of *D. stramonium*; the flower of *D. innoxia*, although much larger than that of *D. stramonium*, is similar in general form and structure to the latter and stands erect upon the plant.

Calyx (Fig. 1, A). The calyx differs from that of *D. stramonium* in being more enlarged towards the base and is cylindrical-ovoid; also it has no projecting ridges over the midribs making it less rigid than the calyx of *D. stramonium*; the lobes measure about 7 to 16 mm. and the tube about 4 to 9 cm. in length. The calyx, although fugaceous, does not separate by abscission; it remains for a longer period and drops off the persistent part by drying and withering away.

Corolla (Fig. 2, A). The corolla is much larger than that of *D. stramonium* and has 10 lobes; the midribs run into the 5 short acuminate lobes which have filiform twisted tips; the remaining 5 alternating lobes are larger, broadly triangular and are folded inwards in the plicate and twisted corolla. The tube measures approximately 12 to 16 cm. in length and the lobes are about 1 cm. long.

Andrœcium (Fig. 2, A). The stamens are very similar to those of *D. stramonium*, except in size; the free part of the filament measures approximately 5 to 6 cm. in length, the adherent part about 9 to 10 cm.; the anther lobes are about 11 to 12 mm. long and 3 mm. wide.

Gynœcium. The *ovary* is similar to that of *D. stramonium* but is larger in size and the spines are longer. The *style* is about 15 cm. in length; the *stigma* (Fig. 2E) has a papillose receptive surface in the form of a band passing over its apex and formed by a proliferation of the central pith as in *D. stramonium*.

Fruit. The fruit is similar in shape to, but much larger than, that of *D. stramonium*; the persistent part of the calyx forms a wider flange which is only slightly recurved. The entire calyx, before dehiscence, bulges out, due to the growth of the fruit and the spines; it remains covering the fruit during the early part of its development until nearly full grown, as also does the withered corolla which hangs on by its vascular tissue and by support from the withered style.

The *seeds* are yellowish to dark-brown, ear-shaped to sub-reniform about 4 to 5 mm. long, 3 to 4 mm. broad, 1 to 1·5 mm. thick and flattened; the surface has uneven shallow depressions and is finely pitted; the margin is somewhat wavy and the edge exhibits a triple ridge.

Histology of the Calyx

Outer (abaxial) epidermis. The cells are similar in form to the corresponding cells of *D. stramonium* but are somewhat larger in size; on the lobes (Fig. 1, Ao) the cells measure approximately L and T*= 30 to **45** to **60** to 135 μ and R = 15 to **24** to 36 μ; at about the middle of the tube they measure approximately L and T = 15 to **24** to **60** to 140 μ and R = 15 to **20** to 30 μ and at the base of the tube (Fig. 1, Co) L and T = 9 to **30** to **45** to 60 μ and R = 15 to **21** to 45 μ.

Stomata, of the cruciferous (anisocytic) type, are frequent over the entire epidermis. The following kinds of *trichomes* are present: *small glandular* trichomes with a multicellular head; numerous *long glandular* trichomes with a unicellular head similar to the typical trichomes of the foliage leaf of *D. innoxia*; also tapering *covering* trichomes more slender than those of *D. stramonium*; the proportion in which these different types of trichomes occur varies in different samples. There are present in many cells of the epidermis especially along the midribs, some exceptional crystals which have rounded ends and a ragged central core (Fig. 1, Bv, c_1).

* When recording measurements, the letters L, T and R are used to indicate measurements in a longitudinal, tangential and radial direction respectively, the directions having reference to the axis of the relevant plant member.

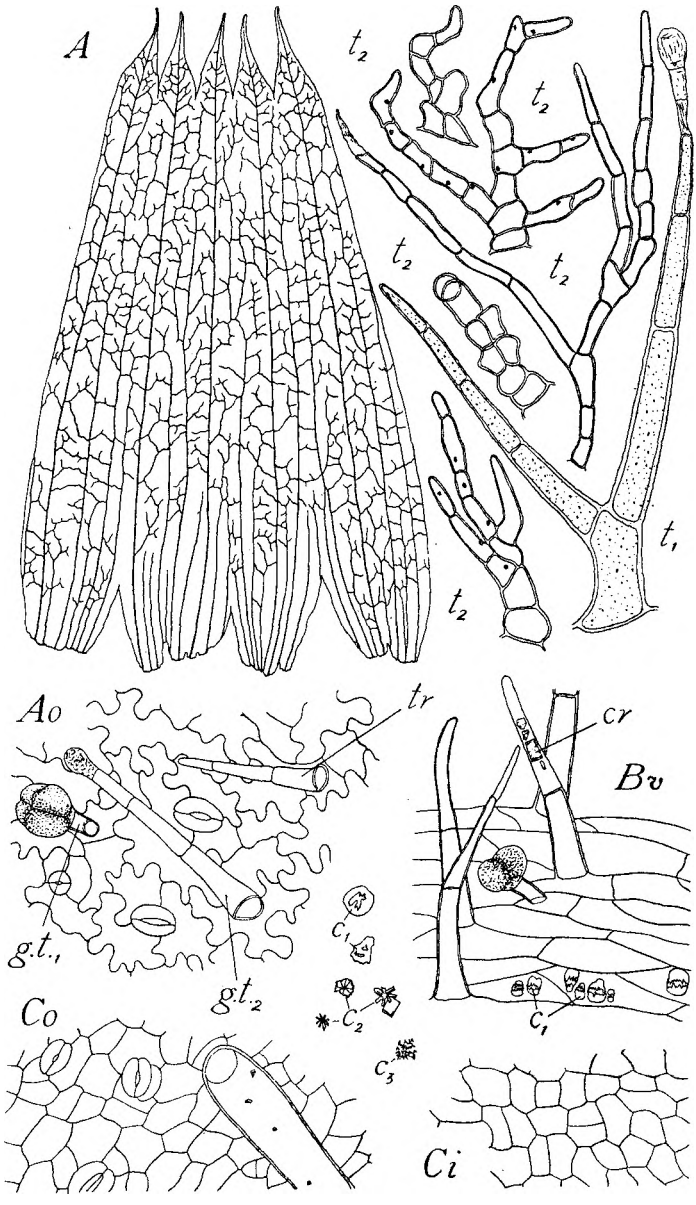


Fig. 1. *D. innoxia* Miller, Calyx. A, calyx spread out showing the form and the position of the widest part at about one-tenth of the distance from the base, and also the venation (× 1). Ao, outer epidermis on the lobe; Bv, outer epidermis on midrib at the middle of the calyx; Co, outer epidermis at the base; Ci, inner epidermis at the base. c_1, peculiar crystals with a ragged core; c_2, crystal aggregates from the mesophyll of the calyx; c_3, micro-sphenoidal crystal-sand from an idioblast; *cr*, crystal in trichome; $g.t._1$, clavate glandular trichome; $g.t._2$, long glandular trichome; t_1, warty branched trichome from the edge of the calyx lobes; t_2, peculiar simple and branched trichomes from the edge of the calyx lobes; *tr*, covering trichome. All × 160.

Inner (*adaxial*) *epidermis*. The cells are similar to the corresponding cells of *D. stramonium*; they measure approximately L and T = 15 to **36** to **60** to 150 μ and R = 18 to 33 μ on the lobes and L and T = 9 to **24** to **60** to 75 μ and R = 15 to 24 μ at the base of the tube (Fig. 1, Ci). *Stomata* are common and more frequent on the tube. *Trichomes*. All three types of trichomes are present in varying proportions and frequency in different samples, but are much less frequent than on the outer epidermis.

Trichomes. (*a*) The *small glandular* trichomes (Fig. 1, Ao, g.t.$_1$) with a unicellular stalk and commonly a 5-celled globular or clavate head, having 4 cells arranged vertically over a platform cell, measure approximately: stalk, 24 to **30** to 36 μ in length and head, 30 to **36** to 45 μ in diameter.

(*b*) *Long glandular* trichomes (Fig. 1, Ao, g.t.$_2$) having a 2- to 5-celled slender uniseriate stalk and an elongated globular or ellipsoid unicellular head, such as are typical of the foliage leaf of *D. innoxia*, are common; they measure about 28 to **280** to 560 μ in length, 15 to **30** to 45 μ in diameter at the base, the diameter of the head being 15 to 21 μ.

(*c*) *Covering* trichomes (Fig. 1, Ao, Bv, tr) are slender and tapering with occasional prismatic and odd-shaped crystals in some cells (Fig. 1, Bv, cr); they measure about 15 to **30** to 90 μ in length, the diameter at the base being 15 to **30** to 45 μ; some trichomes on the lobes have a warty cuticle.

(*d*) *Characteristic covering* trichomes on the edge of the lobes resemble those of *D. stramonium* but often have 2 and sometimes 3 or 4 branches (Fig. 1, t_2); in some samples, however, they are rare and are replaced by conical warty covering trichomes which are sometimes branched (Fig. 1, t_1).

The *Mesophyll* is similar to that of *D. stramonium*.

Venation (Fig. 1, A). The venation is similar to that of the calyx of *D. stramonium* and the midrib shows no particular difference in transverse section except for the absence of the projecting ridge.

Histology of the Corolla

Outer (*abaxial*) *epidermis*. The cells on the lobes resemble the corresponding cells of *D. stramonium* both in form and dimensions (Fig. 2, Ao); they differ, however, in having usually straight or slightly curved anticlinal walls. Certain epidermal cells on the lobes have the outer cell-wall collapsed and appear as brownish patches in surface view (Fig. 2, Ao, c_1). The cells on the tube (Fig. 2, Bo, Co and Eo) show little variation from the corresponding cells on the corolla of *D. stramonium* except that they are larger in size; at a point near the middle of the tube, they measure approximately L = 180 to **255** to 330 μ, T = 15 to **18** to 33 μ and R = 18 to **21** to 30 μ; at the base of the tube, the measurements are L = 150 to **210** to 300 μ, T = 15 to **30** to 45 μ and R = 18 to 30 μ.

Stomata are very rare or absent on the lobes but are frequent on the tube except towards the base; they are usually cruciferous (anisocytic). Clavate glandular *trichomes* are present in small numbers as in

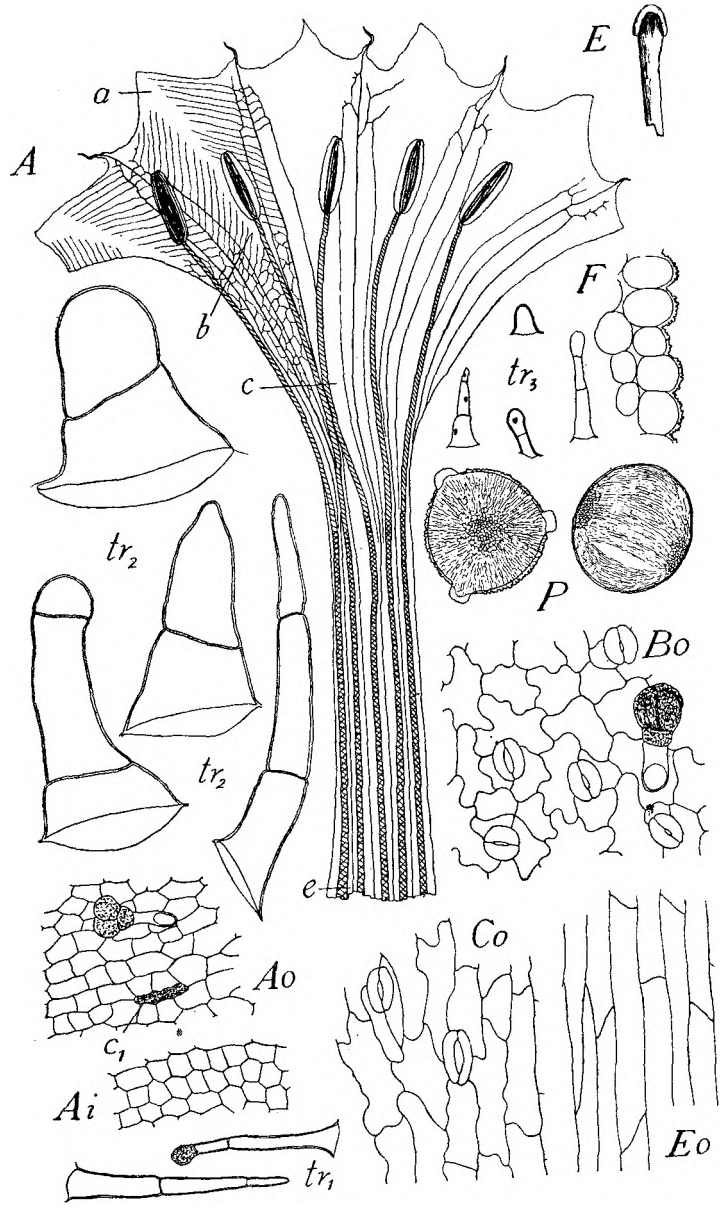


Fig. 2. *D. innoxia* Miller. A, corolla, with epipetalous stamens, spread out, showing venation of one petal (× 1); Ao, outer epidermis of the corolla at *a* (as marked on A); Ai, inner epidermis of the corolla at *a*; Bo, Co and Eo, outer epidermis of the corolla at position *b*, *c* and *e* respectively; E, stigma and upper part of the style (× 2); F, epidermis of style in transverse section showing striated cuticle; P, pollen grains in polar and side view (× 280). c_1, collapsed cell; tr_1, trichomes from the edge of the corolla lobes; tr_2, trichomes on the adnated part of the filaments; tr_3, trichomes from the ovary wall. All × 160 unless otherwise specified.

D. stramonium; slender covering trichomes and typical long glandular trichomes as found on the calyx, are present along the edge of the lobes (Fig. 2, tr_1).

Inner (adaxial) epidermis. The cells in the different regions are in general similar to the corresponding cells on the corolla of *D. stramonium.* On the lobes (Fig. 2, Ai), the cells measure approximately L and T = 15 to **21** to **33** to 45 μ and R = 15 to **21** to 24 μ; the cells on the throat of the tube often bear small papillæ as in the case of *D. stramonium.* The cells on the tube are much larger than those of *D. stramonium*; on the middle of the tube, they measure approximately L = 210 to **270** to 360 μ, T = 15 to **21** to 30 μ and R = 15 to **24** to 30 μ; at the base L = 90 to **120** to 180 μ, T = 21 to 30 μ and R = 18 to 27 μ. *Stomata* are usually absent but occur quite frequently in the tube in certain samples. *Trichomes* are usually absent, but in some samples, some large conical or short covering trichomes with a rounded apex are present in the part of the corolla tube where the filaments are adnated.

Mesophyll. The mesophyll shows no noticeable difference from that of *D. stramonium* in either arrangement or contents of the cells.

Venation. The structure of the midrib in transverse section are similar to that of *D. stramonium.* The venation, however, differs slightly in the upper part of the corolla, since the numerous straight lateral branches from the apical part of the midrib run into the broader intermediate lobes and terminate in the median line of those lobes.

Histology of the Stamens

The *filament* is similar to that of *D. stramonium* except that the epidermal cells are longer. The numerous *trichomes* on the adnated part of the filament resemble those of the adjacent part of the corolla, but are larger in size (Fig. 2, tr_2), their dimensions being often twice or rather more than twice those of the corresponding trichomes of *D. stramonium.*

The *anther* resembles that of *D. stramonium*, the cells being slightly larger in comparison. The fibrous layer is lignified, as in *D. stramonium.*

The pollen grains (Fig. 2, P) are spherical in polar view, but slightly oval in outline when viewed laterally; they have three small indistinct germinal furrows and three pores about 12 to 24 μ in diameter; the grains measure about 54 to **63** to **66** to 69 μ in diameter when mounted in lactophenol and warmed; they measure about 69 to **78** to **81** to 84 μ after boiling in solution of chloral hydrate. The exine is striate, the markings appearing as short, wavy, longitudinal ridges except at the poles where they reticulate. The grains contain oil globules and minute starch grains which measure up to 3 μ in diameter after treatment with a solution of chloral hydrate and iodine.

Histology of the Carpels

The *ovary* shows no appreciable difference from that of *D. stramonium.* The trichomes on the spines (Fig. 2, tr_3) are mostly covering and less of the glandular type; in certain specimens, trichomes are rare or absent.

Style and Stigma. The cuticle on the style is more markedly striate

longitudinally as compared with that of *D. stramonium* (Fig. 2, F). In addition to the two main bundles, one on either side of the oval region of small cells, a few small bundles occur in rare instances.

Powdered Flowers and Summary

Dried flowers of *D. innoxia* when reduced to No. 85 powder, yield a greyish-brown powder with a marked, heavy typical odour which quickly distinguishes it from the powder of the flowers of *D. stramonium*. The powder was examined after mounting it in various reagents and the characters which would help in identification are listed below in order of importance; they form a summary of the most diagnostic histological characters.

1. Pollen grains, which are not very frequent, measuring 54 to 69 μ in diameter in lactophenol and 69 to 84 μ in chloral hydrate solution and having the characteristic streaky striations on the exine, typical of the pollen of *D. innoxia*.
2. A few long glandular trichomes with a unicellular ovoid head characteristic of *D. innoxia*; the trichomes are usually very much broken and difficult to recognise.
3. Fragments of the fibrous layer which often give a slight reaction for lignification.
4. Fragments of the calyx, with small crystals present as prisms or aggregates; but these are of minor importance.

Key for Identification of the Powdered Flowers of the Medicinal Solanaceous Plants

A. Pollen grains, spherical, about 40 to **55** to 60 μ in diameter when mounted in chloral, with three distinct pores and three well-marked long germinal furrows. *Atropa belladonna* or *Hyoscyamus* spp.

(1) Cuticle of calyx without striations. Trichomes; large multicellular ovoid heads (about 10 to 30 cells) of glandular trichomes. Corolla; epidermal cells of the lobes with deep infoldings, cells of the inner epidermis at the base of the tube with pitted anticlinal walls. Pollen grains, exine irregularly pitted. *Hyoscyamus niger*.

(2) Cuticle of calyx striate—

(*a*) Trichomes, often branched, with unicellular globular glandular heads, basal cells covered with transversely striated cuticle. Pollen grains, exine with pits often in rows of 3 to 8. *Hyoscyamus muticus*.

(*b*) Trichomes with unicellular globular or uniseriate glandular head of 2 to 4 cells. Corolla; epidermal cells of the lobes papillose, yielding a pink tinge in chloral; sub-rectangular pits on the cells of the outer epidermis at the base. Pollen grains, exine with pits arranged in long longitudinal rows. *A. belladonna*.

B. Pollen grains, spherical to sub-spherical, about 50 to **65** to **80** to 85 μ in diameter when mounted in chloral hydrate solution, with 3 pores

varying in distinctness and very small furrows, sometimes apparently absent. *Datura* species.

(1) Pollen grains, exine irregularly warty. Trichomes warty. *D. stramonium* and *D. tatula.*
   (*a*) Fragments of corolla and anther yield a pink colour in chloral mounts. *D. tatula.*
   (*b*) No colour patches in chloral mounts. *D. stramonium.*

(2) Pollen grains, exine with longitudinal streaky markings except at the poles. Trichomes, rather slender with unicellular ovoid glandular heads. *D. innoxia.*

Note on Making Microscopical Preparations of Pollen Grains

When pollen grains are mounted in solution of chloral hydrate, the mountant has a remarkable action upon many of the grains if the preparations are boiled or are heated to near boiling for a short time. Since it is customary to boil mounts of powdered drugs in chloral hydrate in order to clear the particles sufficiently, the changes brought about must be noted.

These observations were first made when working with pollen of *D. innoxia.* The grains are first slightly swollen, the pores becoming more distinct, and then the contents escape through a pore, which may be somewhat enlarged by splits in the exine. When specimens which have been stored in solution of ethanol and glycerin are used, the liberated contents still enclosed by the intine, have the form of the pollen grains and show the position of the 3 pores as slight rounded projections; the spherical mass also becomes somewhat enlarged and the surface is smooth, the slightly granular contents being visible through it; if the outline is sharply focussed, no wall is visible. When dried specimens are used, the contents come out in irregular masses. At the same time, the shells which are composed of the exine and are sometimes ruptured and often shrunk, simulate smaller immature pollen grains. A similar phenomenon is observed with the pollen of *D. stramonium* but the action is slower. Mounts made by boiling in lactophenol behave similarly, but the action is less vigorous than with chloral hydrate.

It is therefore necessary to use care in making measurements, so as to avoid errors due to recording the diameters of the extruded interiors when using pickled specimens and of empty shells when using either the pickled or the dried specimens; the slide should also be allowed to cool before the measurements are made.

Pollen of species of *Hyoscyamus* behaves similarly but the action of the mountant is slower than with species of *Datura*, so that the majority of the grains give normal measurements. To what extent these changes occur with pollen generally, it has not been possible to investigate.

References

1. Miller, *The Gardener's Dictionary*, ed. VIII, 1768.
2. Safford, *J. Wash. Acad. Sci.*, 1921, II, 173.
3. Timmerman, *Pharm. J.*, 1927, **118**, 739. (*D. innoxia.*)
4. Gerlach, *Economic Botany*, 1948, **2**, 4, 440.
5. Wallis and Rohatgi, *J. Pharm. Pharmacol.*, 1952, **4**, 243.

THE SPECTROPHOTOMETRIC DETECTION AND ESTIMATION OF PROCAINE HYDROCHLORIDE IN AQUEOUS SOLUTION

By A. I. BIGGS

From the Department of Chemistry, University of Malaya and the Government Department of Chemistry, Singapore

Received May 9, 1952

INTRODUCTION

THE identification and estimation of procaine hydrochloride in aqueous solution can be accomplished by well established methods.[1] Nevertheless the analysis is a lengthy procedure and limited in accuracy by the amount of material available for examination, and samples, such as those from anæsthesia cases where an enquiry is instituted into the state of the anæsthetic, are often insufficient for analysis by the recognised method.

We have described[2] an investigation of absorption spectra in the ultra-violet as an analytical method for substances normally encountered in toxicological work. Elvidge[3] has studied the absorption spectra of procaine hydrochloride; we have redetermined the absorption spectra of this substance and found it to possess pronounced and characteristic absorption bands in the ultra-violet region, suitable for its identification and estimation in aqueous solution. In addition the absorption spectra of solutions of partly decomposed procaine hydrochloride have been examined and the degree of decomposition determined spectroscopically.

EXPERIMENTAL

The absorption spectra in these experiments were determined on a Beckmann spectrophotometer as described in another paper.[2]

Procaine hydrochloride B.P. was dissolved in distilled water and diluted to give a concentration of 0·002 per cent. w/v. The absorption spectrum in the range 2000Å to 3500Å was determined and the results obtained are shown as curve 1 in Figure 1. Several different samples of procaine hydrochloride were examined in this way and the spectral absorption curves were found to be practically identical.

Aqueous solutions of procaine hydrochloride were examined over the concentration range 0·002 to 0·00025 per cent. w/v and Beer's Law was found to hold as the extinction coefficients given in Table I show.

TABLE I

Concentration per cent.	Extinction coefficients			
	2090 (min.)	2210 (max.)	2400 (min.)	2900 Å (max)
0·002	200	320	58	670
0·001	218	315	42	660
0·0005	220	316	60	668
0·00025	236	320	60	668

Procaine hydrochloride was dissolved in water to give a solution of 0·1 per cent. w/v and the solution was divided into 3 parts. The first was examined immediately, the second was ampouled and kept in a refrigerator for 3 months and the third was ampouled and left in diffused sunlight for

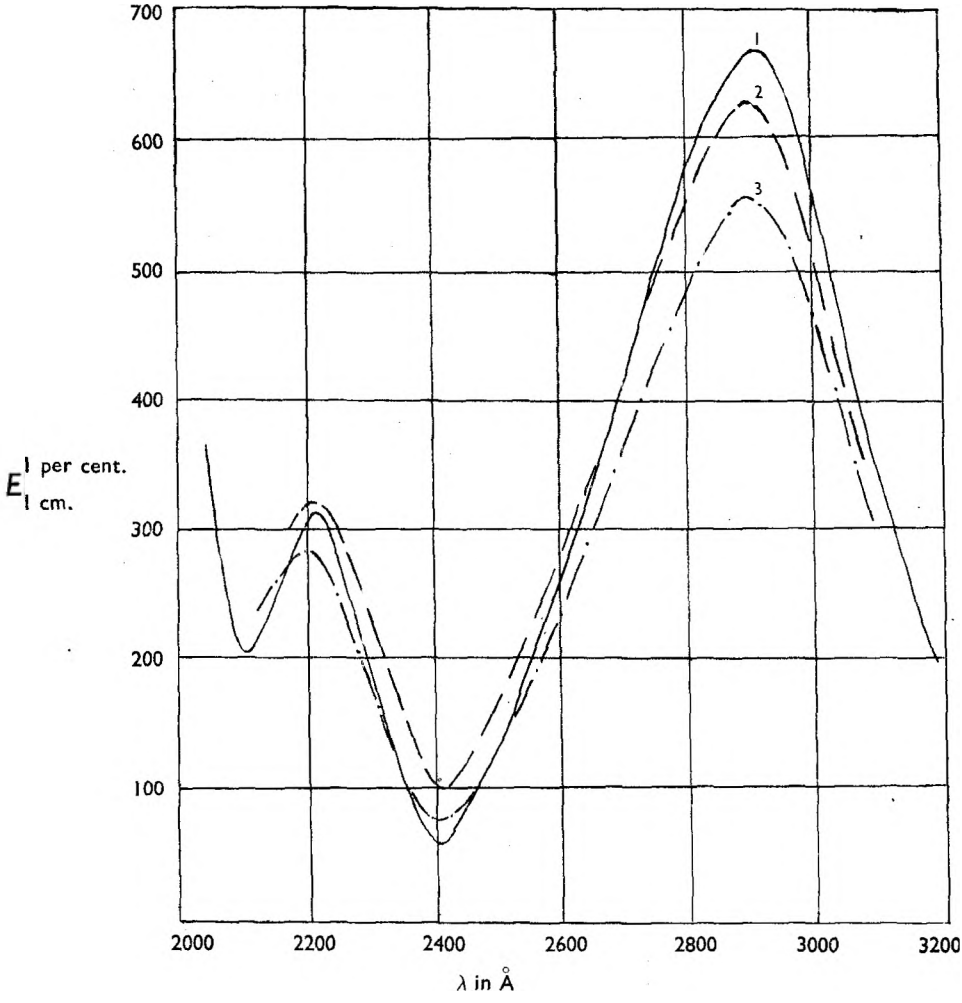


FIG. 1. 0·002 per cent. w/v of procaine hydrochloride in aqueous solution.
1. —— solution freshly prepared.
2. — — solution left in refrigerator for 3 months.
3. ·—·—·—· solution left in diffused sunlight for 3 months.

the same time at room temperature (about 27° C.). After this period the second and third batches were diluted to 0·002 per cent. w/v and examined spectrophotometrically. The absorption curves are shown in Figure 1.

As an additional check on the decomposition of procaine hydrochloride a 0·1 per cent. w/v aqueous solution was ampouled and autoclaved under

the following conditions:—(*a*) 40 minutes at 10 lb. steam pressure; (*b*) 1 hour at 10 lb.; (*c*) 2 hours at 10 lb.; (*d*) 40 minutes at 15 lb. After autoclaving, these solutions were protected from light until ready for examination. The solutions were then diluted to 0·002 per cent. w/v and examined spectrophotometrically: the results are shown in Figures 2 and 3.

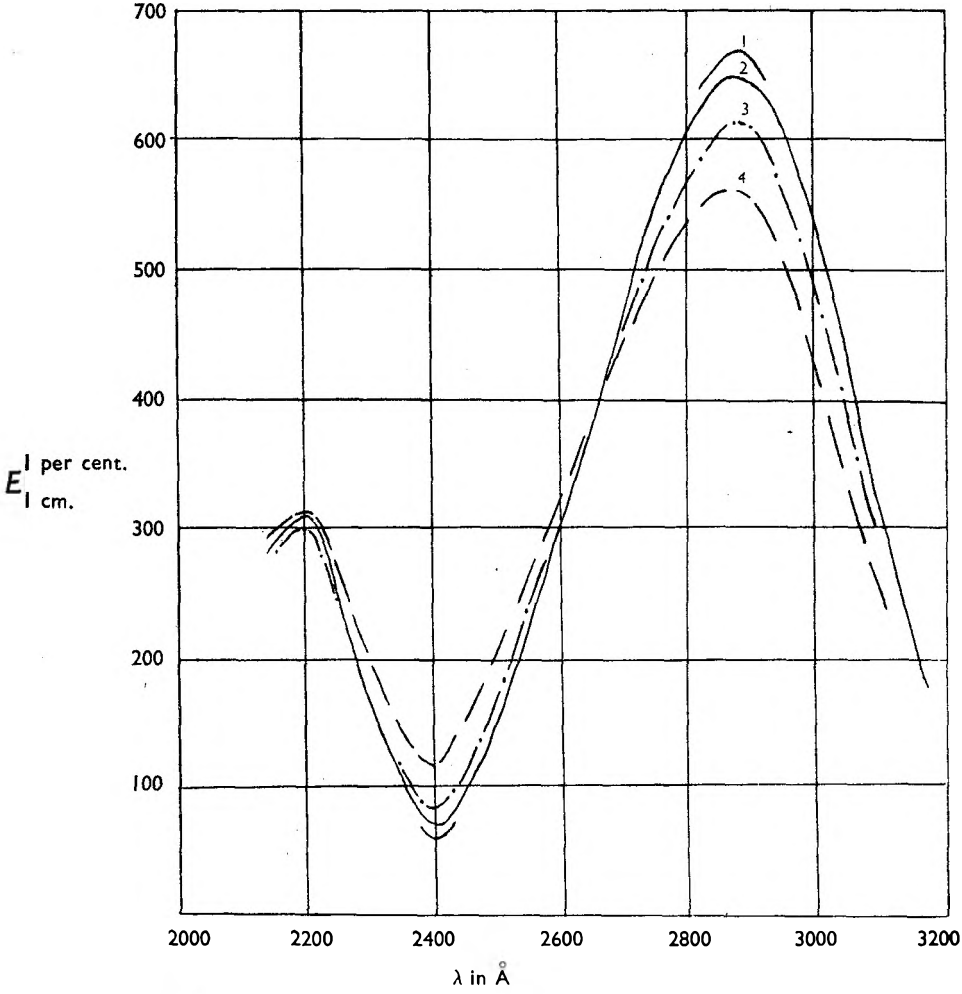


FIG. 2. 0·002 per cent. w/v of procaine hydrochloride in aqueous solution.
1. ——— solution freshly prepared (portion of curve).
2. — — — solution autoclaved at 10 lb. steam pressure for 40 minutes.
3. —·—·—· solution autoclaved at 10 lb. steam pressure for 1 hour.
4. — — — solution autoclaved at 10 lb. steam pressure for 2 hours.

DISCUSSION

The absorption curve for procaine hydrochloride reveals a pronounced absorption band with maxima at 2900Å and 2210Å and minima at 2400Å

and 2090Å. The $E^{1\ \text{per cent.}}_{1\ \text{cm.}}$ extinction coefficients at 2900Å and 2210Å are 670 and 320 respectively. Both these values are extremely high and together with the shape of the absorption curve and its well defined maxima and minima are distinctive of procaine hydrochloride and eminently

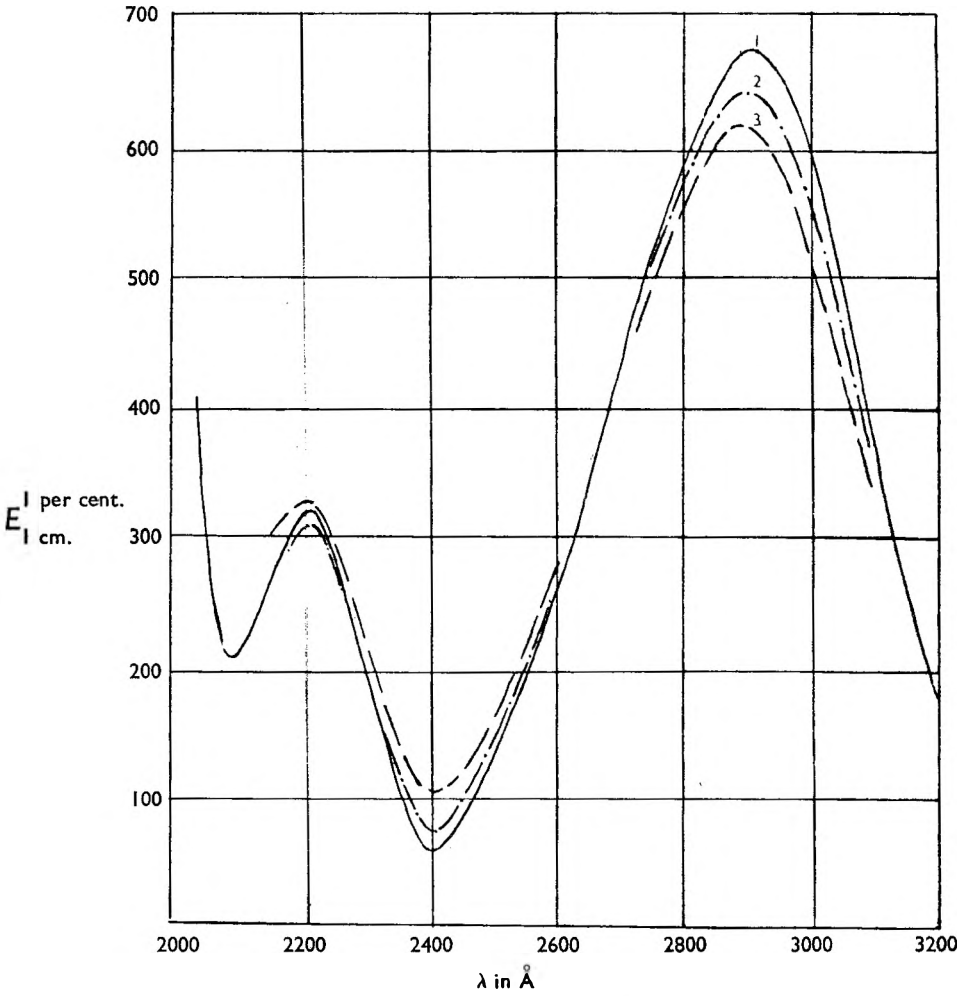


FIG. 3. 0·002 per cent. w/v of procaine hydrochloride in aqueous solution.
1. ——— solution freshly prepared.
2. —·—·—· solution autoclaved at 10 lb. steam pressure for 20 minutes.
3. –– — — solution autoclaved at 15 lb. steam pressure for 40 minutes.

suitable for the identification of this substance. This is particularly true of the maxima at 2900Å which shows a progressive diminution in $E_{max.}$ with increasing severity of treatment of the solution. The fact that Beer's Law is applicable at least between 0·002 and 0·00025 per cent. w/v, suggests that the method has quantitative use.

The absorption curves in Figure 1 show the effect of time and atmospheric conditions on ampouled samples of aqueous procaine hydrochloride solutions. The solution which was kept in the dark and at a temperature of approximately 40° F. was found to be slightly decomposed and that which had been exposed to diffused sunlight at room temperature had decomposed even further. Use was made of the $E_{max.}$ at 2900Å to calculate the degree of decomposition, and the analytical results are shown in Table II.

TABLE II

DECOMPOSITION OF PROCAINE HYDROCHLORIDE SOLUTION

Substance	Decomposition per cent.
Left in refrigerator for 3 months	5·2
Left in open for 3 months	17·0

The absorption curves in Figures 2 and 3 show the results obtained when aqueous solutions of procaine hydrochloride were subjected to varying conditions of steam pressure and time of autoclaving. They indicate that excessive steam pressure and autoclaving times lead to a progressive amount of decomposition of the procaine hydrochloride. The extent of decomposition estimated from the $E_{max.}$ values at 2900Å is recorded in Table III.

TABLE III

EFFECT OF AUTOCLAVING CONDITIONS ON DECOMPOSITION

	Decomposition per cent.
40 minutes at 10 lb. steam pressure	3·7
1 hour at 10 lb.	8·1
2 hours at 10 lb.	15·6
40 minutes at 15 lb.	8·1

As a final experiment a sample of procaine hydrochloride which had been stored in the solid state for 6 years in the tropics was made up to an 0·002 per cent. w/v solution. The following extinction coefficients were measured and the purity of the sample estimated from the values of each maximum:—$E^{1\ per\ cent.}_{1\ cm.}$ at 2210Å, 287 = 90 per cent. $E^{1\ per\ cent.}_{1\ cm.}$ at 2900Å, 595 = 89 per cent.

These experimental data indicate that procaine hydrochloride may be both identified and estimated spectroscopically in amounts as small as 0·06 mg. The same sample may be used for both identification and estimation, the method is quick and reliable and should be of considerable value in analysing small quantities of dilute procaine hydrochloride solutions in water.

SUMMARY

1. Aqueous solutions of procaine hydrochloride both pure and partially decomposed, have been examined spectrophotometrically and the experimental absorption bands recorded.

2. The use of the absorption bands occurring in the far ultra-violet region of the spectrum for identifying and estimating this substance has been discussed.

I am indebted to Professor R. A. Robinson for his interest and assistance in this work and I also wish to thank Mr. A. W. Burtt, Chief Chemist, Singapore, for his help.

REFERENCES

1. *Methods of Analysis of the Association of Official Agricultural Chemists*, Sixth Edition, 697.
2. Biggs, *J. Pharm. Pharmacol.*, 1952, **4**, in the press.
3. Elvidge, *Quart. J. Pharm. Pharmacol.*, 1940, **13**, 219.

THE ULTRA-VIOLET SPECTROPHOTOMETRIC ASSAY OF ALKALOIDS

PART I. STRYCHNINE IN THE PRESENCE OF BRUCINE

BY RABINDRA NATH BHATTACHARYA AND ANIL KUMAR GANGULY
From the Bengal Immunity Research Institute, Calcutta

Received April 28, 1952

SALTS of strychnine are often used parenterally in a very low concentration. The injection prepared from the hydrochloride, is now included in the British Pharmacopœia, 1948. Apart from its use as a single salt, strychnine in combination with other alkaloids and/or other metallic salts is often prescribed and methods of assay for such preparations are required. Following the method of Brownlee[1] work was being carried out in this Institute on the estimation of strychnine in various mixtures. In the course of these studies it was considered advantageous to estimate the alkaloid by ultra-violet spectrophotometry. This work is in progress; but in the meantime a paper by Ridi and Khalifa[2] on the assay of strychnine in galenicals and other preparations came to our notice and we consider it desirable to note down the observations on the same problem so far recorded in our laboratory.

Strychnine or brucine can be estimated when present alone, or after solvent extraction when present together, by titration with standard perchloric acid in acetic acid medium using crystal violet as indicator.[3] Titration methods require somewhat large quantities of bases for accurate determination, and when more than one is present they need to be separated. The accuracy of the results obtained depends mainly on the efficiency of separation and extraction.

EXPERIMENTAL

Pure strychnine and brucine obtained by repeated recrystallisation were used. Dilute standard solutions of the bases were prepared in 0·001 N sulphuric acid. Percentage transmission and optical density of the solutions and their mixtures were determined against 0·001 N sulphuric acid. All dilutions of the stock solutions were made in 0·001 N sulphuric acid: A Beckman spectrophotometer, Model DU, calibrated against hydrogen lines was used for the measurements. Fused silica cells of light path 1·004 cm. were used in all cases.

RESULTS AND DISCUSSION

Figure 1 gives typical transmission-wavelength curves for pure strychnine and pure brucine in dilute sulphuric acid. Strychnine shows maximum absorption in the curves at 252 to 254 mμ while brucine shows maximum absorption at 262 mμ and another transmission minimum at 300 mμ. The wavelengths correspond to those obtained by previous workers.[2,4] Ridi and Khalifa[2] record the wavelengths as 254 mμ,

264 mμ and 301 mμ, although their experiments were made in a different solvent, ethanol. A comparison of the curves obtained by them with those in Figure 1, shows no significant shift with change of solvent from dilute sulphuric acid to ethanol. It is to be observed from Figure 1 that at 300 mμ, there exists a significant absorption by strychnine. A correction is required, or compensation must be made in the method for this absorption by strychnine in solution, when estimating brucine from absorption measurements at 300 mμ. Also when strychnine and brucine

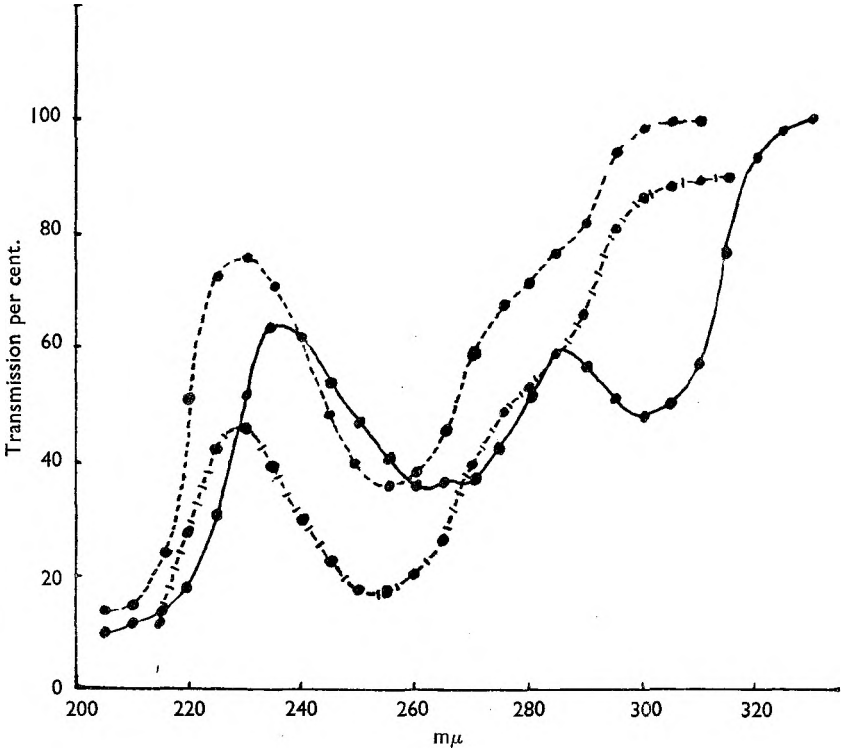


FIG. 1. Transmission-wavelength curves.
———— Brucine 0·0016 per cent. in dilute sulphuric acid.
–|–|–|–|– Strychnine 0·00218 per cent. in dilute sulphuric acid.
– – – – – Strychnine 0·00126 per cent. in dilute sulphuric acid.

are present together in estimable quantities, they influence absorption by each of the bases to some extent (*vide infra*).

In Figures 2A and 2B are given straight line graphs obtained with pure strychnine and pure brucine in dilute sulphuric acid, by plotting the optical densities against their respective concentrations at wavelengths of 300 mμ, 262 mμ and 252 mμ. The ratios of optical density per cm. and concentration in g. per cent. are tabulated as $E_{1\text{ cm.}}^{1\text{ per cent.}}$ in Table I.

Progressive deviation from Beer's law is to be noticed in Figures 2A and 2B in the concentration range above 5 mg. per cent. in the case of strychnine and above 8 mg. per cent. in the case of brucine.

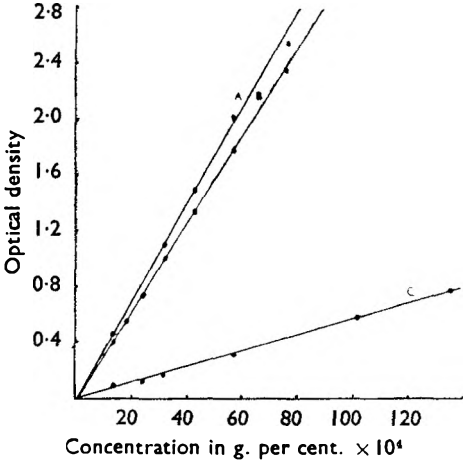


FIG. 2A. Concentration-optical density graphs for strychnine.
A at 252 mμ
B at 262 mμ
C at 300 mμ (optical density magnified 10 times).

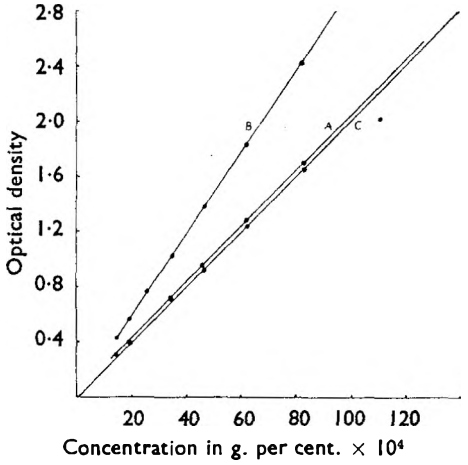


FIG. 2B. Concentration-optical density graphs for brucine.
A at 252 mμ
B at 262 mμ
C at 300 mμ

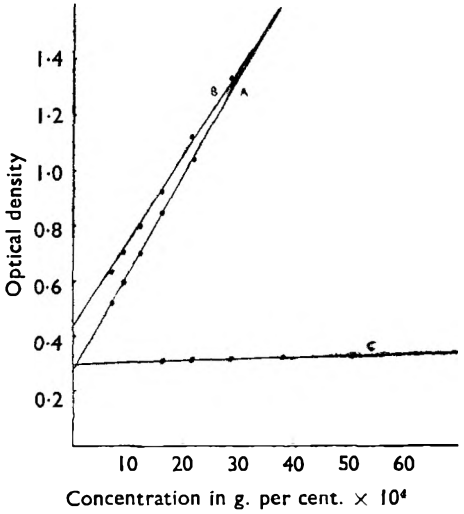


FIG. 3A. Concentration-optical density graphs for strychnine in the presence of a fixed concentration (0·00147 g. per cent.) of brucine.
A at 252 mμ.
B at 262 mμ.
C at 300 mμ.

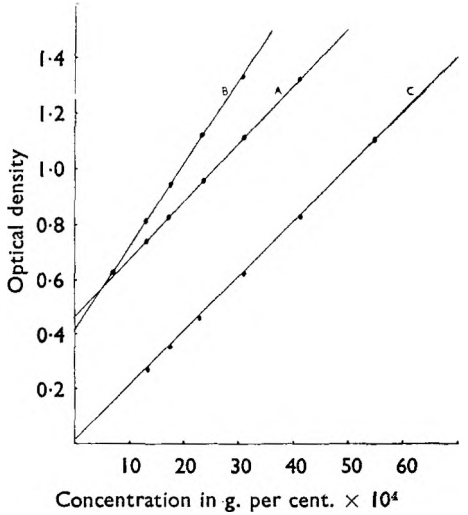


FIG. 3B. Concentration-optical density graphs for brucine in the presence of a fixed concentration (0·00135 g. per cent.) of strychnine.
A at 252 mμ.
B at 262 mμ.
C at 300 mμ.

TABLE I

$E_{1\text{ cm.}}^{1\text{ per cent.}}$ VALUES FOR PURE STRYCHNINE AND PURE BRUCINE

	300 mμ	262 mμ	252 mμ
Strychnine	5·6	310	330
Brucine	199·2	291·4	202·6

The graphs in Figures 3A and 3B were obtained by varying the concentration of strychnine in a solution of "fixed" brucine concentration and *vice versa*. In Table II $E_{1\text{ cm.}}^{1\text{ per cent.}}$ values for the systems at different wavelengths are given. In Table III are recorded for comparison the values, of optical densities for the "fixed" concentrations of brucine and strychnine at different wavelengths (i) as read from the intercept of the straight lines on the optical density axis and (ii) those obtained experimentally with the particular concentrations of pure brucine and pure strychnine in the absence of the other.

TABLE II

$E_{1\text{ cm.}}^{1\text{ per cent.}}$ VALUES FOR STRYCHNINE IN FIXED CONCENTRATION OF BRUCINE (0·00147 g. per cent.)

$E_{1\text{ cm.}}^{1\text{ per cent.}}$ VALUES FOR BRUCINE IN FIXED CONCENTRATION OF STRYCHNINE (0·00135 g. per cent.)

	300 mμ	262 mμ	252 mμ
Strychnine	5·3	309	347
Brucine	195·6	298	207

TABLE III

OPTICAL DENSITIES OF BRUCINE AND STRYCHNINE IN CONCENTRATIONS OF 0·00147 G. PER CENT. AND 0·00135 G. PER CENT. RESPECTIVELY (1) as read from Figure 3 and (2) as obtained directly by measurements in such concentrations

	300 mμ		262 mμ		252 mμ	
	(1)	(2)	(1)	(2)	(1)	(2)
Strychnine	0·009	0·009	0·415	0·415	0·460	0·457
Brucine	0·300	0·302	0·425	0·430	0·290	0·290

From Tables I and II it should be clear that strychnine and brucine when present together produce changes in the slopes of the curves indicating interference. The strict validity of Beer's law in the concentration range of the mixtures and the agreement in the values tabulated in Table III suggest that the interference is not dependent on the concentration ratio of the two bases (in the concentration range used) and that the optical density is additive, i.e., the total optical density is the sum of the densities at zero concentration of the variable constituent and the optical density due to the variable constituent in the mixture. The validity of Beer's law and the agreement in the values of intercepts with the experimentals were checked at other wavelengths

(not cited in the figures) such as at 228 mμ, 236 mμ and 286 mμ and also at concentration ranges of 6 mg. per cent. of strychnine and 8 mg. per cent. of brucine.

In Figure 4 the straight lines were obtained—(i) by plotting concentrations of brucine in mixtures containing brucine and strychnine in a fixed ratio of 0·788, against optical densities of the mixtures determined at 252 mμ, 262 mμ and at 300 mμ. It is important to note that the slope of curve C at 300 mμ (viz. 201) is not the same as that obtained with brucine in fixed concentration of strychnine (viz. 195·6). The curve D is obtained by calculating the absorption due to brucine alone at 300 mμ in the mixtures using the 1·004 $\times$ $E^{1\text{ per cent.}}_{1\text{ cm.}}$ value (196·4) of the base in presence of strychnine. The two straight lines are significantly different, and a straight line practically coinciding with curve C is obtained by plotting the sums of the absorptions by the two bases. It may also be noticed that curve C is also reproduced if the 1·004 $\times$ $E^{1\text{ per cent.}}_{1\text{ cm.}}$ value (200) for pure brucine solutions at 300 mμ is taken to calculate the absorption and no allowance is made for absorption by strychnine (cf. ii) present in them. The latter procedure is at least not theoretically justified when there exists significant absorption by strychnine at 300 mμ.

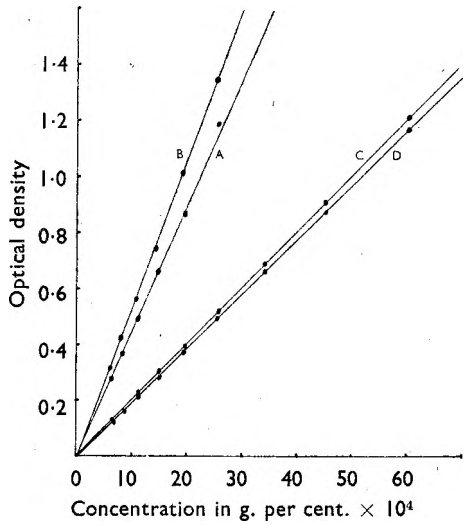


FIG. 4. Brucine concentration-optical density graphs for brucine-strychnine mixture in the weight ratio of 1 : 0·788.

A at 252 mμ.
B at 262 mμ.
C at 300mμ.
D at 300 mμ. for brucine only in the presence of strychnine.

Experimental points on curves A, B and C also correspond to results obtained by calculation from Fig. 3.

(ii) Another straight line (A) coinciding with the 252 mμ line is obtained by plotting the sum of the individual optical densities of the bases at respective concentrations, read from Figure 3 or by calculating the sums of the absorptions by the bases using their 1·004 $\times$ $E^{1\text{ per cent.}}_{1\text{ cm.}}$ values at 252 mμ given in Table II. If the 1·004 $\times$ $E^{1\text{ per cent.}}_{1\text{ cm.}}$ values at 252 mμ for pure solutions be taken for such calculations, a curve widely different from the curve A is obtained, hence as a general procedure the slopes of the curves for the individual bases in presence of one another should be accepted when working with their mixtures. The curve B is obtained by plotting the experimental values of absorption by the bases at 262 mμ and also by plotting the sums of individual absorptions calculated by

the method envisaged. The very close coincidence of the lines obtained at different wavelengths renders obvious the feasibility of estimating strychnine and brucine accurately in presence of one another.

For the determination of strychnine and brucine in an unknown sample, a solution of suitable concentration is prepared. Two determinations of optical densities are required, say one at 252 mμ and the other at 262 mμ. The concentrations of the bases are found by solving the simultaneous equations:—

$$E^{S}_{252}\cdot S + E^{B}_{252}\cdot B = e^{M}_{262}$$
$$E^{S}_{262}\cdot S + E^{B}_{262}\cdot B = e^{M}_{262}$$

Where E^{S}_{252}, E^{S}_{262}, and E^{B}_{252}, E^{B}_{262} are $E^{1\ per\ cent.}_{1\ cm.}$ values for strychnine and brucine present together at 252 mμ and 262 mμ respectively, S (strychnine) and B (brucine) are the concentrations of bases in g./100 ml. of solution and e^{M}_{252} and e^{M}_{262} are the optical densities per cm. of the solution at the two wavelengths. The error involved in this method is usually less than 1 per cent. for both the constituents.

Summary

1. A simple and rapid ultra-violet spectrophotometric method of assay of mixed solutions of strychnine and brucine has been developed with an accuracy within 1 per cent.

2. Change of solvent from water to ethanol does not produce a shift in the maximum and minimum of the ultra-violet absorption curves.

3. The slopes of the straight line graphs—optical density against concentration of the bases are altered when the two are present together.

The authors wish to express their sincere thanks to Dr. U. P. Basu, D.Sc., F.N.I., for suggesting the problem and taking a keen interest in this work.

References

1. Brownlee. *Quart. J. Pharm. Pharmacol.*, 1945, **18**, 163.
2. Ridi and Khalifa, *J. Pharm. Pharmacol.*, 1952, **4**, 190.
3. Herd, *J. Amer. pharm. Ass., Sci. Ed.*, 1942, **31**, 9.
4. Brewstier and Blanc, *Bull. Soc. Chem.*, 1934, **1**, 712.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Dehydrolaudanosoline, Alkaloids Related to. J. Ewing, G. K. Hughes, E. Ritchie and W. C. Taylor. (*Nature, Lond.*, 1952, **169**, 618.) A simple derivative of "dehydrolaudanosoline" of Robinson and Sugasawa (*J. Chem. Soc.*, 1932, 789), was obtained from the bark of *Cryptocarya bowiei* (Hook) Druce, collected in northern Queensland. A water-soluble alkaloid, $C_{20}H_{24}NI$, m.pt. 214° C. (decomp.) $[\alpha]_D^{21°\,C.}$–151° (water) was isolated in about 0·7 per cent. yield; it contained three methoxy groups, and on methylation gave an *o*-methyl ether, $C_{21}H_{26}O_4NI$, m.pt. 153° to 155° C., $[\alpha]_D^{21°\,C.}$–175° (water), which alkali converted to methine-A, $C_{21}H_{25}O_4N$, m.pt. 102° C., $[\alpha]_D^{20°\,C.}$–221° (chloroform). Furthur methylation and alkaline degradation eventually gave optical inactive methine-B, $C_{22}H_{27}O_4N$, m.pt. 111° C., identical with an authentic specimen prepared in the same way from dehydrolaudanosoline iodide. Reactions of the products obtained are given. Bark collected in southern Queensland did not contain this alkaloid, but yielded (1·5 per cent.) a different alkaloid, $C_{19}H_{20}O_4NI$, m.pt. 246° C., $[\alpha]_D^{21°\,C.}$–186° (water), which had one methoxy, one methylenedioxy and one hydroxyl group, *o*-methyl ether, $C_{20}H_{22}O_4NI$, m.pt. 227° C., $[\alpha]_D^{21°\,C.}$–179° (water); this second alkaloid is probably a derivative of dehydrolaudanosoline. R. E. S.

ANALYTICAL

Acid-base Titrations in Non-aqueous Solvents. J. A. Riddick. (*Anal. Chem.*, 1952, **24**, 41.) A review of the subject is given, including historical and theoretical aspects, details of solvents and indicators, and the various standards and methods employed. The solvents used can be divided into two classes; aprotic solvents which are inert, and amphiprotic solvents which take part in the reactions. Aprotic solvents are not believed to take part in neutralisation reactions, having a zero or very small dipole and not readily forming complexes; most of the saturated and benzene-type hydrocarbons and some of the halogenated hydrocarbons, such as carbon tetrachloride and chloroform, belong to this group. Amphiprotic solvents have an appreciable dipole moment, or have groups with dipoles such as *p*-dioxan; lower fatty acids, alcohols and amines belong to this class. The relative acidity of two acids will vary from solvent to solvent, and the variation of the ratio of their activity will be greater the more different are the acids. The choice of a solvent depends almost entirely on the nature of the substance to be determined; there is considerable evidence that mixed solvents may be superior to a single solvent in general solvent power and in sharpness of the indicator colour change or potentiometric break. Most analytical methods have been developed using acetic acid (anhydrous) as the solvent. There is no absolute criterion of acidity or basicity in non-aqueous solutions; acids and bases of unlike nature may undergo a change in the relative position of their respective strengths in different solvents and, generally, a satisfactory method has not been found to determine absolute acid or base strengths in a variety of solvents. As indicators, *p*-aminophenyl benzenesulphonamide, 1-naphthyl-6-sulphonamide, and 1-naphthyl-7-sulphonamide in acetic acid solution fluoresced strongly in ultra-violet light

and could be titrated visually with perchloric acid, using the fluorescent colour change as indicator. Methods for acid-base titrations have been reported which employ the following indicators: modified methyl orange (xylene cyanol), methyl red, thymol blue, methyl violet, 1-naphtholbenzein, bromocresol yellow, crystal violet, phenolphthalein, benzyl auramine, methyl orange, and cresol red. Most of the early potentiometric measurements were made using a lithium chloride bridge; references to many electrode systems are quoted. Perchloric acid is the strongest acid in organic solvents; it is the most suitable acid titrant in acid type amphiprotic solvents and has been used for the determination of a wide variety of substances; the most widely used bases for amphiprotic solvents, particularly the alcohols, are alkali metal alkoxides and alkali metal hydroxides; potassium hydroxide appears to be more suitable than sodium hydroxide. There is no satisfactory acid for use in aprotic solvents; *p*-toluene sulphonic acid in chloroform has been used and trichloracetic, *d*-camphor-sulphonic, and picric acids have been used as titrants to demonstrate the applicability of bromophthalein magenta as an indicator in aprotic solvents. Derivatives of guanidine appear to be the most satisfactory base titrants in aprotic solvents; 1:3-diphenyl-, di-*o*-tolyl-, and di*cyclo*hexylguanidine appear to be the strongest bases in benzene. With reference to the methods used for titrations in acetic acid the titrant must be kept and used at a constant temperature or a temperature correction must be applied; details and references relating to the procedure are given. Among the substances which can be titrated are listed: salts of carboxylic acids, amines, amino-alcohols, oxazolines, amino-acids, amides of carboxylic acids, chlorides, bromides, nitrates, sulphates, quinine, alkylene oxides, nicorinic acid, nicotinamide and related compounds, sulphonamides, and basic nitrogen in oils. For the estimation of weak acids Polit proposed a 1 : 1 mixture of ethylene glycol or propylene glycol and *iso*propanol with perchloric acid as the titrant and methyl red as the preferred indicator; the mixture was regarded as an almost universal solvent for alkali metal salts of monobasic organic acids; alkali metal salts of monocarboxylic acids, amines, mixed acids, salts of inorganic acids, boric acid and alkali in soap were titrated using this solvent. Other solvents which have been used are methanol-benzene, monobutylamine, pyridine, alcohols, and chloroform. Other aspects discussed are: the effect of water, the standards to be used and the accuracy and precision of the method. R. E. S.

Amidone (Methadone), Analytical Studies of. J. Demonceau. (*J. Pharm. Belg.*, 1952, 7, 36.) The addition of powdered sodium perchlorate to a solution of an amidone salt gives an oily precipitate which becomes crystalline on shaking for a few seconds. The sensitivity of the reaction is about 1 in 4000, and the microcrystalline clusters of needles derived from amidone may be distinguished from the polyhedrons produced by phenadoxone or the other microcrystalline forms from morphine, pethidine, codeine, etc. A more sensitive reagent is a mixture of equal volumes of 5 per cent. mercuric chloride solution and 40 per cent. potassium bromide solution. Characteristic crystals are obtained with amidone in concentrations from 1 to 500 to 1 in 30,000. At higher concentrations the precipitates are amorphous. The addition of 2 drops of nitric acid (20 per cent. HNO_3) and 3 to 4 ml. of sulphuric acid to 1 ml. of amidone hydrochloride solution gives a pink to purple-red colour according to the concentration and this reaction will detect 25 μg. of amidone hydrochloride. Potassium nitrate may be used in place of nitric acid. The presence of chloride is necessary for formation of the red colour (amidone base gives an orange colour) but an excess of chloride ions decreases the sensitivity of the test.

Phenadoxone gives the same colour as amidone, morphine and brucine give the colour on the addition of a few drops of reagent but an excess of reagent gives an orange to yellow colour. The following may be used quantitatively. Place 2 ml. of the amidone hydrochloride solution in a large test tube immersed in cold water and add 8 ml. of sulphuric acid containing 0·5 per cent. w/w of potassium nitrate, shaking after the addition of 10 drops. Heat in a boiling water bath for exactly 2 minutes, cool with cold water for 15 minutes and determine the spectrophotometric absorption at 525 mμ. Using 1-mg. samples, errors up to 6·5 per cent. were experienced. G. B.

p-Aminosalicylic Acid, Estimation of m-Aminophenol in. A. Kirschbaum. (*Pharm. Acta Helvet.*, 1952, **27**, 26.) It has been suggested that the amount of *m*-aminophenol present in solutions of *p*-aminosalicylic acid should be estimated by shaking 10 ml. of solution at *p*H 8 to 8·5 six times with 10 ml. of ether, evaporating off the ether and drying *in vacuo* and weighing the residue. The residue is tested for m.pt. 120° to 122° C. and its solution should give a yellow to brown colour with aqueous solution of ferric chloride (*p*-aminosalicylic acid gives a red colour) and if to 2 ml. of solution 1 ml. of saturated solution of potassium iodate and then 2 ml. of glacial acetic acid are added, a characteristic reddish violet colour is formed in a short time, and this can be used for colorimetric estimation. The author investigated the extraction of *m*-aminophenol with ether, using a 20 per cent. solution of anhydrous sodium *p*-aminosalicylate (A) and the same with the addition of 2 per cent. of *m*-aminophenol (B). Solution B was extracted 6 times with ether, but only 80 per cent. of the *m*-aminophenol was obtained, a further 6 extractions only recovered another 15 per cent. and the latter residue was coloured and impure. Commercial ether was used in these experiments; using anæsthetic ether 93 per cent. was extracted in 6 shakings and the residue was much purer, showing that the peroxides present in the commercial ether had caused decomposition. The solutions were allowed to stand 5 months without special precautions and then tested again. Solution A had become reddish-brown and the *p*H was more alkaline and when extracted with anæsthetic ether a residue of 0·060 g. (from 2 g. of original sodium *p*-aminosalicylate) was obtained; this was impure *m*-aminophenol. Solution B had become brownish-black and opaque, ether extracted 0·225 g. as against 0·186 g. for the fresh solution. Although the aqueous solutions were deeply coloured the ether extracts were practically colourless. When solutions were kept in ampoules sealed from air, even when 50 per cent. of the *p*-aminosalicylate was decomposed by heat, no discoloration was caused and no change of *p*H, and extraction with ether gave pure *m*-aminophenol. Decomposition can be approximately measured by acidifying and determining the carbon dioxide evolved, in the cold, from the sodium carbonate formed, or by direct titration of the latter. H. D.

Barium in Calcium Salts, Limit Test for. A. Askgaard and F. Reimers. (*Dansk. Tidsskr. Farm.*, 1952, **26**, 32.) A seeding reagent is prepared by precipitating the barium chloride in 2 drops of a solution containing 100 μg. of Ba^{++} per ml. with 1 ml. of 0·1 M sulphuric acid. To this seeding reagent is added 10 ml. of test solution and after 1 minute's standing the mixture is vigorously shaken. The turbidity produced, after 30 minutes standing, is compared with a standard liquid. If no barium is allowed a blank using the seeding reagent and 10 ml. of water is prepared for comparison. If a positive reaction for barium is allowed the turbidity produced by 4 drops of the barium chloride solution, 1 ml. of 1 M sulphuric acid and 10 ml. of water is recommended

for comparison. The turbidity of this standard is only slightly altered on standing for 30 minutes. J. R. F.

Chloramphenicol, Colour Reaction for. R. Truhaut. (*Ann. pharm. franc.*, 1951, **9**, 347.) Chloramphenicol, in common with trihalogen compounds and a series of halogen derivatives was found to give a colour reaction, when heated with pyridine in an alkaline medium. 5 ml. of a solution of the drug in purified pyridine was heated with 2 ml. of a 50 per cent. w/w solution of potassium hydroxide at 100° C. for 5 minutes. The solution was cooled in iced water for 90 seconds, when a rose to red colouration developed in the pyridine layer, the depth of colour depending on the concentration. Attempts to use this reaction for quantitative estimation and determination of the drug in urine and blood have failed owing to the development of an opalescence. J. R. F.

Salicylates and Derivatives, Estimation of Salicylic Acid in. G. Scandellari (*Boll. chim.-farm.*, 1951, **90**, 387.) The method of assay based on the formation of Lautemann's Red (tetriododiphenylquinone) may be used when there are no other substances present which react in the same way with iodine. It can also be used for estimating acetylsalicylic acid, salol, phenol and salicylamide, but cinchophen, *p*-aminosalicylic acid and sodium gentisate react irregularly. Place about 0·3 g., accurately weighed, of the substance previously dried at 100° C. in a 250 ml. beaker. Add 5 ml. of 2 N sodium hydroxide and 100 ml. of water and heat on a water bath to about 95° C. for 20 minutes. Then add concentrated solution of iodine (iodine 12·70 g., potassium iodide 20 g., water to 100 ml.) until the liquid is deep brown. An abundant precipitate is formed. Leave on the water bath for an hour, keeping free iodine always in excess, and cool. Remove the excess of iodine by cautiously adding sodium bisulphite and collect the red precipitate on a double tared filter paper 11 cm. in diameter. Wash until free from iodide, dry at 100° C. and weigh. 1 g. of the precipitate corresponds to 0·4017 g. of salicylic acid. Sodium salicylate showed 86·40 and 86·31 per cent. of salicylic acid (theory 86·25 per cent.); acetylsalicylic acid, 76·25 per cent. (theory 76·66 per cent.); salol, 125·22 per cent. (theory 64·48 per cent. for one molecule and 128·95 for two molecules); phenol, 147·79 per cent. (theory 146·86 per cent.); cinchophen does not form a red precipitate; sodium *p*-aminosalicylate showed 41·59 per cent. (theory 78·41 per cent.); sodium gentisate gave a black crystalline precipitate equivalent to 12·05 per cent. of salicylic acid (theory 77·97 per cent.); salicylamide, 74·90 per cent. (theory 100·72 per cent.). H. D.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Cardiac Glycosides, Aglycones and Acetates, Paper Chromatography of. E. Heftmann and A. J. Levant. (*J. biol. Chem.*, 1952, **194**, 703.) Data are given relating to the relative mobility in benzene-formamide and toluene-propylene glycol systems and on the sensitivity to Tollens' and trichloroacetic acid reagents of a series of cardiac glycosides, aglycones and acetates. Details of procedure are quoted for the preparation of the chromatographic cabinet, the preparation of the filter paper and for the development. After completion of the development, the sheets were dried and sprayed with either Tollens' reagent or trichloroacetic acid solution. Tollens' reagent gave black or dark brown spots after use of benzene-formamide and reddish-brown spots after toluene-propylene glycol; trichloroacetic acid gave either yellow or green spots, which turned brown on prolonged heating, visible in daylight and fluorescing

around the edges in ultra-violet light or invisible in daylight but fluorescing brightly in ultra-violet light. The sensitivities of the spot tests are recorded for 22 cardiac glycosides, aglycones and acetates. The results are discussed in an attempt to correlate structure and chromatographic behaviour. R. E. S.

ORGANIC CHEMISTRY

Saccharin, Hydrolytic Stability of. O. DeGarmo, G. W. Ashworth, C. M. Eaker and R. H. Munch. (*J. Amer. pharm. Ass. Sci. Ed.*, 1952, **41**, 17.) A method for the determination of the amount of decomposition in saccharin depends upon measurements of the optical densities of solutions in 0·1N sodium hydroxide solution. Under these conditions, saccharin and its hydrolytic decomposition products, *o*-sulphamylbenzoic acid and (ammonium *o*-sulpho) benzoic acid exhibit absorption maxima at 267·3 mμ, the absorption coefficients of the two decomposition products being about equal and much lower than that for saccharin itself. Using optical density measurements at 267·3 mμ the decomposition of saccharin at temperatures used in cookery was shown to be negligible.

DECOMPOSITION OF SACCHARIN SOLUTIONS

Solvent	*p*H	Percentage decomposition after 1 hour at		
		100° C.	125° C.	150° C.
Water	2·0	2·9	8·5	18·6
Buffer	3·3	0	1·0	1·9
Buffer	7·0	0·3	0·3	1·6
Buffer	8·0	0	0	0

G. B.

TOXICOLOGY

Amphetamine in Viscera, Determination of. E. Rathenasinkam. (*Analyst*, 1952, **77**, 135.) A scheme is described for the isolation, identification and determination of amphetamine; the isolation is first achieved by extracting with ethanol acidified with tartaric acid according to the Stas-Otto process. The residue is steam-distilled from alkaline solution, the distillate evaporated to low bulk made alkaline with sodium hydroxide and the base extracted into chloroform; the hydrochloride is then produced by extraction into hydrochloric acid. Methods of identification are reviewed and a further colour reaction of the nitro-compounds formed by the nitration of amphetamine is described. A number of processes for the determination of the substance are discussed and a new method depending on the precipitation of the amphetamine as oxalate is given; a comparison of the results obtained by this method with those obtained by a volumetric method is shown. R. E. S.

Blood Stains, Spectrophotometric Detection of. A. A. Khalifa and M. K. Salah. (*Nature, Lond.*, 1952, **169**, 461). A test for the identification of blood stains is described depending on the fact that potassium cyanide, with hæmoglobin, methæmoglobin or hæmatin, gives cyanohæmoglobin which is characterised by an absorption curve with a maximum at 422 mμ and smaller bands at 360 and 550 mμ respectively; the maximum at 422 mμ has 10 times the intensity of the visual band at 550 mμ making the ultra-violet examination more sensitive than the visual. The test was applied by soaking a suspected stain (as small as 2 sq. mm.) in 0·5 per cent. potassium cyanide solution for 15 minutes, filtering, and determining the absorption curve from 300 to 600 mμ. The method can be adopted for quantitative estimation. The test has been applied to blood stains obtained

from a variety of materials; leather, painted wood, walls, soil, sand and stains from cloth either washed with soap and water or contaminated with vegetable juices or rust. A graph is given of absorption curves obtained. R. E. S.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Dextran Sulphate, A Synthetic Heparin Analogue. C. R. Ricketts. (*Biochem. J.*, 1952, **51**, 129.) A series of sulphuric esters of dextran, differing widely in molecular weight and in the number of sulphate groups, were prepared. Three main molecular weight types of dextran sulphate were prepared, two from unfractionated hydrolysates and the third from the low molecular fraction of a hydrolysate with intrinsic viscosity 0·24. Intrinsic viscosity was used as a measure of molecular weight, the average molecular weight of the first two dextrans being about 200,000 and 20,000; allowing for the introduction of 1·3 sulphate groups per glucose unit, the average molecular weight of the dextran sulphates would then be very approximately 300,000 and 30,000. The third type of dextran sulphate had an estimated molecular weight of less than 20,000 allowing for the introduction of 1·3 sulphate groups per glucose unit. Biological testing of the preparations showed that the third series of dextran sulphates (lowest molecular weight) were free from toxic effects although the higher molecular weight series were found to be toxic. Details are given of the preparation of a non-toxic dextran sulphate. When prepared the dextran sulphate decomposed on boiling in aqueous solution with the formation of inorganic sulphate and reducing substances; such decomposition, for example, on autoclaving, could be prevented by buffering the solution with sodium chloride and sodium bicarbonate. Maximum blood anticoagulant activity was found when the number of sulphate groups exceeded an average of 1·3 per glucose unit. R. E. S.

Pancreatin with High Enzymatic Activity. C. W. Bauer and A. J. Vazakas. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 552.) Pancreatin prepared by defatting fresh pancreas with acetone and drying with ether, according to the method of Willstätter and Waldschmidt-Leitz, had a high lipase content and contained 3 times the amount of trypsin and twice the amount of amylase required by the U.S.P. The lipolytic activity was destroyed by exposure to an acidity equal to that of the stomach. The lipase content of fresh hog pancreas was reduced by immersion in ethanol ranging in strength from 20 to 95 per cent. for 11 days. Hog intestine was investigated as a source of lipase by cutting into several sections, grinding a piece from each section with sand and water and assaying the resulting suspensions; all samples gave low results. When a portion of the intestine was ground together with a piece of fresh pancreas, the lipase content of the resulting suspension was found to be no greater than the sum of the lipase contents of the constituents, indicating that if the intestine contained a lipase precursor, it was not activated by fresh pancreas.

G. R. K.

***Saccharomyces cerevisiae*, Effects of Cocaine on the Metabolism of.** B. E. Ryman and E. O'F. Walsh. (*Biochem. J.*, 1952, **50**, 570.) The glucose fermenting properties of *Saccharomyces cerevisiæ* at *p*H 7 have been studied in the presence of cocaine and added vitamin B_1. *S. cerevisiæ* grows quite well in the presence of cocaine, provided that the concentration is not greater

than 0·004 M (0·005 M inhibits growth completely); the organism appears to acquire a tolerance for the drug, and yeast which has been grown in the presence of cocaine grows quite well in its absence. Experiments with vitamin B_1 indicated that changes occurred in the cells as a result of growth in the presence of cocaine; a difference in behaviour towards added vitamin B_1 between the normal yeast and the cocaine-grown yeast was noted. Cocaine, in concentrations sufficient to arrest growth (0·005 M) at *p*H 7, inhibited completely the carboxylase system of *S. cerevisiæ*; the carboxylase systems of yeast grown in the presence and in the absence of cocaine were inhibited by cocaine to a similar extent. Growth in the presence of cocaine at *p*H 7 did not show any quantitative adaptation of the carboxylase system of *S. cerevisiæ*. R. E. S.

Streptomycin, A New Colour Reaction of. W. J. Halliday. (*Nature, Lond.*, 1952, **169**, 335.) When streptomycin and diacetyl are mixed in aqueous alkaline solution, a pink colour is produced by virtue of the guanidine groupings in the antibiotic. This colour can be greatly intensified by the further addition of α-naphthol, and the modification increases the sensitivity only. The following facts are noted: (1) the colour was slow to develop, and faded after reaching a maximum intensity; (2) oxygen was necessary both for colour development and for the fading process; (3) strong absorption was found at the shortest wavelengths of visible light, with a characteristic band in the green region (peaks at 504 mμ for the first reaction and at 515 mμ for the modification). The sensitivity was limited to about 50 μg. of free base in a total of 15 ml. Many guanidine derivatives interfered with the test, and dihydrostreptomycin gave a similar reaction. A. H. B.

Terramycin, Fluorimetric Behaviour of. M. Serembe. (*Arch. Ital. Sci. Farmacol.*, 1951, **1**, 244.) Terramycin in the solid state gives a yellow fluorescence under Wood's light. In solution, with *p*H 1 to 5 there is no fluorescence, in distilled water (*p*H 5 to 6) there is a yellow fluorescence which becomes brighter as the *p*H increases up to a maximum at *p*H 9 to 10. On standing, the fluorescence becomes greenish and this appears more rapidly with greater alkalinity and higher temperature. Lithium chloride, disodium hydrogen phosphate, calcium chloride and magnesium chloride increase the fluorescence notably, other salts have less action. The yellow fluorescence can be used for estimating the amount present. With biological material the solubility of terramycin in *n*-butanol can be used for removing other substances which fluoresce. 1 ml. of blood serum, pulped organs, urine or bile is brought to *p*H 9 to 10 with 0·1N sodium hydroxide and shaken thoroughly with 2 ml. of *n*-butanol for serum or pulped organs and 1 ml. for urine or bile, and then centrifuged. The intensity of fluorescence in the butanol solution is proportional to the amount of terramycin present within the limits of 5 mμ and 50 mμ per ml. Aureomycin also gives a fluorescence, which can be distinguished from that of terramycin as it is yellow at *p*H 6 to 8·5 and blue at higher figures. It is also blue in the presence of lithium chloride, sodium bicarbonate, sodium sulphate and disodium hydrogen phosphate. The change of colour of the fluorescence of terramycin to greenish and aureomycin to blue is accompanied by loss of activity. H. D.

Terramycin in Serum and Urine after Ingestion of Terramycin Base. J. D. Carlile, A. C. Kester, C. B. McDonald and C. F. Clancy. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 535.) 15 males were given a single dose of 2 g. of terramycin base on a fasting stomach. After 1 hour the average serum level was 0·9 μg./ml., although not all subjects showed a measurable level.

After 2 hours the level was 1·8 μg./ml., all subjects having a demonstrable level of drug. The highest level, 2·4 μg./ml., was reached after 4 hours; the drug had disappeared by the 24th hour. The levels in 5 control subjects receiving terramycin hydrochloride followed a similar course except that the highest level, also reached after 4 hours, was 4·8 μg./ml. In a similar series of experiments, in which the drugs were given in doses of 0·5 g. every 6 hours, some of the subjects receiving the base showed demonstrable serum levels after 8 hours; after 24 hours the test and control groups showed 1·1 and 1·6 μg./ml. respectively. Urine analyses after the administration of a single dose of 2 g. of base and hydrochloride showed that all subjects were excreting the drug by the end of the first hour and also after 24 hours. In general, the levels attained in subjects receiving the hydrochloride were greater than those in subjects receiving the base. It appears that the rate of absorption of terramycin from the alimentary tract is independent of whether the base or the more soluble hydrochloride is administered, but that after ingestion of the base the concentration in the urine is less than that following ingestion of the hydrochloride.

G. R. K.

Thiochrome Solutions under Various Lighting Conditions. L. J. DeMerre and M. A. Seibold. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 566.) Direct sunlight and ultra-violet light produced total destruction in thiochrome solutions. Natural daylight also produced total destruction but at a slower rate. Exposure to dim daylight in intensities not higher than 10 foot candles produced no destruction in 120 minutes. Artificial light from an incandescent bulb had a marked destructive effect for intensities above 600-foot candles. With monochromatic light, destruction was greater in the short wavelength region. In the green region (485 mμ) and above, no destruction occurred. G. R. K.

BIOCHEMICAL ANALYSIS

Ethanol in Blood or Urine, Determination of. F. J. Scandrett. (*Analyst*, 1952, **77**, 132.) A method is described for the micro-determination of ethanol in blood or urine by means of a new micro-diffusion procedure; after numerous trials the method and the quantities and concentrations of reagents used by Widmark were found to be the most suitable. A new micro-diffusion apparatus that permits the separate temperature control of the two chambers, is illustrated and described in detail; a lower vessel, which contains the sample for determination, has a flat base and is attached to the chimney part of a "mushroom" which is surrounded by a simple form of condenser that allows water at 50° C. to circulate over and under it. Accurate results were obtained over the range of 80 to 300 μg. of ethanol per 0·1 ml. of blood and urine, the standard deviation being less than ± 3·0 μg. per 0·1 ml. of blood. It is claimed that the method reduced the time for a single diffusion to 30 minutes, and that as a result of the large excess of water in the "mushroom" receiver there is no possibility of loss of the liberated iodine, that the end-point is not so abrupt and the subsequent titration and shaking are, therefore, easy to control and manipulate. An increase of the temperature gradient of the absorption made possible an increase in the range over which diffusion techniques could be used; experiments with blood containing 300 mg. of ethanol per 100 ml. stored at room temperature for one week, did not show any diminution of ethanol concentration. R. E. S.

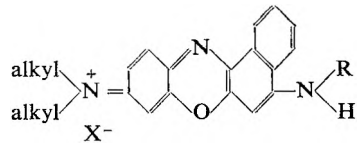
Penicillin, Effect of Sugars on Plate Assay of. C. R. Bond. (*Analyst*, 1952, **72**, 118.) An examination was made on the effect of dissolved substances in penicillin test solutions; such substances often yield larger zones, giving

fictitiously high assay results, if normal simple solutions of penicillin are used as standards. Preliminary investigation of this phenomenon showed that the causal agent was sucrose; work was carried out to determine more precisely the effects of the commonly used sugars (sucrose, lactose and dextrose), on the size of the zones of inhibition, and on the definition of the zone edges. Sucrose produced the largest effect and dextrose a somewhat smaller effect, while up to 0·4 per cent. of lactose was almost without effect on the size of the zones. In the assay of penicillin lozenges or other samples containing sugars it was considered that the standards used must be compensated by addition of the appropriate sugars. The presence of sucrose in assay solutions gave improved zone edges in an ordinary nutrient agar media, this also being obtained by incorporating 0·1 per cent. of sucrose into the nutrient agar. The accuracy of the method could thus be increased and the curvature of the regression line for zone diameter on the logarithm of the concentration in the sucrose medium was sufficiently small over the range 2 to 8 units per ml. to allow calculation of results on a theoretically linear relationship without introducing serious error (maximum about 3 per cent.). In the assay of penicillin lozenges the concentrations of sugars in the standards must be the same as in solutions under test. R. E. S.

Urinary 17-ketosteroids, Estimation of. E. R. Cook. (*Analyst*, 1952, **77**, 34.) A method is described for the rapid estimation and fractionation of urinary 17-ketosteroids based on the routine procedure of Reiss, Hemphill, Gordon and Cook, by which the crude extract from 250 ml. of urine may be rapidly purified and partitioned into 3(α)-hydroxy- and 3(β)-hydroxy-17-ketosteroids. Attempts were made to reduce the experimental work to a minimum by separating smaller quantities of material and by a direct estimation of the β-ketosteroid component as its digitonide. Details of the method and of the precautions necessary to obtain quantitative results are given. Known amounts of pure 17-ketosteroids added to urine gave recoveries of at least 93 per cent.; the procedure is recommended as a rapid and satisfactory routine determination for use in the estimation of fractional 17-ketosteroids. R. E. S.

CHEMOTHERAPY

Chemotherapeutic Dyes. I. 5-Aralkylamino-9-alkylaminobenzo[a]phenoxazines. M. L. Crossley, P. F. Dreisbach, C. M. Hofman and R. P. Parker. (*J. Amer. chem. Soc.*, 1952, **74**, 573.) A series of 5-amino and 5-aralkylamino-9-(mono- and di-)-alkylaminobenzo[a]phenoxazonium salts of general formula I—



where R = a benzyl or ring-substituted benzyl group and X is the anion of a salt, were prepared. Their differential tissue staining and tumour growth-retarding actions were investigated, and the effect of structural modifications upon these actions are discussed. Some of the derivatives showed a marked differential fat-staining effect when administered orally either to normal mice or to tumour-bearing mice. In the latter case, the tumour was stained blue

and the fatty tissue red. Many of the compounds exhibited strong antituberculous activity when administered orally to infected mice, and 20 members of the series possessed activity equivalent to, or greater than, that of streptomycin given subcutaneously at optimum dosage. Compounds with an unsubstituted amino group in the 5-position were inactive. The presence of one, or preferably two, alkyl radicals on the 9-amino nitrogen seems to be necessary for activity. Maximum effect was obtained in the 9-di-*n*-propylamino and 9-di-*n*-butylamino series. The presence of a substituent in the benzyl ring is unnecessary for high activity. An acidic group in the molecule lowers the antituberculous effect.

A. H. B.

Chemotherapeutic Dyes. II. 5-Arylamino-9-dialkylaminobenzo [a] phenoxazines. M. L. Crossley, R. J. Turner, C. M. Hofmann, P. F. Dreisbach and R. P. Parker. (*J. Amer. chem. Soc.*, 1952, **74**, 578.) A series of 5-aryl amino-9-(mono- and di-)-alkylaminobenzo[a]phenoxazines was prepared and the compounds tested for tumour growth-retarding action and antituberculous action. The alkyl groups were varied from methyl to *n*-hexyl, and maximum antituberculous activity resulted when two propyl groups were present on the 9-amino group. Substitution by chloro or methyl groups in the 5-position of the aryl ring maintained or increased activity, but larger alkyl groups decreased activity. Introduction of electronegative substituents such as nitro, carboxy, carbethoxy or acetyl produced a sharp decrease in activity. 26 of the compounds of this series, when administered orally to mice, possessed activity equivalent to or greater than that of streptomycin administered subcutaneously at optimum dosage.

A. H. B.

Chemotherapeutic Dyes. III. 5-Heterocyclicamino-9-dialkylaminobenzo[a]-phenoxazines. M. L. Crossley, C. M. Hofmann and P. F. Dreisbach (*J. Amer. chem. Soc.*, 1952, **74**, 584.) A series of 9-dialkylamino-5-heterocyclicaminobenzo[a]phenoxazines was prepared and tested for tumour growth-retarding and antituberculous action. Those compounds possessing a 9-diethylamino group showed a growth-retarding effect on tumour transplants, the most active compound being the 9-diethylamino-5-(2-pyridylamino)benzo[a]phenoxazine derivative. None of the compounds of the series possessed significant antituberculous activity.

A. H. B.

PHARMACY

NOTES AND FORMULÆ

Choline Gluconate. (*New and Nonofficial Remedies, J. Amer. med. Ass.*, 1952, **148**, 744.) Choline gluconate is 2-(hydroxyethyl)trimethylammonium D-gluconate. It is commercially available as a 58 to 62 per cent. aqueous solution. The following tests and standards apply to a 95 per cent. sample, which occurs as a straw-coloured highly viscous mass with an amine-like odour and a bitter taste; soluble in water, sparingly soluble in ethanol, very slightly soluble in ether and practically insoluble in benzene and chloroform; the *p*H of a 50 per cent. solution is between 5·0 and 6·0. Choline gluconate gives a pale yellow precipitate with mercuric potassium iodide, and an emerald-green colour with cobaltous chloride and potassium ferrocyanide. The yellow colour of an aqueous solution is intensified by ferric chloride. The crystals of gluconic acid phenylhydrazide formed by heating a 10 per cent. solution with glacial acetic acid and phenylhydrazine melt at 199° to 202° C. Choline gluconate

contains less than 5 per cent. of water, when determined by the Karl Fischer method, and yields less than 0·05 per cent. of ash. It is assayed spectrophotometrically by measuring the light absorption at 5260 Å of a solution in acetone of the reineckate obtained by treating an aqueous solution with ammonium reineckate. The content of choline gluconate is obtained from a standard curve prepared with Choline Chloride U.S.P. Reference Standard, and is not less than 95·0 and not more than 105·0 per cent. G. R. K.

Cyclocumarol (Cumopyran). (*New and Nonofficial Remedies, J. Amer. med. Ass.*, 1952, **148**, 939.) Cyclocumarol is 3 : 4-dihydro-2-methoxy-2-methyl-4-phenyl-2H,5H-pyrano[3,2-c][1]-benzopyran-5-one and occurs as a white crystalline powder with a slight odour, m.pt. 164° to 168° C., insoluble in water, and slightly soluble in ethanol. A 0·015 per cent. solution in ethanol exhibits ultra-violet absorption maxima at 2710 and 2820 Å and a minimum at 2760 Å. The ratio of the extinction coefficients at 2760 and 2820 Å is not less than 0·856 and not more than 0·956. It loses not more than 1·0 per cent. of its weight when dried at 105° C. for 2 hours, yields not more than 0·2 per cent. of ash, and complies with a test for heavy metals. It is assayed by dividing the extinction coefficient at 2820 Å of a 0·015 per cent. ethanolic solution by the factor 396; it contains 97·0 to 103·0 per cent. of cyclocumarol. Cyclocumarol is an anticoagulant. G. R. K.

PHARMACOGNOSY

Colchicum, Alkaloid Content of. A. Mastnak-Regan. (*Acta pharm. Jug.*, 1951, **1**, 67.) After the seeds and corm, the flower has the highest content of alkaloids, while the leaves have the lowest. In the dried plant the corm is the richest part, followed by the leaves, the seeds, the fruit and the flower. During growth the percentage of alkaloids in the corm is lowest in the autumn; in old corms, the percentage is greatest in the spring. In the stem the alkaloidal content diminishes during May and June when the leaves begin to wither. Unripe seeds contain less alkaloid than ripe seeds. Fresh corm and seeds are poorer in alkaloidal content than dried corm and seeds. The iodimetric method of assay was used in these determinations. The method of the U.S.P. XIII, P. Helv. V and D.A.B. VI for the extraction of the alkaloids often produced strong emulsions, whereas the method of P. Ned. V modified by Uffelie was free from this objection. Results obtained by the iodimetric method were often very low. Those obtained by the P. Ned. V and the U.S.P. methods were in agreement. G. R. K.

Digitalis Leaf Glycoside Yields, Effects of Freezing Temperature Storage on. H. W. Youngken, Jr., E. H. Djao and D. P. N. Tsao. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 569.) First year digitalis leaves stored at −18° to −12° C. for two years packed in manila envelopes or cloth bags were of excellent green appearance and had dried considerably; those stored in the envelopes had dried more than those stored in the bags, but in both the moisture content was still considerably more than the U.S.P. maximum. Tinctures made by the U.S.P. method modified to allow for the higher moisture content had glycoside contents consistently higher (usually more than 20 per cent.) than tinctures made from leaves from the same source which had been dried immediately after collection by heating at 29° to 38° C. in a current of air. When the frozen material was dried in the same way before preparation of the tincture, all samples but one gave lower yields than the undried material, indicating that

the method of drying destroyed part of the glycosides, although the loss was less when the material had been previously frozen. The disadvantages of storage at low temperature are the longer time taken to prepare official tinctures due to the necessity of determining the moisture content and adjusting the composition of the solvent, and the larger storage space required. G. R. K.

***Digitalis purpurea*, Effects of Season, Temperature and Plant Age on Glycoside Production in.** D. P. N. Tsao and H. W. Youngken, Jr. (*J. Amer. pharm. Ass. Sci. Ed.*, 1952, **41**, 19.) Plants were raised from seeds of known strains of *Digitalis purpurea* L. and grown in carefully controlled field experiments or in hydroponic culture in greenhouses. Dried leaves were assayed for glycoside content against the U.S.P. reference standard, by a modified Knudson–Dresback method using a spectrophotometer. Some samples were also assayed by the U.S.P. pigeon method. In field experiments the highest glycosidal yield was obtained at the end of 141 days' growth after which the glycoside content declined. This maximum corresponded to collection of the plants in August. For plants grown for 6 to 7 weeks in the greenhouse, the glycoside content was highest in August-September and January-March. The highest glycosidal yield was achieved after 120 to 136 days' growth in hydroponic culture. Discrepancies between the colorimetric and biological assays were observed, the colorimetric method giving higher and more consistent results.

G. B.

PHARMACOLOGY AND THERAPEUTICS

Antibiotics: *in vitro* Effect on Typhus Rickettsiæ. A. Karp and J. C. Snyder. (*Proc. Soc. exp. Biol., N.Y.*, 1952, **79**, 216.) Aureomycin and terramycin in concentrations of 100 to 300 μg./ml. were found to inhibit markedly the respiration of murine and epidemic typhus rickettsiæ *in vitro*, as measured by the rate of oxygen uptake by the Warburg method. Chloramphenicol in concentrations up to 300 μg./ml. produced only slight inhibition. The inhibition of respiration was correlated with a decrease in the number of viable rickettsiæ as determined by toxicity and infectivity for white mice. S. L. W.

Blood Coagulation, Influence of Drugs on. D. I. Macht. (*J. Amer. med. Ass.*, 1952, **148**, 265.) A number of widely used drugs have been found to exert a thromboplastic or coagulation promoting influence on the blood of animals and humans. The digitaloid drugs and the antibiotics, penicillin, streptomycin and aureomycin, are amongst these; also included are the mercurial diuretics, metallic antisyphylitics, amphetamine and some opium derivatives. Although there is usually a wide margin of safety for accommodating fluctuations in coagulation time, attention is drawn to the possible deleterious effects of the administration of these drugs. J. R. F.

Citrovorum Factor in the Treatment of Megaloblastic Anæmia. L. S. P. Davidson and R. H. Girdwood. (*Lancet*, 1951, **261**, 1193.) A good clinical and hæmatological response was obtained in 6 cases of Addisonian pernicious anæmia and 2 cases of the megaloblastic anæmias of pregnancy from the administration of leucovorin (a synthetic form of citrovorum factor, stated to have the structure 5-formyl-5:6:7:8-tetrohydropteroyl-glutamic acid). Good results were obtained from administration either by mouth or by intramuscular injection of a dose of 3 to 12 mg. daily, the dosage schedule being varied according to the response. The effects produced in one case of idiopathic steatorrhœa were much less striking. S. L. W.

Colchicine, Natural Resistance of the Golden Hamster to. M. W. Orsini and B. Pansky. (*Science*, 1952, **115**, 88.) Rats and mice were killed by the intraperitoneal injection of 0·25 and 0·5 mg. of colchicine per 100 g. of body weight, respectively. In similar experiments, hamsters survived 7 mg. per 100 g. and showed no toxic effects. Their fertility and normal weight increase continued. When the animals were fed on a diet containing colchicum seed, 33 per cent., mice and rats died but hamsters survived and increased in weight. This resistance resembles that shown by rabbits towards aconite and it is suggested that it may have been acquired from a close association with *Colchicum* sp. in the animal's normal habitat, where the plants may possibly be eaten as food by hamsters. G. B.

Dihydrostreptomycin; Deafness following use in Tuberculous Meningitis. J. T. Naismith. (*Brit. med. J.*, 1952, **1**, 796.) The results obtained in a series of 26 cases of tuberculous meningitis treated with streptomycin are compared with those in a series of 51 cases treated with dihydrostreptomycin. While the latter showed a 67 per cent. recovery rate as compared with 50 per cent. in the former, 50 per cent. of the survivors (i.e., 17 out of 34) in the second series were deaf, whereas only 1 out of 13 survivors in the first series had impairment of hearing. In both series of cases the drug was given intramuscularly and intrathecally, though in the second series the intrathecal dosage was increased. The average time of onset of deafness was 5 months from beginning of treatment and most patients appeared to have fully recovered or to be steadily improving when their hearing began to be impaired. It is unlikely therefore that the deafness is caused by the meningitis, but rather that it is a toxic manifestation—apparently irreversible—of the drug used. While dihydrostreptomycin appears to be at least as effective as other forms of streptomycin in the treatment of tuberculous meningitis, there is enough evidence to warrant caution in its use and to indicate the desirability of a controlled investigation of its advantages and disadvantages compared with other forms of streptomycin. S. L. W.

Dimercaprol in Lead Poisoning. E. C. Vigliani and N. Zurlo. (*Brit. J. industr. Med.*, 1951, **8**, 218.) An investigation was carried out of the effect of injections of dimercaprol in 27 patients with occupational lead poisoning of whom 14 were suffering from acute lead colic on admission to hospital. The average lead content of the blood before treatment was 122 μg. per cent. The greatest reduction in the blood level was attained 8 to 10 hours after the injection of 2 to 5 mg./kg. of body weight, the average value being about 50 μg. per cent. 24 hours after the injection, the amount of lead in the blood had returned to near the previous level. A single injection of 50 mg. of dimercaprol raised the urinary excretion during the first hour from 6 to 25 times the rate before the injection. After the second hour, the rate of excretion fell rapidly. A series of 1 to 3 injections daily for several days, the total daily dose being 50 to 450 mg., produced an increase in urinary lead for the first 3 to 5 days, followed by a reduction to pre-treatment levels. On stopping treatment, both blood and urinary lead tended to return to the initial levels. In some cases, administration during lead colic aggravated the symptoms and caused them to re-appear even after they had disappeared for several days. The principal pharmacological effect of dimercaprol is the transfer of lead from the blood and certain tissues to other tissues. The authors conclude that the de-leading effect is relatively small when the unfavourable effects of changes in the internal distribution of lead are taken into consideration. H. T. B.

αα-Diphenyl-γ-dimethylaminovaleramide hydrogen sulphate, a Synthetic Atropine Substitute. A. Wollum and H. M. Pollard. (*J. Lab. clin. Med.*, 1951, **38**, 238.) This report is a summary of studies undertaken to evaluate the effect of αα-diphenyl-γ-dimethylaminovaleramide hydrogen sulphate (BL-139) on certain phases of fasting gastric secretion and gastric and small bowel motility. It is very similar to atropine in its effect on gastric secretory volume, free hydrochloric acid, and *p*H. The effect of both this drug and atropine on gastro-intestinal motility (gastric as measured by balloon studies and roentgenogram, and small bowel as measured by roentgenogram) appears similar, that is, a moderate but not complete or sustained inhibition of motility. Clinical side-reactions are similar to those of atropine, and the incidence of these side-reactions suggests that the dosage in clinical use should be similar to that of atropine in the majority of patients, namely, 0·5 mg. The available evidence does not suggest this is a better parasympatholytic agent than atropine.

S. L. W.

Hydroxyethylcarbamyl Group, Pharmacology of Compounds containing. R. Hazard, J. Cheymol, P. Chabrier, Y. Gay and M. P. Muller. (*Ann. pharm. franc.*, 1951, **9**, 390.) The authors give methods of preparing compounds containing the β-hydroxyethylcarbamyl group from benzylamine, phenylethylamine and phenyl*iso*propylamine and also compounds in which the carbamyl group is replaced by an amido group. They tested their pharmacological actions as to:—(1) toxicity to white mice, (2) local anæsthetic action on the cornea of the rabbit, (3) effect on the nervous system of the rat (excitement, depression, sleep), (4) changes in the arterial pressure of the chloralised dog. Compared with the amines the β-hydroxyethyl carbamates have less toxicity, possess a very marked anæsthetic action, produce depression instead of excitement and reduce instead of increasing the blood pressure. The anæsthetic action is notable, β-hydroxyethyl benzylcarbamate ($C_{10}H_{13}O_3N$) has an anæsthetic action equal to that of procaine, with a toxicity of one quarter, β-hydroxyethyl phenylethyl carbamate ($C_{11}H_{15}O_3N$) has twice the anæsthetic action and half the toxicity, and β-hydroxyethyl-phenyl *iso*propyl carbamate has twice the anæsthetic action and two-thirds of the toxicity. They are soluble in water and lipids and are neither basic, nor ionisable nor hydrolysable like esters. They have considerable chemical stability, and their hypotensive action is less than that of procaine. The esterification of the carbamates by succinic or maleic acids noticably increases the toxicity and diminishes their anæsthetic action, suppresses the action on the central nervous system and weakens the hypotension. The sodium salts of the corresponding succinamides show reduction in the toxicity and complete disappearance of anæsthetic action and of effects on the central nervous system and on blood pressure. H. D.

Mepacrine Hydrochloride in Tæniasis. W. A. Sodeman and R. C. Jung. (*J. Amer. med. Ass.*, 1952, **148**, 285.) The use of mepacrine hydrochloride in the elimination of *Tænia saginata* in 11 cases of tæniasis has been investigated. A dose of 0·6 to 0·8 g. was administered by giving 2 tablets of 0·1 g. every 5 minutes, with water, until the entire dose was taken. If the drug had previously produced nausea and vomiting, sodium bicarbonate was added to the water and the medication repeated. The drug was effective in 10 patients on initial trial, and in the 11th when treatment was repeated. The only toxic reactions of importance were the nausea and vomiting which in general were easily controlled. J. R. F.

Mephenesin Carbamate, Pharmacology of. P. E. Dresel and I. H. Slater. (*Proc. Soc. exp. Biol., N.Y.*, 1952, **79**, 286.) Mephenesin carbamate, 3-*o*-toloxy-2-hydroxypropyl carbamate (MC2303) is similar to mephenesin in potency and activity, but appears to have a longer duration of action, using the effectiveness of the two compounds against the extensor phase of maximal electric shock pattern as a criterion. Studies in animals were made to ascertain whether the mode and site of action of the compounds were comparable. In mice given the drug the pinna reflex disappeared before the corneal, a sign considered characteristic of mephenesin-like activity, and anti-convulsant actions in mice appeared similar. In spinal and anæsthetised cats both compounds showed a selective depression of the multisynaptic flexor reflex. In rabbits doses of both compounds causing a smooth reversible paralysis caused comparable slowing in the brain-wave frequency with an increase in amplitude. The action is not considered to be the result of degradation to mephenesin. S. L. W.

Mephenesin, Effect of on Spastic Paralysis. D. R. Laurence. *Lancet*, 1952, **262**, 178.) Mephenesin given by mouth, in the form of an elixir containing 1 g. in 15 ml., to the limit of tolerance was of benefit to only 2 out of 27 patients with various types of spastic paralysis, though it altered neurological signs in many cases without improving the patients' performance. Mephenesin intravenously, in a dose of 1 g. in 5 per cent. solution, produced good muscular relaxation of short duration in each of 10 cases of spastic paralysis without diminishing voluntary power, but it is useless for therapeutic purposes not only because of the brevity of its action but also because of the side-effects such as sleepiness and dizziness which are severe enough to prevent the patient taking advantage of his temporary release from spasticity. Nystagmus was also invariably present for a few minutes after each injection. S. L. W.

Mercurial Diuretics, Pharmacology of New. C. A. Handley, D. Chapman and J. H. Moyer. (*Proc. Soc. exp. Biol., N.Y.*, 1951, **78**, 433.) The compounds selected for investigation from a new series of diuretics were 3-chloromercuri-2-methoxypropylurea (compound I), 3-carboxymethylmerapto-mercuri-2-methoxypropylurea (II) and 3-(α-carboxyethylmercaptomercuri)-2-methoxypropylurea (III). Diuretic activity and effect on sodium excretion were studied on dogs after intravenous injection, the urine being collected from a retention catheter at 20 minute intervals. Meralluride (mercuhydrin) was used as the standard diuretic. Sodium determinations were made by a flame photometer. Acute cardiac effects were measured by the electrocardiogram. Chronic toxicity was investigated by measuring the glomerular filtration rate at monthly intervals after doses of 1 mg. of Hg./kg. of body-weight 3 times weekly. Tubular function was determined from the maximal rate of tubular reabsorption of dextrose. Diuretic activity of each compound was 3 to 4 times as much as that of meralluride; the maximum effect was slightly delayed but the duration of action was about the same. The rates of excretion of mercury approached that of meralluride. The chronic toxicity and the rate of excretion were about the same for the four compounds. Of 6 animals given large doses of compound III, one developed ventricular fibrillation but the other 5 showed no significant cardiac changes. 2 out of 6 animals given compound I also developed ventricular fibrillation and all showed some cardiac effects. Dogs given compound II showed no cardiac changes and all the compounds had a lower acute toxicity than meralluride. H. T. B.

Methoxamine, Clinical Observations on. M. H. Nathanson and H. Miller. (*Amer. J. med. Sci.*, 1952, **223**, 270.) Methoxamine, a synthetic

sympathomimetic compound is β-(2:5-dimethoxyphenyl)-β-hydroxy*iso*propylamine hydrochloride. It differs from adrenaline, noradrenaline and most other pressor amines in that it does not induce ventricular fibrillation or other arrhythmias even in the *cyclo*propane-sensitised heart of the experimental animal. This study was undertaken to determine its action on the rhythmic function of the human heart. Its effect was studied in 6 patients in whom it was possible to induce cardiac standstill of 5 seconds or longer by compression of the right carotid sinus, in 6 patients with complete heart block, and in 20 patients with sinus rhythm. The drug was given by intravenous injection in a dose of 5 to 10 mg. and the rhythm was studied from the electrocardiogram. It was concluded that methoxamine in doses producing a pronounced pressor reaction does not increase the rhythmic function of the human heart. This is shown by the following: (*a*) it does not prevent the carotid sinus-induced cardiac standstill; (*b*) it does not increase the ventricular rate in heart block; (*c*) there is a definite slowing of the heart rate following intravenous administration of the drug. The bradycardia is abolished or prevented by atropine. The pronounced vagal effect induced by the drug can be employed in the treatment of supraventricular tachycardia. Because of the absence of a cardiac stimulating action it may be of value in the treatment of certain hypotensive states.

S. L. W.

Nisin in Experimental Tuberculosis. E. M. Bavin, A. S. Beach, R. Falconer and R. Friedmann. (*Lancet*, 1952, **262**, 127.) Crude and purified preparations of nisin, the antibiotic produced by *Streptococcus lactis*, were dissolved in 0·02N hydrochloric acid, and the solutions after adjustment to *p*H 4 were administered by injection in the treatment of experimental tuberculosis in mice. Previous work had shown that *in vitro* nisin compares favourably with streptomycin against *Mycobacterium tuberculosis* under the conditions of the experiments. The results of the experimental *in vivo* tests were assessed by the increase in survival time and by the protection against corneal inoculation of *Myco. tuberculosis* when the antibiotic was given by intraperitoneal injections twice daily, each of 25,000 units, for 18 days. Only one of 5 groups of 10 animals showed any significant increase in survival time and in none of 18 mice was any protection demonstrable by the corneal method. Further tests were carried out *in vivo/in vitro* by the Brownlee method. Guinea-pigs were given a large dose and when signs of serious distress were apparent, or after 2 hours, blood was taken from the heart and part was assayed for nisin, the remainder being tested against *Myco. tuberculosis* by serial dilution. The supernatant liquors from preparations of lungs, spleen and liver homogenised with 0·02N hydrochloric acid were also assayed. In these tests the heart blood suppressed the growth of *Myco. tuberculosis* in only one instance; the blood contained 500 units/ml. and this was sufficiently toxic to kill the animal. The tissue extracts contained only 50 to 70 units/ml. The authors conclude that the activity of nisin is reduced by serum so that *in vitro* results are not repeated *in vivo*. The best nisin-producing organism so far obtained produces only about one-thirtieth of the quantity of antibiotic produced by organisms yielding penicillin and streptomycin. As the cost is appreciably higher, nisin is unlikely to find a place in therapeutics.

H. T. B.

Pheniodol; Elimination in Normal Subjects. H. O. Bang and J. Georg. (*Acta Pharmacol. Toxicol.*, 1951, **7**, 321.) Experiments on three healthy persons showed that pheniodol given orally (3 g.) is very readily absorbed from the intestine and then mainly excreted in the urine. The major part is excreted

in a few days but the excretion continues for several days. In this it differs from iodophthalein, which is excreted mainly in the fæces. The reason for this difference is not clear, though it may be that attachment to the plasma protein has some bearing on the problem, iodophthalein being completely bound to the proteins, whereas in these experiments about 14 per cent. of pheniodol was found ultrafiltrable. After intravenous injection of pheniodol (1 mg./kg. of body weight was given to 7 healthy persons) a plasma concentration of about 2 mg./100 ml. was obtained, which decreased to about half this value in 4 to 6 hours. Measurable amounts of pheniodol were present for 48 to 96 hours after injection. Quantitative balance tests were made in 2 healthy persons given 3 g. pheniodol orally and in 1 given 500 mg. intravenously. After oral administration 64 and 72 per cent. were recovered, after intravenous injection 80 per cent. S. L. W.

Phenylboric Acid, Toxicity of. F. Caujolle, C. Franck, P. Gayrel and G. Roux. (*Thèrapie*, 1951, **6**, 366.) Phenylboric acid may be prepared by the condensation of butyl borate with phenyl magnesium bromide, hydrolysis with dilute acid and purification to remove products of side reactions (for example, diphenyl). The LD50 for mice was found to be 560 mg./kg. and for guinea pigs, 284 mg./kg., both by intraperitoneal injection. For dogs, the toxicity by slow intravenous injection depends upon the speed with which the injection is given. The lethal dose administered during 1 hour was 450 mg./kg. Phenylboric acid has previously been shown to augment the action of hypnotics such as barbiturates and thiobarbiturates. It appears that its toxicity is small enough to permit its pharmacological use. G. B.

Prantal, A New Parasympathetic Blocking Agent, Pharmacology of. S. Margolin, M. Doyle, J. Giblin, A. Makovsky, M. T. Spoerlein, I. Stephens, H. Berchtold, G. Belloff, and R. Tislow. (*Proc. Soc. exp. Biol., N.Y.*, 1951, **78**, 576.) Prantal is the methyl sulphate of *N*:*N*-dimethyl-4-piperidylidene-1:1-diphenylmethane, one of a new series of quaternary compounds having autonomic ganglion blocking properties and therefore of possible interest in the management of peptic ulcer. Prantal appears to act primarily upon that portion of the parasympathetic nervous system which is associated with gastric secretion and motility. In doses eliciting this selective action it produces no mydriasis in animals and rarely produces mydriasis or xerostomia in man, even in doses inhibiting gastric motility for several hours. The intravenous sympathetic ganglion blocking dose is 50 to 100 times that required for parasympathetic ganglion blocking. After oral administration to dogs it delays the gastric emptying time more effectively and for a longer period than methantheline bromide; it also reduces the volume and titratable total acid of the gastric secretion. In rabbits, the minimum mydriatic concentration on topical application to the eye is 2 per cent. whereas 0·1 per cent. of methantheline bromide is mydriatic. Intravenously, doses of up to 8 mg./kg. of body weight produce no mydriasis in rabbits whereas methantheline bromide is mydriatic in doses of 1 mg./kg. of body weight. H. T. B.

Streptomycin, *p*-Aminosalicylic acid and Thiacetazone in Genito-Urinary Tuberculosis. J. Cosbie Ross, J. G. Gow and C. A. St. Hill. (*Lancet*, 1951, **260**, 1033.) The effects of streptomycin alone, streptomycin and *p*-aminosalicylic acid, and streptomycin, *p*-aminosalicylic acid and thiacetazone on tuberculosis of the genito-urinary tract were investigated. Genito-urinary tuberculosis was divided into 5 types, according to the Medical Research

Council classification, and the criteria for diagnosis and control of disease (as opposed to cure) were carefully laid down. Since streptomycin resistance develops rapidly in an acid medium, efforts were made to keep the urine alkaline with potassium citrate, although this proved difficult. Streptomycin alone in this series was given in a total dosage of 90 g. as 0·5 g. twice daily for 90 consecutive days. Results were disappointing as were those when streptomycin, in the same dosage, and *p*-aminosalicylic acid, 15 g. daily, were given. There was a slight improvement when streptomycin and *p*-aminosalicylic acid were given alternately for 6 months, each being given for a month alone, streptomycin 0·5 g. twice daily, and *p*-aminosalicylic acid 15 g. daily. 6 patients with the most severe type of genito-urinary tuberculosis were treated with streptomycin, *p*-aminosalicylic acid and thiacetazone combined. Streptomycin and *p*-aminosalicylic acid were given alternately as before and thiacetazone 50 mg. daily initially, gradually increasing to 200 mg. daily, the whole course lasting 6 months. Results with this regime showed an encouraging improvement and the authors intend to use it for all future cases. 5 g. of potassium iodide was added to the alkaline mixture given with the streptomycin, following reports of its value in increasing the effectiveness of streptomycin against the tubercle bacillus in laboratory animals and in pulmonary lesions. The three drugs mentioned should be used as an adjunct, not a substitute, for surgery. Every case should have sanatorium treatment, and surgery should be performed under streptomycin cover. Tabulated results are given of the various regimes employed.

G. R. B.

Streptomycin in Ophthalmology. A. Sorsby, J. Ungar and N. L. Bailey. (*Brit. med. J.*, 1952, 1, 119.) The clinical results obtained with streptomycin in 53 cases of ocular infection seen during the past 3 years are recorded. In 6 cases of acute conjunctivitis due to penicillin-resistant organisms, mainly *Proteus vulgaris*, local applications including drops, 0·5 g./ml. used half-hourly, ointment, 0·1 g./g., and subconjunctival injection, 0·5 g. in 10 ml. with 0·3 ml. adrenaline solution, rapidly effected a cure. Several cases of infected corneal ulcer were successfully treated with daily subconjunctival injections of 0·5 g. of streptomycin in a solution containing atropine sulphate 1·6 mg., cocaine hydrochloride 8 mg., water to 0·6 ml., 0·3 ml. of adrenaline solution being added immediately before the injection was given. One case each of tuberculous conjunctivitis and of dacryocystitis due to *Pr. vulgaris*, and 3 of tuberculous keratitis were cured by systemic treatment with streptomycin. Among the conditions believed but not proved to be of tubercular origin, Eales's disease, iridocyclitis and scleromalacia perforans were not significantly affected by systemic streptomycin. In phlyctenular ophthalmia, systemic streptomycin cut short severe attacks and appeared to reduce the frequency and the severity of relapses. Experimental evidence from the use of subconjunctival injections in the treatment of *Pseudomonas pyocyanea* infection of the eye in rabbits showed that the intraocular streptomycin levels are higher and are maintained for a longer period if the injection contains adrenaline.

H. T. B.

Terramycin in Treatment of Gonorrhœa. R. R. Willcox. (*Brit. med. J.*, 1951, 2, 527.) Only 2 relapses were observed in 19 cases given 2 oral doses, each of 1 g., of terramycin spaced 6 hours apart. Single doses of 1 to 2 g. of terramycin orally were unsuccessful however in 3 out of 6 cases. In this small series terramycin, in double doses, was shown to be not quite as effective as penicillin at its best, though more prolonged schedules remain to be tried. While it may not perhaps replace penicillin in the treatment of gonorrhœa,

it is likely to have a considerable application for those who dislike, or are unable to obtain, injections, for cases resistant to penicillin, and as an "emergency kit" for both treatment and prophylaxis of travellers and soldiers. Like penicillin, however, it may mask undeclared syphilis. S. L. W.

Triethylene Melamine in the Treatment of Neoplastic Disease. D. A. Karnofsky, J. H. Burchenal, G. C. Armistead, Jr., C. M. Southam, J. L. Bernstein, L. F. Craver and C. P. Rhoads. (*Arch. intern. Med.*, 1951, **87**, 477.) Triethylene melamine (2:4:6-triethylenimino-*s*-triazine) is a new compound which closely resembles the nitrogen mustards both in its effects on normal and neoplastic tissues and in the essential chemical grouping necessary for its specific biological activity. It is a white, crystalline powder, readily soluble in water. Solutions should be prepared in water or normal saline solution, since triethylene melamine reacts with many substances to lose its pharmacological activity. In contrast to methyl-bis-(2-chlorethyl) amine, the nitrogen mustard in general use, it is relatively stable in an alkaline medium, but it will react rapidly at an acid *p*H. Intravenous injection of triethylene melamine produced evidence of transient improvements similar to those obtained with nitrogen mustard in Hodgkin's disease, lymphosarcoma and chronic lymphatic and myelogenous leukæmia. The daily intravenous dose was about 2 to 3 mg., diluted with normal saline to a concentration of 0·5 mg./ml., immediately before use, and a total course of treatment for an adult was 5 to 20 mg. In this dosage it rarely caused nausea or vomiting, or venous thrombosis. Careful control of treatment is necessary to prevent depression of hæmopoietic function. Oral administration produced temporary clinical improvement in Hodgkin's disease, lymphosarcoma, chronic lymphatic and myelogenous leukæmia and mycosis fungoides. It produced slight nausea with infrequent vomiting or no gastro-intestinal symptoms. Dosage varied widely but a course of triethylene melamine usually consisted of 20 to 40 mg. given in a 3 to 5 week period. If properly used, triethylene melamine orally appears to be a valuable addition to the chemical palliatives now being used for the treatment of certain types of neoplastic disease. G. R. B.

Tromexan, Clinical Experience with. M. R. Bronstein and E. Witkind. (*Amer. J. med. Sci.*, 1951, **222**, 677.) Tromexan was used in 50 cases of hæmorrhagic disease. Doses 6 times as high as for dicoumarol were required to reach therapeutic levels in 24 hours. Wide fluctuations in prothrombin time occurred especially in the first 7 days, and for this reason daily determination of the prothrombin time was essential. It is recommended that a reliable thromboplastic substance such as thromboplastin A be used and that results be expressed as prothrombin times in seconds. A single daily dose is generally satisfactory, but no correlation could be observed between dose and body weight. Cumulative phenomena were observed but were more rapidly dissipated than for dicoumarol, either by withdrawing the drug or by administering a water-soluble derivative of vitamin K. Tromexan should not be given when there is renal insufficiency, and resistance to the drug occurs occasionally. G. B.

Tromexan, Toxicology and Anticoagulant Action of. C. M. Gruber, Jr., K. S. Lee, Z. T. Lasziczenko and C. M. Gruber. (*Arch. int. Pharmacodyn.*, 1951, **87**, 402.) Oral and intraperitoneal administration of lethal doses of the drug produced death in mice, rats and some rabbits within 12 hours (immediate death). In other rabbits death was delayed, taking between 12 and 72 hours.

Immediate deaths were not characterised by hæmorrhagic complications in the three species, but by primary cardiac arrest. Delayed death in rabbits was due apparently to hæmorrhage into the cæcum. The dose and route of administration appear to influence the type of death in rabbits. Orally the drug produced immediate and delayed deaths. Intraperitoneally, with one exception, death was immediate. No sign of chronic toxicity developed in rats or dogs when the drug was given daily by the oral route over 3 months. In dogs, production of hypoprothrombinæmia and return to normal prothrombin levels was more rapid with tromexan than with bishydroxycoumarin. No absolute evidence that vitamin K_1 and menaphthone sodium bisulphite antagonise the hypoprothrombinæmia produced by tromexan was obtained. J. R. F.

Unsaturated Carbinols, a New Class of Hypnotics. S. Margolin, P. Perlman, F. Villani and T. H. McGavack. (*Science*, 1951, **114**, 384.) The simple unsaturated aliphatic alcohols were found to possess high hypnotic activity, desirable duration of action, and low toxicity, and 3-methyl-pentyne-ol-3 was considered suitable for extensive pharmacodynamic, biochemical, and clinical study. The hypnotic effect was characterised by the reaction pattern—sedation, loss of righting reflex, and sleep. Its hypnotic activity was compared with that of amylene hydrate, paraldehyde, phenobarbitone, phenobarbitone sodium, and presidon when oral doses were given to mice, dogs and man. A high selectivity of hypnotic action was shown by 3-methyl-pentyne-ol-3, because neither analgesic, anæsthetic nor antispasmodic activity could be demonstrated. It did not depress respiration, and caffeine given parenterally caused rapid recovery from the deep hypnotic state induced by overdoses. No undesirable after effects were observed on animals following overdoses, and the drug was also shown to possess low toxicity. The results of metabolic studies are recorded. In a clinical study of 134 subjects it was found to be highly active, without toxic effect, and free from undesirable side effects. A number of patients were given daily doses of the compound for more than 6 months without any untoward effects. Clinical laboratory tests indicated the absence of any pathological changes attributable to the drug. A. H. B.

Veratrine, Veratridine and Cevadine, Cardiac Stimulation by. M. de V. Cotten and R. P. Walton. (*Arch. int. Pharmacodyn.*, 1951, **87**, 473.) Injection of the alkaloids into open-chest vagotomised dogs produced cardiovascular effects which resembled those due to adrenaline discharged from the adrenals. Large doses of dibenamine hydrochloride blocked, and adrenalectomy reduced, these effects. Several successive doses of the alkaloids caused the death of some animals by ventricular fibrillation. Veratrine showed the greatest activity in this respect. A dose of 0·35 mg./kg. of veratrine, 0·07 mg./kg. of veratridine and 0·5 mg./kg. of cevadine produced similar effects to a dose of 3 μg./kg. of adrenaline and was called the "heart stimulant dose." With single "heart stimulant doses" each alkaloid produced strong skeletal muscle spasms despite the condition of deep barbiturate anæsthesia. Hypotensive effects may be produced with initial injections, but do not usually occur with repeated dosage. Single "heart stimulant doses" of veratrine and cevadine in intact dogs produce convulsive effects, parasympathetic manifestations and depression of external respiratory movement. Adrenaline-like effects without cardioacceleration were produced on isolated rabbit heart indicating a direct action by the drugs. The heart stimulant effects, however, are only obtained by doses which produce marked side effects and prospective clinical application seems remote. J. R. F.

LETTER TO THE EDITOR

The Determination of Santonin in Artemisia—Solubility Correction

SIR,—With reference to the letter of Mr. J. Isaacs published in your June number, I have to express regret for the mistake in the solubility correction to be added in the assay of santonica as described in my paper.[1] The figure should have been 0·046 g., and not 0·0064 g. as recorded. It was a clerical mistake, which unfortunately escaped my notice. In my earlier publication[2] the solubility correction was rightly recorded as 0·046 g., and this figure was used in later papers.[3,4] Minute traces of santonin are absorbed by the mixture of animal charcoal and kieselguhr used for removing the resinous colloidal impurities in the final purification of the santonin. Taking into consideration the solubility at 15° to 17° C. and the adsorption factor, I suggest 0·046 g. as the correction factor to be added to the final weight of refined crystals of santonin. I am most grateful to Mr. Isaacs for pointing out the error.

N. A. QAZILBASH.

Department of Botany,
Islamia College,
Peshawar.

June 6, 1952.

REFERENCES

1. Qazilbash, *J. Pharm. Pharmacol.*, 1951, **3**, 105.
2. Qazilbash, *Bull. Sci. Pharm.*, 1935, **42**, 133.
3. Qazilbash, *Quart. J. Pharm. Pharmacol.*, 1942, **15**, 328.
4. Qazilbash, *Ind. J. Pharm.*, 1943, **5**, 58.

ABSTRACTS (*Continued from page* 510)

Vitamin B_{12} from Fish, Hæmopoietic Effect of. K. Hausmann and K. Mulli. (*Lancet*, 1952, **262**, 185.) Concentrates of high microbiological vitamin B_{12} activity were prepared from fish solubles and parenterally administered to 4 patients with pernicious anæmia in relapse. The doses were equivalent to 100 and 120 μg. of vitamin B_{12} as microbiologically determined. There was no improvement in the clinical condition of any of the patients and no increase in the numbers of reticulocytes and red cells; the megaloblastic state of the bone marrow remained unchanged. After being treated with potassium cyanide for 8 days the concentrates became completely soluble in butanol and yielded the absorption spectra of vitamin B_{12}. Administration to 3 of the patients of doses equivalent to 50 and 60 μg. of vitamin B_{12} from this preparation resulted in rapid clinical improvement in the clinical condition, high reticulocytosis and increase in the number of red cells to normal levels within 3 weeks; the megaloblasts and giant myelocytes of the bone marrow disappeared. It is concluded that the hæmopoietically inactive red pigments are peptide conjugates of vitamin B_{12} which can be utilised for the growth of lactic acid bacteria but not in the intermediate metabolism of patients with pernicious anæmia, and that treatment with potassium cyanide releases vitamin B_{12} from peptide linkage. S. L. W.

PHARMACOPŒIAS AND FORMULARIES

SUPPLEMENT 1952 TO THE BRITISH PHARMACEUTICAL CODEX 1949.

By LLOYD C. MILLER, PH.D.

Director of Revision, The United States Pharmacopeia

The Supplement, 1952, to the B.P.C. 1949 is a commendable reflection of the prodigious rate of advancement of modern therapeutics. Like the parent volume, it is divided into 7 parts. Chemotherapeutic substances and antibiotics form the greater part of the 36 additions to Part I. Most of these added drugs are now in wide use, but apparently were not sufficiently proved to warrant their inclusion in the B.P.C. 1949. Of the new monographs, 14 have been added to bring the Codex into conformity with the British Pharmacopœia Addendum 1951. All but one of this latter group relate to substances developed in recent years, the exception being methacholine hydrochloride. Injection of Globin Zinc Insulin is the only long-established preparation given a monograph in the 22 other additions. The new non-pharmacopoeial substances include an antibiotic (aureomycin), 3 muscle-relaxants (gallamine triethiodide, mephenesin and decamethonium iodide), Vitamin B_{12} (cyanocobalamin), and two absorbable hæmostatics (oxidised cellulose and absorbable gelatin sponge). The comprehensive paragraphs on "Actions and Uses" will be of great use to those to whom the Codex is an everyday working tool. The vaccines for acne, dysentery (Flexner), staphylococcus and tuberculin were named in the British Pharmacopœia, 1948, but the Addendum, 1951, did not include the monographs for them. Hence, Part II of the Supplement deletes these vaccines in accordance with official policy. In Part III the monographs on human blood preparations, introduced in the 1949 Codex, having now been made official in the 1951 Addendum to the B.P., are here suitably amended to conform with the official requirements. Part V of the Supplement, Surgical Dressings, permits the use of rayon-cotton mixtures in some of the dressings where cotton only was allowed hitherto. A new bandage, "Rayon and Rubber Elastic Bandage," is included. Many changes appear in Part VI (The Formulary). In the formulation of the guttae the percentage of medicament has been altered to give a round figure in the metric system with the consequence that the Imperial formulæ involve quantities quite awkward to weigh. A good example is Guttæ Atropinæ Sulphatis, which now requires $4\frac{2}{3}$ gr. of alkaloid sulphate to make 1 fl. oz. as against 4 gr. (0.91 g.) before. Attention given to palatability and presentation of preparations for children answers questions which have arisen in the Symposium Sessions of the last two British Pharmaceutical Conferences. The book includes in its 148 pages new data on the densities of a number of volatile oils and galenicals and also modifications of certain tests and assay processes for inorganic chemicals. It is well bound, thoroughly indexed, and deserves attention not only as the complement to the B.P.C. 1949 but in its own right for the information it provides.