The Journal of
PHARMACY
and
PHARMACOLOGY

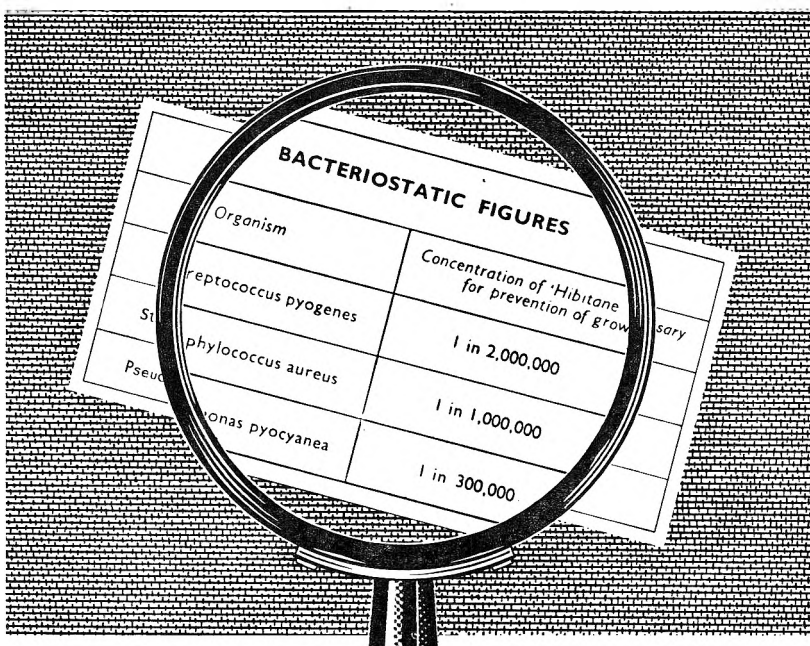
VOLUME VII No. 7 JULY, 1955

Published by Direction of the Council of

THE PHARMACEUTICAL SOCIETY OF GT. BRITAIN

33 BEDFORD PLACE, LONDON, W.C.1

An entirely NEW anti-bacterial agent for local application



'Hibitane'

Trade Mark **(Chlorhexidine)**

'Hibitane' Concentrate 2%

Specially prepared to be diluted for ordinary antiseptic purposes. Mildly detergent and anti-rusting.

'Hibitane' Powder

For the preparation of pure aqueous and alcoholic solutions, incorporation in creams, ointments, etc.

'Hibitane' Antiseptic Cream 1%

For lasting disinfection of the hands and prevention of cross infection. Also as a beneficial skin application.

'Hibitane' Obstetric Cream 1%

A non-irritant antiseptic lubricant for vaginal examinations. Combines rapid and complete antibacterial action with persistent effect.

References:

1 *Brit. Med. J* (1955) *i.* 81. *ibid* (1955) *i.* 985

2 *Brit. J. Pharmacol. Chemother.* (1954) 9. 192

IMPERIAL CHEMICAL (PHARMACEUTICALS) LIMITED · Wilmslow · Manchester

A subsidiary company of Imperial Chemical Industries Ltd.

Ph.539

The Journal of

PHARMACY *and* PHARMACOLOGY

Successor to The Quarterly Journal of Pharmacy and Pharmacology

33 BEDFORD PLACE, LONDON, W.C.1

Telephone: CHAncery 6387

Telegrams: Pharmakon, Westcent, London

Editor: George Brownlee, D.Sc., Ph.D., F.P.S.

Annual Subscription **50s.** Single Copies **5s.**

Vol. VII. No. 7 **July, 1955**

CONTENTS PAGE

[*Continued on page ii*

CONTENTS

PAGE

Research Papers—*continued*

Abstracts of Scientific Literature

REVIEW ARTICLE

STEREOISOMERISM AND BIOLOGICAL ACTION

By A. H. Beckett, B.Sc., Ph.D., F.P.S., F.R.I.C. and
A. F. Casy, B.Sc., F.P.S., A.R.I.C.

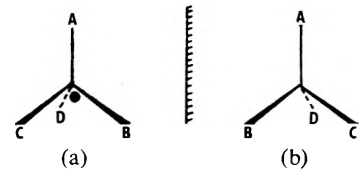
*Chelsea School of Pharmacy, Chelsea Polytechnic, London, S.W.*3

The relation of chemical structure to biological activity has long been the subject of extensive chemical and pharmacological investigations. That stereochemical factors also play an important role in the determination of biological action is evident from the existence of so many stereoisomeric pairs of compounds in which the members of each pair differ considerably in the magnitude of their biological effects. The purpose of this review is to discuss the features which can result in biological discrimination between isomers, and to present information concerning classes of compounds in which activity is greatly dependant upon stereochemical factors.

Stereoisomers are defined as isomeric substances which differ only in the geometrical arrangement (configuration) of their atoms or groups. Stereoisomerism may be subdivided into optical and geometrical isomerism.

Optical isomerism

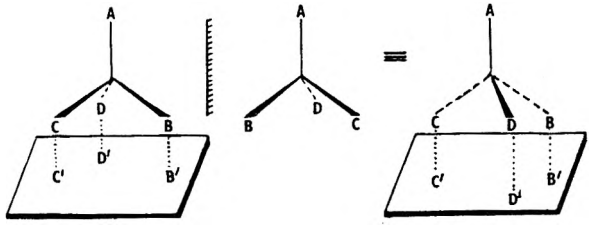
Optical isomers (enantiomorphs) may be defined as stereoisomers in which the atoms or groups comprising the compound are arranged in two different ways to form two molecular species which differ from one another only as an object differs from its mirror image (see Fig. 1 in which A, B, C and D represent groups or atoms attached to a carbon atom (asymmetric carbon atom); (a) and (b) are thus enantiomorphs. Such isomers commonly arise from the presence of an asymmetric centre within the molecule, although this feature is not essential for molecular dissymmetry. Optical isomers differ in their action on plane-polarised light. They have identical chemical properties except in their reactions with other optically active molecules. Furthermore, the combination of enantiomorphs with another optically active substance gives two products (diastereoisomers) which are not related as object to mirror image, and may exhibit large differences in physical properties such as solubility, partition coefficients and reactivity. One other important feature arises from the difference in the arrangement of groups in enantiomorphs as follows. If three of the groups attached to an asymmetric carbon atom are aligned towards a particular surface, then



(a) (b)

Fig. 1. —— represents in the plane of the paper, ——— represents pointing towards and - - - represents pointing away from the observer.

a similar alignment of the same three groups is not possible in the enantiomorph (see Fig. 2). The value or direction of the optical rotation of enantiomorphs has almost no significance from a configurational and thus an "alignment to a receptor surface" point of view.

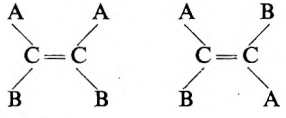


(a) (b) (b)

FIG. 2. Alignment of enantiomorphs a and b to a receptor surface. C, D and B represent groups in the enantiomorphs and C' D' and B' represent their points of alignment at the surface.

Geometrical isomerism

Geometrical isomers may be defined as stereoisomers in which the molecular species are not related as object to mirror image. This type of isomerism may result when rotation within the molecule is restricted, for example by double bonds (Fig. 3) or by rigid (or semi-rigid) ring systems (Fig. 4). Usually, certain groups in a geometrical isomeric pair of compounds are separated by different distances.

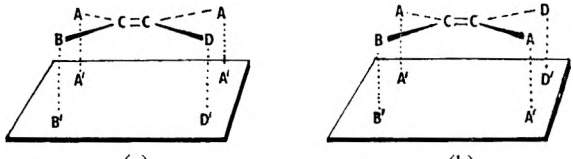


cis-isomer *trans*-isomer

FIG. 3

FIG. 4

Geometrical isomers may differ greatly in physical properties such as solubilities, partition coefficients, dissociation constants, etc. Frequently, differences in chemical properties are also very marked, especially those involving the participation of the groups which are separated by different



(a) (b)

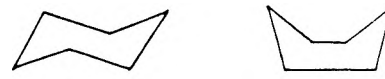
FIG. 5. Alignment of geometrical isomers a and b to a receptor surface. A, B and D represent groups in the isomers and A', B' and D' represent their points of alignment to the surface.

distances in the isomers., e.g., maleic acid with two carboxyl groups in proximity to each other readily forms an anhydride whereas its geometrical isomer, fumaric acid, does not. The difference in the relative positions of certain groups results in the failure of geometrical isomeric

pairs to orient such groups similarly to a receptor surface. For example, if the *cis*-isomer (A groups *cis*) (Fig. 5a) is held at a particular surface by forces involving both A groups, then the *trans*-isomer (*b*) cannot present these groups in a similar manner, and differences in the adsorption of the isomers will be apparent. However, both the isomers shown can present groups A and B in similar ways. It follows that a "three-point" reception of three of the four groups will involve discrimination in the adsorption of the isomers.

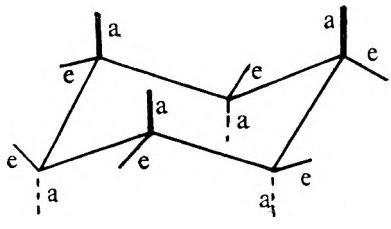
Conformational considerations

In recent years, a further aspect of stereoisomerism which has important implications in the observed differences of the biological activities of geometrical isomers has received considerable attention, namely the conformational aspect (see Barton[1], Klyne[2] and Orloff[3] for detailed accounts). Conformational analysis is the study of the different arrangements in space of atoms or groups in a single classical organic structure (configuration), e.g., the chair (I) and boat (II) conformations of *cyclo*hexane. The energy barriers between the various conformations are not sufficiently high, in the examples so far examined, to allow of the isolation of two separate conformations of the same classical configuration.



(I) (II)

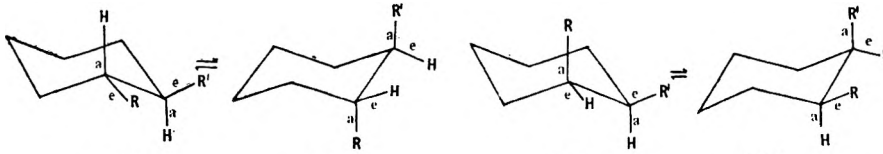
In general, for the 6-membered carbocylic or heterocyclic ring structures, the chair conformation is the more stable one. This obtains because, although the boat and chair forms are equally strain free in the classical sense, the mutual repulsions of neutral non-bonded atoms results in the chair form being the more stable since the distance between the non-bonded atoms is at a maximum. The C—H bonds of the chair conformation of *cyclo*hexane are of two different types, 6 bonds which lie approximately in the general plane of the ring and are designated equatorial (e) bonds, and 6 bonds which are perpendicular to the general plane of the ring, 3 pointing in one direction and 3 in the other; these are designated axial (a) bonds (see III).



(III)

In the absence of strong electrostatic effects, the most stable conformation of a molecule composed of six-membered alicyclic rings will be built up of chair forms with the larger groups in equatorial positions, e.g., a *trans*-1:2-disubstituted *cyclo*hexane isomer will have the chair conformation (IV) rather than the alternative chair form (V) in which the non-bonded interactions are greater. [Conformations (IV) and (V) can be interconverted by the rotation and twisting (not the breaking of bonds).]

A *cis*-1:2-isomer, in which R′ is a larger group than R, will have the conformation (VI) rather than (VII).

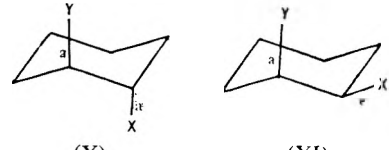


(IV) (V) (VI) (VII)

Many facts concerning the thermodynamic stability and the reactivity of epimeric alicyclic compounds (i.e., geometric isomers in which inversion occurs by the interchange of positions of an H atom and another group —see (VIII) and (IX)—can be explained in terms of the equatorial and axial conformations of the groups concerned. For example, equatorial hydroxyl groups are thermodynamically more stable than axial ones, and when a polycyclic secondary alcohol is heated with alkali ("equilibrated"), the isomer with an equatorial hydroxyl group is present in larger amount in the reaction mixture; ecgonine (axial carboxyl group) is isomerised by alkali to ψ-ecgonine (equatorial carboxyl group). However, in a consideration of the possible explanation of the differences in biological actions of isomers, the effects of conformations upon the course and rate of reactions are of greater interest. The differences in the reactions of various conformations are due to differences in the accessibility of the groups concerned, or differences in the steric requirements of the reactions which may be satisfied to a greater or less extent by the particular conformation.

(VIII) (IX)

A few of the observed differences in reactions of axial and equatorial groups which may explain differences in biological action of certain isomers are here enumerated. Axial groups are subjected to greater steric hindrance (from the axial hydrogen atoms or groups on the β-carbon atom) than the corresponding equatorial ones. This is reflected in the following differences in reactions: equatorial hydroxyl groups are more readily esterified than the corresponding axial ones; equatorial acyloxy groups are more readily hydrolysed than axial groups; equatorial carboxyl groups are more readily esterified and the product more readily hydrolysed than the corresponding axial groups; axial secondary alcoholic groups are more readily oxidised (attack upon the C—H bond is the rate determining step) than corresponding equatorial groups.



(X) (XI)

The observed differences between the reactions of various conformations upon the demand of a reaction with steric requirements is illustrated in the following example. Bimolecular elimination reactions will only proceed readily if the four centres of importance in the reaction lie in one plane. This condition is satisfied by a 1:2-*trans*-disubstituted *cyclo*hexane in which both groups are axial or are able to adopt the

axial conformation (X), but not by the corresponding *cis*-compound (XI), in which one group must necessarily be axial and the other equatorial.

Before considering the implications of the above differences between enantiomorphs and between geometrical isomers and their various conformations upon biological activities, a brief consideration of the various factors which may influence the latter activities is included (see Albert[4], Sexton[5] and Danielli[6] for a detailed treatment).

Biological Action of Molecules

Biologically active compounds may be broadly classified into those which are *structurally non-specific* and those which are *structurally specific*. The first type have a general non-selective action upon tissues or enzyme systems, e.g., the depressant action of chloroform, bromoform and trichloroethylene; compounds of this type usually can give the same biological response although differing greatly in chemical constitution—their mechanism of action is probably a physico-chemical one. The second type probably act at specific receptor sites in tissue or enzyme systems to form reversible complexes, the dissociation constants of which are affected by the closeness of fit of the drug to the receptor.

The biological response upon the presentation of a reagent to a living organism is dependent upon (*a*) the access of the molecule to the site of action, and (*b*) the reaction of the molecule at the site, and many factors may influence the result. Some of the more important of these factors which may be affected by stereochemical features of molecules are given below.

Penetration of membranes. The membranes surrounding all cells are composed of layers of lipoids and proteins and are strongly charged. Ions, because of their charge and their relatively greater size due to hydration, penetrate these membranes less readily than the corresponding neutral molecules.

Adsorption at surfaces. Adsorption may be indiscriminate or specific. Adsorption of soaps which are adsorbed on any surface irrespective of its chemical nature is an example of indiscriminate adsorption; in this type, neutral molecules are usually held more firmly than ions. The specific type of adsorption usually involves the mutual attraction of unlike charges (cation for anion and *vice versa*) reinforced by the short range van der Waals forces which require close fitting complementary surfaces to be effective.

Chemical reactivity. Not only will this factor influence the attack upon tissue or enzyme system by which a biological response may be mediated, but it will affect the metabolism and the distribution of a molecule within an organism.

Degree of ionisation. At a given *p*H value, the proportion of ions to neutral molecules is dependent only upon the pKa of the ionisable groups. Because ions and neutral molecules usually behave differently in their penetration of membranes, their adsorption at surfaces, their lipoid solubility and sometimes their chemical reactivity, any stereo-chemical factor which can influence the pKa of a group of a molecule which is partially

ionised at physiological *p*H may have a profound effect upon the biological action.

Lipoid solubility. This factor will not only influence the penetration of membranes by a reagent, but will influence the degree of localisation of a substance. It has a great effect especially upon structurally non-specific agents.

Steric factors. Such factors can influence the chemical reactivity of a group within the molecule and affect especially the specific adsorption of molecules at surfaces.

Differences in Behaviour of Stereoisomeric Pairs of Compounds

The individuals of stereoisomeric pairs of compounds can exhibit features which may influence the above factors differently and so lead to a discrimination in the biological responses. The differences in the summation of the above factors for the isomers may be considered as leading to (1) differences in the distribution of the isomers, (2) differences in the properties of the isomer-drug receptor combination and (3) differences in the strength of attachment or "fit" of the isomers to a complementary drug receptor surface.

1. *Differences in the Distribution of the Isomers*

Two isomers which inherently possess identical biological effects if presented in equal concentrations to the site of action, will give an overall difference in biological response in the whole organism if concentration differences are produced before the molecule arrives at the site, whether the compound be structurally specific or structurally non-specific.

Optical isomers. A difference in the distribution of enantiomorphs may result from their combination with another optically active substance to give diastereoisomers of differing solubilities which will affect the penetration of membranes or the solubilities in various tissues.

Distribution will also be affected by preferential destruction or metabolism of one of the enantiomorphs by a dissymmetric enzyme system, e.g., after the administration of racemic mepacrine, optically active mepacrine can be detected in the urine[7]; (−)-5-ethyl-5-phenylhydantoin is stated to have a more powerful "anæsthetic" action than the (+)-isomer[8] and this may result from the greater rate of metabolism of the latter since it has been shown that it disappears more rapidly from rat plasma than its enantiomorph[8]; enzymes which are present in pig and rat kidney hydrolyse the acyl-L-amino-acids much more readily than their enantiomorphs[9].

It is also possible to explain the above observations in terms of preferential adsorption (or absorption) of one isomer at a surface in the organism (see later) leading to concentration differences. This phenomenon is well known *in vitro*. Porter and others[10] showed (+)-isomers of certain dyes were held more strongly by wool than their enantiomorphs. More recently, Bradley and Easty[11] have shown that wool and casein selectively absorb (+)-mandelic acid and (+)-α-naphthylglycollic acid from aqueous solutions of their respective racemic mixtures. Resolution of (±)-*p*-phenylenebisiminocamphor and "Tröger's base" by the selective adsorptive action of lactose has been reported[12,13].

Geometrical isomers. Large differences in physical and chemical properties exhibited by geometrical isomers can readily affect their distributions. Explanations of many of the former differences can be given in terms of spatial separation of groups or accessibility of groups and it may well be that these effects can be correlated with discriminations in biological responses. For example, the fact that the pKa_1, of ψ-ecgonine is greater than that of ecgonine, but the pKa_2 of ψ-ecgonine is lower than that of ecgonine can be explained in terms of the closer proximity of the amino and carboxyl groups in ψ-ecgonine[14]. Such differences could have great effects upon the distribution of geometrical isomeric compounds if the isomers were partially ionised at physiological *p*H.

The difference in the conformation of epimers with its consequent effect upon the rate of chemical reactivities could also result in concentration differences, e.g., *epi*cholesterol (axial-OH) is more readily oxidised than cholesterol (equatorial—OH)[15]; only those cyclitols with axial hydroxyl groups are oxidised by *Acetobacter suboxidans*[16].

Geometrical isomers, unlike optical ones, can readily be separated by adsorption on optically inactive materials, e.g., chromatography upon alumina. Consequently, differences in the adsorption of the isomers could lead to concentration differences at sites of action. The differences in the adsorptive forces upon the isomers is sometimes explicable in terms of conformations of groups, e.g., in the chromatography of steroids it has been shown that the epimers with an equatorial hydroxyl group are adsorbed more strongly than the corresponding axial epimers[17].

2. *Differences in the Properties of the Drug-receptor Combination*

Many biological responses are now attributed to the reversible "combination " of the molecule in question with one or more "receptors" in tissue or enzyme system. It is not implied that combination alone produces the response, but that a suitable combination may initiate, modify or block a series of interdependent chemical processes. Ionic forces, hydrogen bonding and van der Waals forces are probably involved in the "combination" of drug and receptor.

Optical isomers. It is possible for enantiomorphs to be held equally strongly to a receptor site (e.g., Fig. 1—assume that the groups C and B only involved in the "combination," or alternatively C, B and D but that the receptor "combines" with D equally strongly although differently orientated to the receptor surface in the two isomers) and yet nevertheless to exhibit differences in biological action due to the differences in properties of the two drug-receptor combinations. This explanation of the difference in effects of enantiomorphs was developed by Cushny[18]. He suggested that the two combinations were analogous to diastereoisomeric forms and thus would be expected to exhibit different properties of solubility, etc., as for instance the combinations of (+)- and (−)-tartaric acid with (+)-cinchonidine. In the case of the constrictor action of (−)- and (+)-adrenaline on the vessels of the conjunctiva, it may be considered that the more active (−)-isomer readily produces a

reaction at the neuro-effector cell junction, whereas the less active (+)-isomer only does so when present in a higher concentration than the former[18].

The wide differences in properties exhibited by geometrical isomers renders unlikely the possibility that they could be held equally strongly at a receptor site, and consequently the difference in properties of the drug-receptor combination is unlikely to be a factor worthy of consideration in an explanation of any observed discrimination in biological response.

3. *Differences in the "Fit" to a Complementary Receptor Surface*

It is possible to account for the difference in biological activities of certain enantiomorphs in terms of a "three-point" combination of groups attached to an asymmetric carbon atom with three areas of the receptor surface. Figure 2 illustrates the fact that only one enantiomorph could present the three groups in the correct relative positions; the other isomer could only have two groups correctly aligned. All the groups do not need to be ionic or reactive, e.g., the "three-point" attachment groups for analgesic drugs have been postulated[19] as a flat aromatic ring, a basic group (ionised) and a projecting hydrocarbon moiety.

The differences in the biological effect of enantiomorphs in a series of compounds only becomes of established significance in terms of alignment to areas in a specific receptor surface, if the configurations of the biologically more active enantiomorphs of the series are shown to be identical.

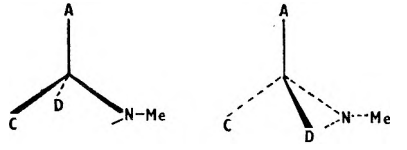
It is possible that the biological discrimination between enantiomorphs can be accomplished in a number of ways involving the presentation of three "groups" to the surface, and these are outlined below.

1. All three groups are essential for the binding of the molecule to the receptor site, the areas of the latter being so orientated that only one enantiomorph fits correctly. It follows that the other enantiomorph will be inactive, and since it does not "fit" the surface, will not antagonise the action of the active one even if presented in higher concentrations than the latter.

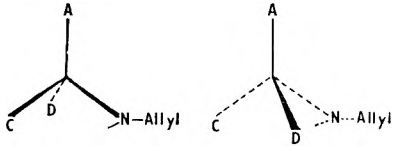
2. Three groups are involved, but the intensity of the biological action is dependent solely upon the ease of combination of the enantiomorphs with the receptor; one enantiomorph "fits" better than the other. This hypothesis was emphasised by Easson and Stedman[20]. The less active one will not antagonise the biological effect of the more active one. (However, if the nature of the drug-receptor complex is also involved in the biological action, it would be possible for the lesser active enantiomorph, in high concentrations, to antagonise the more active one in lower concentrations). It is possible that slight modifications of the highly active isomer might yield compounds of antagonistic action because they could fit the surface correctly, but fail to evoke the reaction sequence which gives the particular biological response. Assume, for

example, that an N—Me group was one of the groups involved and demethylation occurred in the reaction. An N—Allyl derivative of the active enantiomorph could "fit" the receptor correctly but would fail to evoke the reaction sequence and, in suitable concentrations, could block the site and thus act as an antagonist. A similar derivative of the less active enantiomorph would act only as a weak antagonist. (See Fig. 6).

3. Two groups are directly involved in the "combination" of the molecule with the receptor (Fig. 7a and b; C and B), and the "third group" (Fig. 7a and b; Me), improves the combination when correctly,

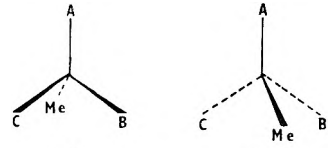


active enantiomorph — less active enantiomorph

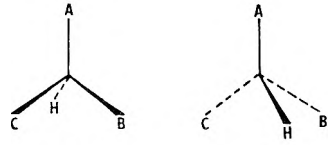


strong antagonist — weak antagonist

FIG. 6



(a) (b)



(c) (d)

FIG. 7

but hinders when incorrectly, orientated; consequently the isomers have different activities. It is unlikely that the less active isomer would antagonise the activity of the more active one. The replacement of the "third group" by one of the same type which did not alter the physical properties such as lipoid solubility or dissociation constant of the molecule, e.g., replacement of $-CH_3$ by $-H$ or $-C_2H_5$ in certain circumstances, would be expected to yield compounds (Fig. 7c and d) with activities less than the more active enantiomorph, but greater than the less active one.

It is recognised that the above subdivisions of the type of "three-point" reception are somewhat arbitrary. In practice, the actual mechanism involved in the mediation of the biological effect may result from a combination of the above types to a greater or lesser extent, complicated by the effect of the nature of the drug-receptor combination.

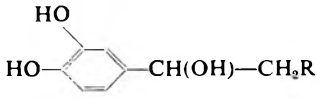
Geometrical Isomers. In Figure 5 it was shown that there were differences between the isomers in the orientation of groups to a receptor surface if three groups were involved. Consequently, arguments apply similar to those used above. The differences in the distance between two groups in the isomer can also lead to discrimination if these have to "fit" two centres in the receptor, e.g., Baldridge *et al.*[21] have used the

enzymatic responses to *cis*- and *trans*-isomers in the *cyclo*hexane series to provide information concerning the distance between the esteratic and anionic sites of the acetylcholinesterase surface.

In the remainder of this review, attention will be given to series of compounds, the members of which exhibit a common biological action which has been shown to be greatly dependent upon the stereochemical features of the molecules concerned.

Sympathomimetic Agents

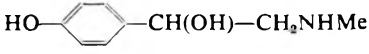
Sympathomimetic substances mimic the effects of stimulation of the sympathetic nerve fibres. An important member of the group is adrenaline (XIIa) which possesses one asymmetric carbon atom and occurs naturally as the (−)-isomer. The (+)-form, obtained by synthetic methods, has been shown to be from 12 to 20 times less active than the (−)-isomer[22]. The relative effects upon systolic blood pressure of the enantiomorphs of both noradrenaline (XIIb) and isoprenaline (XIIc) have been determined and, in each case, the (−)-isomer is found to



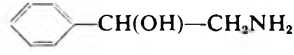
(XII)

R = —NHMe (a), —NH_2 (b), —NH*iso*C_3H_7 (c)

possess the greater activity[23,24]. Replacement of the 3:4-dihydroxyphenyl moiety of adrenaline by the 4-hydroxyphenyl or the phenyl group leads to a reduction in pressor activity. In two compounds of this type (XIII, synephrine, and XIV), the (−)-isomers are again found to be more active than their antimers[25,26].



(XIII)●



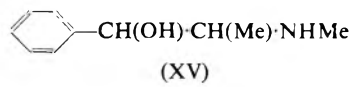
(XIV)

Easson and Stedman[20] accounted for the different activities of the adrenaline antimers in terms of a difference in their ease of attachment to a receptor surface. They suggested that only the (−)-isomer can come into complete contact with the surface, and that the (+)-isomer behaves as if one of the groups necessary for maximum activity is missing. In adrenaline and related compounds, three structural features appear to be essential for maximum pressor activity, namely, a basic centre, a phenyl group and an alcoholic hydroxyl group. The above hypothesis is supported by the fact that (+)-adrenaline has an activity approximating to that of desoxyadrenaline, in which one essential group, the alcoholic hydroxyl group, is missing. Further support has been provided by Schaumann[27] who found that the (+)-isomer of corbasil [1-(3:4-dihydroxyphenyl)-2-aminopropan-1-ol] has approximately the same activity as desoxycorbasil [1-(3:4-dihydroxyphenyl)-2-aminopropane], both compounds being about 160 times less active than (−)-corbasil.

Furthermore, both the (+)-isomer and the desoxy-compound exhibit the same qualitative differences from (−)-corbasil.

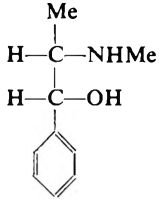
Although configurational studies have not been carried out in this series, Dalgliesh[28] has presented much indirect evidence indicating that (−)-adrenaline and (−)-noradrenaline possess the same configuration.

Ephedrine (XV) and related compounds possess pharmacological actions that are similar to those of adrenaline but which differ in duration and mechanisms of action. The stereochemistry of ephedrine is more complex than that of adrenaline in that the former molecule possesses one additional asymmetric carbon atom and exists in two diastereoisomeric forms (ephedrine and ψ-ephedrine). As with adrenaline, there are marked differences in the biological activities of the various enantiomorphs[29]. Natural (−)-ephedrine is approximately 3 times as active as the (+)-form, whereas the (+)-form of ψ-ephedrine possesses 7 times the activity of its antimer. The (−)-form of norephedrine has 1·5 times the pressor activity of the (+)-isomer, the enantiomorphic ψ-norephedrines being equal in activity[30,31].

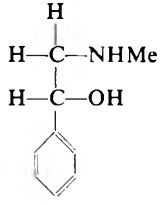


(XV)

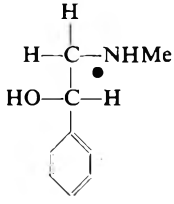
Considerable investigations have been carried out to establish the configurations of the two asymmetric centres of the enantiomorphs of this series[32,33]. It is significant that those enantiomorphs which exhibit the greater pressor activity possess identical configurations with respect to the carbon atom bearing the basic group. On the basis of the *lævo*-rotation of both natural adrenaline and (−)-ephedrine, Freudenberg[34] assigned the configuration (XVI) to (−)-adrenaline. Dalgliesh[28], however, from indirect chemical evidence, considers (−)-adrenaline to be related in configuration to L-(+)-mandelic acid (XVIII) and thus possesses the opposite configuration to that proposed by Freudenberg.



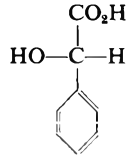
(−)-ephedrine



(XVI)



(XVII)

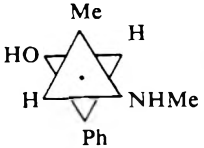


(XVIII)

If configuration (XVII) be subsequently confirmed, the difference between the configurations at the hydroxyl bearing carbon atom of (−)-adrenaline and (−)-ephedrine may be of significance in the interpretation of the pharmacological results.

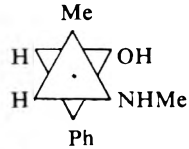
Fodor *et al.*[35] have shown the rate of acyl-migration in *N*-benzoyl-(±)-ψ-ephedrine to be far greater than the rate in the corresponding ephedrine compound. They conclude that, in the latter compound, the —OH and —NHMe groups are relatively distant from one another, whereas in ψ-ephedrine they are relatively close. Similar results were obtained, and like conclusions drawn, for norephedrine and ψ-norephedrine.

Close[36] has presented further chemical evidence to support these contentions and has proposed (XIX) and (XX) as the most probable conformations of (−)-ephedrine and (+)-ψ-ephedrine that represent the resting states of the molecules.



(−)-ephedrine

(XIX)

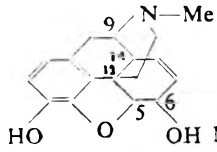


(+)-ψ-ephedrine

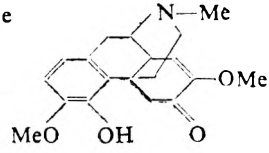
(XX)

Analgesics

(*a*) *Morphine and related compounds.* The structure of morphine (XXI) proposed by Gulland and Robinson[37] and recently conclusively confirmed by synthesis[38], possesses five asymmetric centres ($C_{(5)}$, $C_{(6)}$, $C_{(9)}$, $C_{(13)}$ and $C_{(14)}$). The naturally occurring (−)-isomer has not been compared in activity with its (+)-enantiomorph, now potentially available by the synthesis of Gates and Tschudi[38]. Certain morphine derivatives have, however, been obtained in their enantiomorphic forms by synthesis



(XXI)

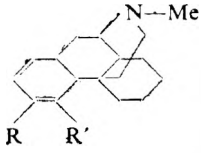


(XXII)

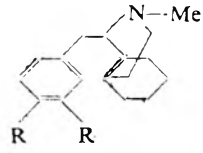
from the alkaloid sinomenine (XXII), a substance which, apart from the absence of the oxygen bridge, possesses a ring system identical to that of morphine. Moreover, the configurations of the asymmetric centres $C_{(9)}$, $C_{(13)}$, $C_{(14)}$ and $C_{(5)}$ (when generated), are the reverse to those of morphine, and on this account, sinomenine affords a route to substances that bear a mirror image relationship to the corresponding morphine derived compounds. Thus Takebe and Kitasato[39] have prepared (+)-dihydrocodeinone, (+)-dihydrothebainone, (+)-tetrahydrodesoxycodeine, (+)-dihydrothebainol, (+)-1-bromo-sinomeninone and (+)-dihydrosinomeninone, all of which are found to be active convulsants that do not show the analgesic action of the corresponding morphine derivatives. Goto and Arai[40] report (+)-dihydromorphine, derived from sinomenine, to be equally powerful a narcotic as morphine, but do not specify its analgesic properties.

(*b*) *Morphinan and related compounds.* Racemic *N*-methyl-morphinan (XXIII; R = R′ = H), prepared by Grewe and Mondon[41] by cyclisation of compound (XXIV; R = H), represents a synthetic analgesic that possesses the same molecular skeleton as morphine. Furthermore, its steric identity with morphine has been shown by Grewe *et al.*[42] in the synthesis of tetrahydrodesoxycodeine (XXIII; R = OMe, R′ = OH) by cyclisation of compound (XXIV, R = OMe), the (−)-isomer being identical with (−)-tetrahydrodesoxycodeine, prepared from codeine. The relative activities of the enantiomorphic forms of the potent analgesic

3-hydroxy-*N*-methylmorphinan (Dromoran, racemorphan XXIII, R = OH, R′ = H), prepared by a similar cyclisation process, have been studied. The (−)-isomer (levorphan) has approximately the same toxicity but a higher analgesic action than the racemic compound, while



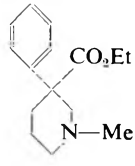
(XXIII)



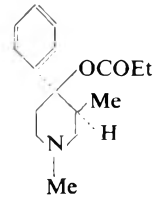
(XXIV)

the (+)-isomer (dextrorphan) is less toxic and inactive[43]. Levorphan is also a greater respiratory depressant than dextrorphan. The (+)-, (−)- and (±)-methyl ethers of Dromoran (XXIII, R = OMe, R′ = H) exhibit parallel analgesic characteristics, although they are less potent and more toxic than the parent compounds[44].

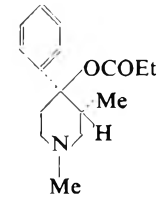
(*c*) *Pethidine and related compounds.* Pethidine itself is a symmetrical molecule but several asymmetric modifications have been obtained during



(XXV)



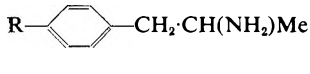
(XXVI)



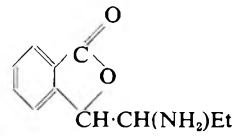
(XXVII)

the synthesis of pethidine type compounds. Macdonald *et al.*[45] reported that racemic nor-*iso*pethidine (XXV) is one quarter, and the (−)-isomer one half as active as pethidine, while the (+)-isomer is inactive. The highly active analgesic 1:3-dimethyl-4-phenyl-4-propionyloxypiperidine has been obtained in two geometrically isomeric forms, in which the propionyloxy and methyl groups are respectively *cis* and *trans* (configurations assigned only provisionally[46]). Pharmacological results on rats show the *cis*- form (XXVI) to be from 5 to 6 times more potent than the *trans*-modification (XXVII). Furthermore, the *cis*-form has been resolved and the (−)-isomer found to be more than twice as active as its antimer[47].

(*d*) *Aralkylamines.* A systematic investigation of aralkylamines by Fellows and Ullyot[48] resulted in several examples of asymmetric compounds in which the various forms showed differences in analgesic activity. (±)-Amphetamine (XXVIII, R = H) is found to possess weak analgesic properties while the (−)-isomer is inactive. The (±)- and (+)-forms of the corresponding *p*-hydroxy derivative (XXVIII, R = OH) possess marked analgesic properties, the (−)-isomer showing only slight activity. Ullyot and his colleagues[49] showed that 1-amino-1-phthalidylpropane (XXIX) possesses considerable activity. This compound,



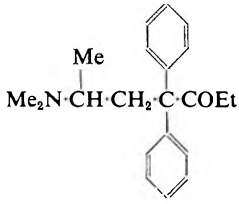
(XXVIII)



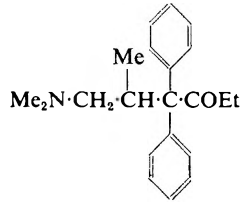
(XXIX)

which contains two asymmetric centres, was separated into two racemic mixtures, one of which was found to be more active than the other.

(*e*) *Methadone and related compounds.* Methadone (XXX) itself possesses one asymmetric centre and its resolution was first reported by Thorp, Walton and Ofner[50] who found the (−)-isomer to be the more active form. This fact was soon confirmed by other workers and the (−)-isomer shown to be approximately 20 times as active as the (+)-form[51,52]. The optical isomers of *iso*methadone (XXXI), obtained by Larsen *et al.*[53], show parallel differences in activity. The enantiomorphic forms of ethyl 4-dimethylamino-2 : 2-diphenylpentanoate (XXXII) have

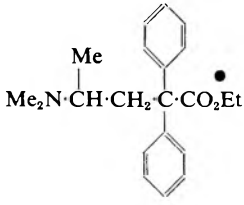


(XXX)

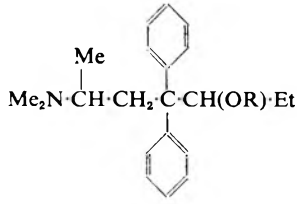


(XXXI)

been prepared from (+)- and (−)-3-dimethylamino-1 : 1-diphenylbutyl cyanide respectively. Chen[54] reports the (+)-isomer to be 7 times as active as the (−)-form. Reduction of the ketonic group of methadone



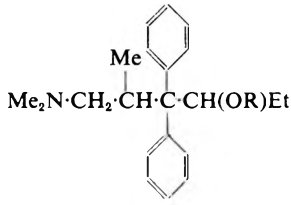
(XXXII)



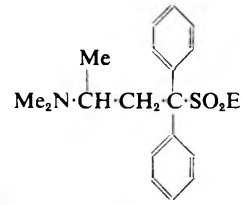
(XXXIII)

to a secondary alcohol introduces a second asymmetric centre. Catalytic hydrogenation, or treatment with lithium aluminium hydride, gives only one of the two possible racemic mixtures, but reduction with sodium and propanol gives both forms (α- and β-methadol, XXXIII, R = H). Reduction of (+)- and (−)-methadone has made available the four possible optical isomers of methadol. The pharmacology of these isomers has been studied by Eddy *et al.*[55] who found α-(−)- and β-(−)-methadol to be from 7 to 8 times as active as their respective enantiomorphs. The corresponding *O*-acetyl derivatives (XXXIII, R = Ac) show greater analgesic properties than the parent compounds. α-(+)- and

β-(−)-acetylmethadol are respectively 6 and 10 times as active as their enantiomorphs[55]. It is significant that, of the four enantiomorphic pairs, the most active member is derived, in three cases, from analgesically active (−)-methadone. The enantiomorphic forms of the *iso*methadols (XXXIV, R = H), and their acetyl derivatives (XXXIV, R = Ac), also exhibit differences in analgesic properties[56]. It



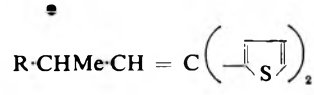
(XXXIV)



(XXXV)

is to be noted that, in this series, the three compounds showing significant analgesic activity, namely, β-(+)-*iso*methadol, β-(−)- and α-(+)-acetyl*iso*methadol, are all derived from (−)-*iso*methadone. Thus, in both the methadol and *iso*methadol series, the more analgesically active isomers are derived, with the exception of α-(−)-methadol, from the more analgesically active enantiomorph of the parent compound. Replacement of the —COEt group of methadone by the ethyl sulphone group (—SO_2Et) gives a compound of comparable analgesic activity[57]. The sulphone (XXXV) has been resolved and the (−)- found to be 20 times as active as the (+)-isomer[58].

(*f*) *The Dithienylbutenylamines.* This most recently developed group of analgesics, comparable in activity with methadone, also provides examples of enantiomorphic pairs which differ in their analgesic activity. (+)-3-Dimethylamino (XXXVI, R = NMe_2), (+)-3-diethylamino (XXXVI, R = NEt_2) and (+)-3-pyrrolidino-1 : 1-di-(2′-thienyl)-but-1-enes have been shown to be more active than their corresponding (−)-antimers[19,59].



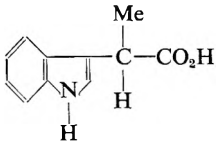
(XXXVI)

The importance of spatial configuration in analgesics is most clearly demonstrated in those compounds possessing one asymmetric carbon atom. In order to obtain evidence of the stereochemical requirements of analgesics, the present authors carried out configurational studies among a group of analgesics of this type[60]. (−)-(XXX), (−)-(XXXV), (+)-(XXXVI, R = NMe_2) and (+)-(XXXVI, R = NEt_2), each substance representing the more analgesically active member of an enantiomorphic pair, were correlated by unequivocal stereospecific routes and shown to possess identical configurations related to D-(−)-alanine. It was shown[19] that these relationships, together with the evidence of analgesic antagonists, provide support for the hypothesis that a substance must possess an overall optimum spatial arrangement of groups in order to show analgesic activity. Evidence was presented for the probability of the activity exhibited by analgesics and their antagonists being due to

association with a specific receptor site, and the differences in analgesic activity between members of enantiomorphic pairs explained in terms of this hypothesis. Highly active analgesics were shown to possess structures which allow of their association with a proposed "analgesic receptor surface," the essential features of which were described.

Plant Growth Substances

It has long been established that substances exist in the tips of growing seedlings which cause the cells to elongate with consequent increase in size of the plant. In 1934, Kögl[61] isolated a substance with pronounced plant growth regulating activity identified as 3-indoleacetic acid. Stereochemical aspects soon received attention when it was found that (+)-α-(3-indole)-propionic acid (XXXVII) was about 30 times more active than the (−)-isomer[62]. Koepfli, Thimann and Went[63], as a result of structure-activity studies, proposed the following generalisations as requirements for an active molecule.



(XXXVII)

(1) a ring system containing at least one double bond,

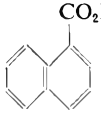
(2) a side chain possessing a $-CO_2H$ group (or a group easily converted into a $-CO_2H$ group),

(3) at least one carbon atom between the ring and the $-CO_2H$ group,

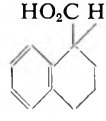
(4) a particular spatial relationship between the ring system and the $-CO_2H$ group.

The latter requirement, of special interest from a stereochemical viewpoint, is exemplified by the variation in activity found among certain *cis-trans* isomeric pairs. Thus, *cis*-cinnamic acid is active, while the *trans*-isomer is inactive[64]. *cis*-2-Phenyl-*cyclo*propane-1-carboxylic acid and *cis*- 1:2:3:4-tetrahydronaphthylidene-1-acetic acid are plant growth stimulating substances, the *trans* isomers in both cases being without activity[65,66].

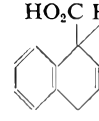
In the examples cited, molecular models reveal the ring and carboxylic acid groups to be almost planar in the *trans* and non-planar in the *cis* isomer. Veldstra[66,67] considered that the *cis* forms owed their biological activity to this factor and explained the increase in growth regulating activity which results on hydrogenating α-naphthoic acid (XXXVIII) to the 1:2:3:4-tetrahydro analogue (XXXIX) in the same terms[68]. This



(XXXVIII)



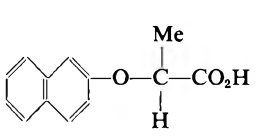
(XXXIX)



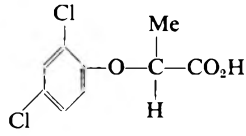
(XL)

hypothesis became inadequate, however, when it was found that the activity of the racemic compound (XXXIX) resided almost entirely in the (+)-isomer[68]. Likewise, the report of Mitsui[69], that the (+)-isomer of 1:4:-dihydro-1-naphthoic acid (XL) is more active than its enantiomorph, was unaccountable on these grounds. Wain and his

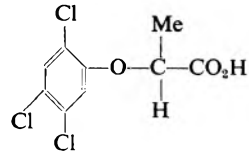
colleagues[70,71] have shown the (+)-isomers of α-(2-naphthoxy)-(XLI), α-(2:4-dichlorophenoxy)-(XLII) and α-(2:4:5-trichlorophenoxy) (XLIII)-propionic acids to be highly active growth substances, while the



(XLI)

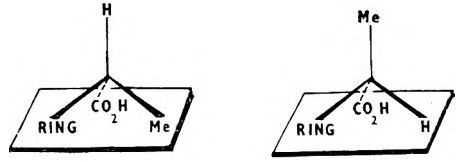


(XLII)



(XLIII)

corresponding (−)-isomers possess little or no activity. Wain[72] explained these results in terms of differences in the "fit" of enantiomorphs at a receptor site. The workers at Wye College have demonstrated the essential nature of the α-hydrogen atom in substances possessing growth stimulating activity, and Wain pointed out that an α-hydrogen atom, an unsaturated ring system, and a carboxyl group, make up three essential structural requirements for compounds of this type. These features must be orientated in a specific configuration in order that the molecule may "fit" the receptor surface. In aryloxycarboxylic acids, all three groupings are attached to an asymmetric centre and it follows that only one enantiomorph will be able to present the three groups in the correct relative positions to the surface and so initiate a growth response. (See Fig. 8: (a) represents the biologically active and (b) the inactive isomer).



(+)-isomer (a) (−)-isomer (b)

FIG. 8

Support for this theory has been provided both by the establishment of configurational identity among the more biologically active members of enantiomorphic pairs, and also by demonstration of the antagonism of certain optically active plant growth stimulating substances by their corresponding inactive enantiomorphs. Much of the evidence has been provided by Fredga and Matell[73,74] in the course of an intensive study of acids of type (XLIV), made with a view to establishing the stereochemical specificity of optically active plant growth substances. Many variants of the basic formula were prepared, and the configurations of the enantiomorphs determined, mainly by the method of quasi-racemates,

$$Ar—X—CHR—CO_2H$$

(XLIV)

Ar = Aromatic Moiety, R = alkyl group, X = O, S, NH or CH_2

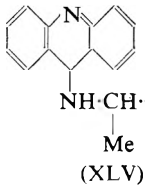
and related to optically active alanine and lactic acid. Thus (+)-α-phenoxy-, (+)-α-(4-chlorophenoxy)-, (+)-α-(2:4-dichlorophenoxy)-, (+)-α-(3:4-dichlorophenoxy)-, (+)-α-(2:4:5-trichlorophenoxy)-, (+)-α-

(2-methyl-4-chlorophenoxy)-, (−)-α-(1-naphthoxy)-, (+)-α-(2-naphthoxy)- and α-(1-chloro-2-naphthoxy)-propionic acids, (+)-α-phenoxy, (+)-α-(2:4-dichlorophenoxy)-, (−)-α-(1-naphthoxy)- and (+)-α-(2-naphthoxy)-butyric acids and (+)-α-(2-naphthoxy)-*n*-caproic acid, each substance being the more biologically active member of an antimeric pair, are all related to D-alanine. Although the configurations of the more active isomers of α-(1-naphthylmethyl)-, α-(2-naphthylmethyl)- and α-(2-naphthylsulphide)-propionic acids were not determined, the Swedish workers considered it very probable that these isomers also were related to D-alanine. Correlation between biological activity and configuration has similarly been reported by Mitsui[75] for (XL) and (XXXIX), and by Veldstra[68] for the latter acid and α-allylphenylacetic acid.

Evidence from antagonism studies has been obtained by Wightman[71] who examined (+)-α-(2-naphthoxy)-, (+)-(2:4-dichlorophenoxy)- and (+)-α-(2:4:5-trichlorophenoxy)-propionic acids in the presence of increasing amounts of their corresponding inactive enantiomorphs. In each case, he found that the inactive (−)-isomer could reduce and, with a high molar ratio, even eliminate the activity of the (+)-isomer in this test. Åberg, in the course of biological examination of enantiomorphic α-phenoxypropionic and α-naphthoxypropionic acids, tested their antagonistic action against 2:4-dichlorophenoxyacetic acid and demonstrated the antagonistic properties of (−)-α-(2-methyl-4-chloro-phenoxy)-, (−)-α-(2:4:5-trichlorophenoxy)-, (−)-α-(2-naphthoxy), (−)-α-(1-chloro-2-naphthoxy)-, (+)-α-(1-naphthylmethyl)-, (−)-α-(2-naphthylmethyl) and (−)-α-(2-naphthylsulphide) propionic acids, (−)-α-(2-naphthoxy)-*n*-butyric acid and (−)-α-(2-naphthoxy)-*n*-caproic acid[73]. Steward[76] has found a synergistic action to exist between the cocoanut-milk growth factor and plant growth stimulating substances. He showed that (+)-α-(2:4:5-trichlorophenoxy)- and (+)-α-(2-naphthoxy) propionic acids were highly active in this respect, whereas both of the corresponding (−)-isomers were completely inactive[72]. Furthermore, the inactive (−)-isomers antagonised the synergistic action of their enantiomorphs[72].

Antibacterials

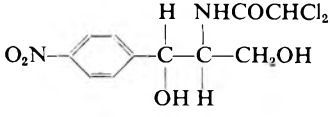
Many antibiotics have been shown to prevent the growth and reproduction of micro-organisms by interference with cell synthesis. It is possible that this action might be achieved by the introduction into the cells of "unnatural" D-amino-acids, such as are present in certain antibiotic polypeptides (e.g., gramicidin-D, tyrocidine and gramicidine-S). Support for this contention is provided by the antibiotic activity of synthetic penicillin derived from D-penicillamine (ββ-dimethylcysteine) and the inactivity of material obtained from the corresponding L-isomer[77]. Linnell and Smith[78] resolved compound (XLV), comprising acridine and an amino-acid moiety, and found the (+)-isomer to be twice as effective as the (−)-isomer in inhibiting the growth of *Staph. aureus* and *Strept. pyogenes* (configurations were not elucidated). Work[79], however, has



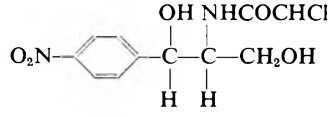
(XLV)

pointed out that the lack of evidence of the inhibitory effects of D-amino-acids upon protein synthesis, renders the above view of the role of these acids in antibiotic action unlikely. He considers it more probable that the configurations of the component amino-acids are of importance in determining "fit" of the antibiotic molecule at a specific receptor surface within an enzyme system.

Conclusive evidence of the stereospecificity of antibiotics has been provided by study of chloramphenicol and related substances. The four optical isomers of 1-*p*-nitrophenyl-2-dichloroacetamido-1 : 3-propanediol



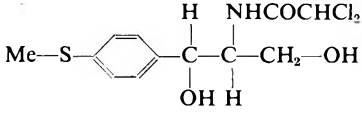
threo



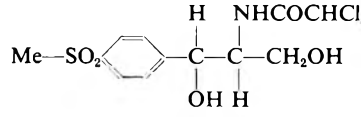
erythro

(XLVI)

(XLVI) have been obtained, and their configurations related to norephedrine and *ψ*-norephedrine[80,81]. While the D-(−)-*threo*-isomer (chloramphenicol) is a potent antibacterial agent, the L-(+)-*threo*, D-(−)-*erythro* and L-(+)-*erythro* isomers possess negligible activity[82]. Furthermore, the antibacterial activities of the racemic compound (XLVII), and its sulphone analogue (XLVIII), have been shown to



(XLVII)



(XLVIII)

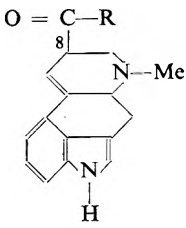
reside, in both cases, in the (+)-isomers, designated D-*threo* on the basis of rotational analogies with chloramphenicol[83,84].

Hahn, Wisseman and Hopps[85], in a study of the mode of action of chloramphenicol, pointed out the antipodal relationship between the D-(−)-*threo* isomer and the L-polypeptides whose formation is inhibited. If this relationship be significant, the D-(−)-*threo* isomer should have no influence on D-polypeptide synthesis; the latter should be inhibited, however, by the L-(+)-*erythro* analogue of chloramphenicol in virtue of an analogous but converse relationship. Hahn *et al.*[85] confirmed these conclusions by experiment. Formation of D-(−)-glutamyl polypeptide by *B. subtilis* was inhibited specifically by the L-(+)-*erythro* analogue of chloramphenicol but not by the antibiotic itself. The same stereoisomer had little effect on the growth of the test organism while chloramphenicol completely suppressed its growth at a low concentration. The D-(−)-*erythro* and L-(+)-*threo* isomers had no effect on either growth or polypeptide formation.

Miscellaneous

Physiologically active ergot alkaloids are all derivatives of lysergic acid (XLIX, R = OH); they occur in association with inactive stereoisomers, derived from *iso*lysergic acid. The parent acids are epimeric

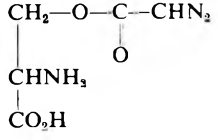
and differ only in the $C_{(8)}$-configuration, the latter, therefore, having a critical influence upon physiological activity. The configuration of the non-lysergic acid moiety (XLIX, R) does not appear to play such an important role in the determination of activity. Stoll[86] found ergometrine (XLIX, R = NH·CH(Me)·CH_2OH) derived from L-alaninol and material obtained from D-alaninol to possess equal potencies. However, when (+)-lysergic acid is combined with norephedrine, the compound with the natural (−)-form is found to be 20 times more active than that with the (+)-isomer.



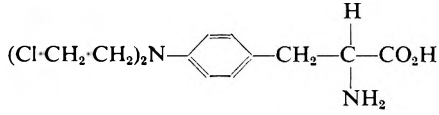
(XLIX)

Reports on the relative physiological activities of (−)- and (+)-thyroxine, although somewhat conflicting, establish the (−)-isomer to be the more active in a wide variety of tests., e.g., oxygen consumption and weight curves of rats[87]; metamorphosis of tadpoles[87,88]; reduction of hyperplastic thyroids[88]; prevention of pituitary basophil changes[89]. In contrast, Salter *et al.*[90] found the so-called calorigenic activities of (−)- and (+)-thyroxine in persons suffering from myxœdema to be nearly equal.

The amino-acid derivatives, *O*-diazoacetylserine (L) (L-isomer is azaserine, isolated from a *Streptomyces*) and *p*-di-(2-chloroethyl)amino-



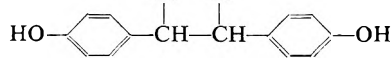
(L)



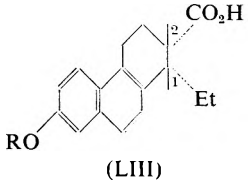
(LI)

phenylalanine (LI) possess antitumour activity; maximum activity has been shown to reside, in both cases, in the L-isomer[91,92].

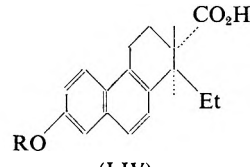
γδ-Bis-(4-hydroxyphenyl)-*n*-hexane exists in two optically inactive forms; the *meso* compounds (LII, hexœstrol), a potent œstrogen, and the much less active racemic mixture (*iso*hexœstrol). The latter has been resolved and the (+)-found to be 10 times more potent than the (−)-isomer[93]. Alkali fusion of (+)-equilenin gives rise to (−)-α- and (+)-β-bisdehydrodoisynolic acids. High œstrogenic activity depends on



(LII)



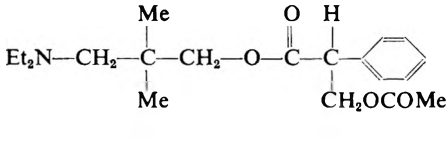
(LIII)



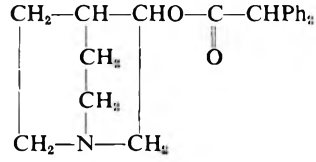
(LIV)

cis-orientation of $C_{(1)}$—Et/$C_{(2)}$—CO_2H, (LIII, R = H) possessing high activity while (LIV, R = H) is inactive[94]. The (−)-α-7-methylether

(LIII, R = Me) is equally active, the corresponding (+)-isomers of both α-compounds possessing virtually no œstrogenic properties[95].



(LV)



(LVI)

The antimeric forms of the synthetic spasmolytics (LV) and (LVI) show differences in activity; in both cases the (−)-isomer possesses the greater action[96,97]. Blood pressure depressants show similar differences; (+)-adenocarpine produces a greater fall in blood pressure in the cat than the (−)-form[98]; the depressant action of (±)-carnosine (formed from histidine and β-alanine) is due entirely to the L-isomer[99]. D-Cysteine is not as effective as L-cysteine in preventing the leucopenia and neutropenia induced by nitrogen mustard[100]. The nicotinolytic activity of (−)-Parsidol [N-(2-diethylaminopropyl)-phenothiazine] is twice that of the corresponding (+)-base[101].

The above examples of stereochemical specificity in biological action serve to indicate the importance of stereochemical investigations of biologically active structurally specific compounds. Although a particular biological response to a compound may be influenced by many factors, studies of the actions of enantiomorphs in which so many properties are identical, enables some of the fine structure of receptor surfaces to be delineated when similar configurations can be established for the more active members of enantiomorphic pairs exhibiting a particular biological action. The use of geometrical isomers which are dissymmetric also offers an approach to the elucidation of both the distances, and the orientation of specific receptor areas of the receptor site, if the differences in the physical properties of the geometrical isomers are not great. The combination of such studies with the investigation of the antagonistic action of stereoisomers, and the change in the biological effect upon alterations of the groups necessary for "combination" at the receptor, has great potentialities in the search to unravel the complexities of the mechanisms by which biological responses are mediated.

References

1. Barton, *J. chem. Soc.*, 1953, 1027.
2. Klyne, *Progress in Stereochemistry* I, Butterworth, 1954, p. 36.
3. Orloff, *Chem. Rev.*, 1954, **54**, 347.
4. Albert, *Selective Toxicity*, Methuen and Co. Ltd., 1951.
5. Sexton, *Chemical Constitution and Biological Activity*, Spon Ltd., 1953.
6. Danielli, *Cell Physiology and Pharmacology*, Elsevier, 1950.
7. Hammick and Chambers, *Nature, Lond.*, 1945, **155**, 141.
8. Butler and Waddell, *J. Pharmacol.*, 1954, **110**, 120.
9. Fu and Birnbaum, *J. Amer. chem. Soc.*, 1953, **75**, 918; Birnbaum, Levinton, Kingsley and Greenstein, *J. biol. Chem.*, 1952, **194**, 455; Fodor, Price and Greenstein, *ibid.*, 1949, **178**, 503.
10. Porter and Hirst, *J. Amer. chem. Soc.*, 1919, **41**, 1264; Porter and Ihrig, *ibid.*, 1923, **45**, 1990.

11. Bradley and Easty, *J. chem. Soc.*, 1951, 499; 1953, 1519.
12. Hendersen and Rule, *ibid.*, 1939, 1568.
13. Prelog and Wieland, *Helv. Chim. Acta*, 1944, **27**, 1127.
14. Beckett and Walker, unpublished.
15. Vavon and Jacubowicz, *Bull. Soc. chim. Fr.*, 1933, **53**, 581.
16. Magasanik and Chargoff, *J. biol. Chem.*, 1948, **174**, 173.
17. Savard, *Recent Progress in Hormone Research*, 1954, **9**, 197.
18. Cushny, *Biological Relations of Optically Isomeric Substances*, Balliére, Tindall and Cox, 1926.
19. Beckett and Casy, *J. Pharm. Pharmacol.*, 1954, **6**, 986.
20. Easson and Stedman, *Biochem. J.*, 1933, **27**, 1257.
21. Baldridge, McCarville and Friess, *J. Amer. chem. Soc.*, 1955, **77**, 739.
22. Tainter, *J. Pharmacol.*, 1930, **40**, 43.
23. Tainter, Tullar and Luduena, *Science*, 1948, **107**, 39.
24. Lands, Luduena and Tullar, *J. Pharmacol.*, 1954, **111**, 469.
25. Tainter and Seidenfeld, *ibid.*, 1930, **40**, 23.
26. Alles and Knoefel, *Univ. Calif. Publications in Pharmacology*, 1938, **1**, 101.
27. Schaumann, *Medicine in its Clinical Aspects*, 1938, **3**, 361.
28. Dalgliesh, *J. chem. Soc.*, 1953, 3323.
29. Chen, Wu and Henriksen, *J. Pharmacol.*, 1929, **36**, 363.
30. Swanson, Scott, Lee and Chen, *ibid.*, 1943, **79**, 329.
31. Jarowski and Hartung, *J. org. Chem.*, 1943, **8**, 564.
32. Freudenberg, Schoeffel and Braun, *J. Amer. chem. Soc.*, 1932, **54**, 234.
33. Leithe, *Ber. dtsch. chem. Ges.*, 1932, **65**, 660.
34. Freudenberg, *Stereochemie*, Deuticke, Leipzig, 1932, p. 697.
35. Fodor, Bruckner, Kiss and Ohegvi, *J. org. Chem.*, 1949, **14**, 337.
36. Close, *ibid.*, 1950, **15**, 1131.
37. Gulland and Robinson, *Mem. Proc. Manchester Lit. Phil. Soc.*, 1924–5, **69**, 79.
38. Gates and Tschudi, *J. Amer. chem. Soc.*, 1952, **74**, 1109.
39. Takebe and Kitasato, *Arch. exp. Med.*, 1934, **11**, 48.
40. Goto and Arai, *Liebigs Ann.*, 1941, **547**, 194.
41. Grewe and Mondon, *Ber. dtsch. chem. Ges.*, 1948, **81**, 279.
42. Grewe, Mondon and Nolte, *Liebigs Ann.*, 1949, **564**, 161.
43. Fromherz, *Arch. int. Pharmacodyn.*, 1951, **85**, 387.
44. Benson, Stefko and Randall, *J. Pharmacol.*, 1953, **109**, 189.
45. Macdonald, Woolfe, Bergel, Morrison and Rinderknecht, *Brit. J. Pharmacol.*, 1946, **1**, 4.
46. Ziering and Lee, *J. org. Chem.*, 1947, **12**, 911.
47. Randall and Lehmann, *J. Pharmacol.*, 1948, **93**, 314.
48. Fellows and Ullyot, *Medicinal Chemistry*, Vol. I, Suter, 1951, p. 390.
49. Ullyot, Taylor and Dawson, *J. Amer. chem. Soc.*, 1948, **70**, 542.
50. Thorp, Walton and Ofner, *Nature, Lond.*, 1947, **160**, 605.
51. Bochmühl and Ehrhart, *Liebigs Ann.*, 1948, **561**, 52.
52. Pohland, Marshall and Carney, *J. Amer. chem. Soc.*, 1949, **71**, 460.
53. Larsen, Tullar, Elpern and Buck, *ibid.*, 1948, **70**, 4194.
54. Chen, *Ann. N.Y. Acad. Sci.*, 1948, **51**, 83.
55. Eddy, May and Mosettig, *J. org. Chem.*, 1952, **17**, 321.
56. May and Eddy, *ibid.*, 1952, **17**, 1210.
57. Klenk, Suter and Archer, *J. Amer. chem. Soc.*, 1948, **70**, 3846.
58. Tullar, Wetterau and Archer, *ibid.*, 1948, **70**, 3959.
59. Green, *Brit. J. Pharmacol.*, 1953, **8**, 2.
60. Beckett and Casy, *J. chem. Soc.*, 1955, 900.
61. Kögl, Haagen-Smit and Erxleben, *Z. Physiol. Chem.*, 1934, **228**, 90.
62. Kögl and Verkaaik, *ibid.*, 1944, **280**, 167.
63. Koepfli, Thimann and Went, *J. biol. Chem.*, 1938, **122**, 763.
64. Haagen-Smit and Went, *Proc. Koninkl. Akad. Wetenschap*, Amsterdam, 1935, **38**, 852.
65. Veldstra and van de Westeringh, *Rec. trav, Chim. Pays-Bas*, 1951, **70**, 1127.
66. Veldstra, *Enzymologia acta Biocatalytica*, 1944, **11**, 137.
67. Veldstra, *Biochem. Biophys. Acta*, 1947, **1**, 364.
68. Veldstra and van de Westeringh, *Rec. trav. Chim. Pays-Bas*, 1951, **70**, 1113.
69. Mitsui, *J. agric. Chem., Soc. Japan*, 1951, **25**, 186.
70. Smith and Wain, *Proc. Roy. Soc.* (B), 1951, **139**, 118.
71. Smith, Wain and Wightman, *Nature, Lond.*, 1952, **169**, 883; *Ann. appl. Biol.*, 1952, **39**, 295.

72. Wain, *Royal Institute of Chemistry Monograph*, 1953, No. 2.
73. Matell, *Stereochemical Studies on Plant Growth Substances*, Almquist and Wiksells, Boktryckeri Ab, Uppsala, 1953.
74. Matell, *Arkiv. Für Kemi*, 1954, **7**, 437.
75. Mitsui, *J. agric. Chem. Soc. Japan*, 1952, **25**, 526.
76. Steward and Caplin, *Science*, 1951, **113**, 518.
77. du Vigneaud, Carpenter, Holley, Livermore and Rachele, *Science*, 1946, **104**, 431.
78. Linnell and Smith, *J. Pharm. Pharmacol.*, 1949, **1**, 28.
79. Work, *Biochem. Soc. Sympos.*, 1948, **1**, 73.
80. Rebstock, Crooks, Controulis and Bartz, *J. Amer. chem. Soc.*, 1949, **71**, 2458.
81. Fodor, Kiss and Sallay, *J. chem. Soc.*, 1951, 1858.
82. Maxwell and Nickel, *Antibiotics and Chemotherapy*, 1954, **4**, 289.
83. Cutler, Stenger and Suter, *J. Amer. chem. Soc.*, 1952, **74**, 5475.
84. Rebstock and Bambas, *ibid.*, 1955, **77**, 186.
85. Hahn, Wisseman and Hopps, *J. Bact.*, 1954, **67**, 674.
86. Stoll, *Experientia*, 1, 250.
87. Gaddum, *J. Physiol.*, 1930, **68**, 383.
88. Reineke and Turner, *Endocrinol.*, 1945, **36**, 200.
89. Griesbach, Kennedy and Purves, *Nature, Lond.*, 1947, **160**, 192.
90. Salter, Lerman and Means, *J. clin. Invest.*, 1935, **14**, 37.
91. Stock, Clarke, Reilly, Rhoads and Buckley, *Nature, Lond.*, 1954, **173**, 71.
92. Bergel and Stock, *J. chem. Soc.*, 1954, 2409.
93. Wessley and Willeba, *Ber. dtsch. chem. Ges.*, 1941, **74**, 777.
94. Shoppee, *Ann. Rep.*, 1947, **44**, 190.
95. Rometsch and Miesher, *Helv. Chim. Acta*, 1946, **29**, 1231.
96. Fromherz, *Arch. exp. Path. Pharmak.*, 1933, **173**, 86.
97. Sternbach and Kaiser, *J. Amer. chem. Soc.*, 1952, **74**, 2215.
98. Bayo Bayo, *Arch. inst. farmacol. exptl.*, 1953, **5**, 156.
99. Hunt and du Vigneaud, *J. biol. Chem.*, 1936, **115**, 93.
100. Weisberger and Storaasli, *J. Lab. clin. Med.*, 1954, **43**, 246.
101. Genzer, Lewis, Freeman, McMillan and King, *J. Amer. chem. Soc.*, 1953, **75**, 2506.

RESEARCH PAPERS

THE ESTIMATION OF FLUORESCEIN IN DILUTE SOLUTIONS

By E. Shotton and A. F. S. A. Habeeb

From the School of Pharmacy, University of London, 17 Bloomsbury Square, W.C.1

Received March 18, 1955

In a previous paper we have described the use of fluorescein as an indicating substance for the entrainment of liquid during the distillation of water[1]. The work described below was carried out to determine the conditions for the estimation of fluorescein in very dilute solutions.

The intensity of the fluorescence of an aqueous solution of fluorescein has been found to be constant from 0° to 80° C., the wavelength of the emitted light increasing with increasing temperature[2] the intensity of the fluorescence being maximum at room temperature[3]. The presence of the following ions I^-, Br^-, Cl^-, CNS^-, $S_2O_3^-$, SO_3^-, Ag^+, Cu^{++}[4,5,6] may cause quenching of the fluorescence but the cations Na^+, K^+, Ca^{++} and Ba^{++} are without effect. Oxygen has also been found to have no effect[7,8].

Boutaric and Roy[9] and Boutaric and Maraux[10] found that the fluorescence of fluorescein solutions was greatest at *p*H 8. According to Volmar[11] fluorescein gives an intense green fluorescence at *p*H 4·3 or above and a slight blue fluorescence below *p*H 3·8.

Concentration quenching is a general property of fluorescent substances and deviations from linearity occur if data are collected over a sufficiently wide range of concentration. Calibration curves are made to allow for concentration quenching. Cohen[12] obtained a linear relation for fluorescein solutions containing 0·125 to 6 μg./ml.

Fluorescein solutions exposed to direct sunlight undergo decomposition[13] seen by a decrease in the intensity of the fluorescence and a change in the absorption spectrum.

Experimental

The Spekker Fluorimeter (model H760)[14,15] was used with a Woods glass filter[16] on either side of the mercury vapour lamp. A heat absorbing filter was also inserted between the lamp and the left hand photocell but not on the right hand side as this reduced too greatly the intensity of the incident light and therefore the intensity of the fluorescence. With small galvanometer deflections a neutral filter (H.508) was inserted in front of the left hand photocell. Readings on the transmission scale of the drum were recorded.

The fluorescein solutions were prepared from Fluorescein Sodium B.P.C. dried at 105° C. to constant weight. The blank reading[17,18] for the cuvette filled with buffer solution was obtained by comparison with a solution of fluorescein containing 0·125 μg./ml. The blank with respect to the other standards was calculated and deducted from each observed fluorescence to give the net fluorescence. One cell was used throughout for the standard and another for the test solutions.

The effect of the p*H of the solution*

A series of solutions were prepared containing 1 μg./ml. of fluorescein buffered at the following *p*H values 4·4, 5, 5·5, 6, 6·5, 7, 8 and 9. Potassium chloride was omitted from the buffer at *p*H 9 to avoid the quenching effect of the chloride ion[19]. The *p*H of the fluorescein solutions was checked with a glass electrode. Preliminary experiments indicated that the solution at *p*H 6 gave the greatest fluorescence and this solution was therefore used as a standard to compare the other solutions.

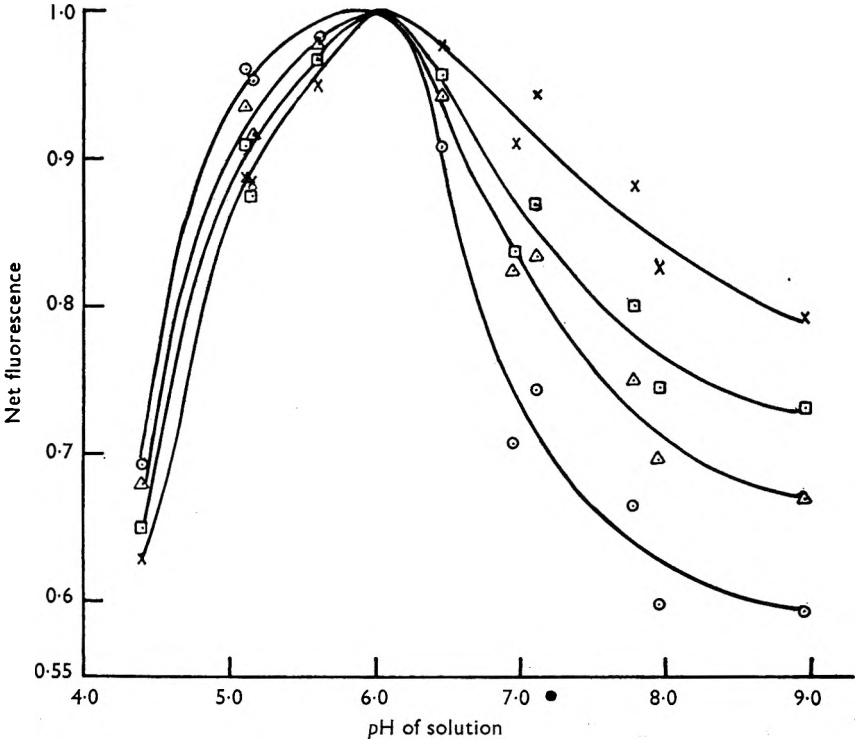


FIG. 1. Effect of *p*H on the fluorescence of fluorescein solutions.
× Kodak Filter F_6 maximum transmission at 5200Å°
○ ,, ,, F_7 ,, ,, ,, 5400Å°
△ Chance Filter OG_1 ,, ,, ,, 5300Å°
⊡ ,, ,, OB_2 ,, ,, ,, 4800Å°

Bertrand[20] reported that the fluorescence spectrum of fluorescein solutions exhibits a maximum at $\lambda = 5490$ Å for 0·01 molar solution and $\lambda = 5200$ Å for 0·0001 molar solution. Filters with a maximum transmission at these wavelengths were inserted between the fluorescing solution and the photocells.

The results are given in Fig. 1.

The gelatin filters F6 and F7 transmit only a fraction of the fluorescent radiation compared with the glass filters resulting in smaller galvanometer deflection and consequently greater errors in the determinations.

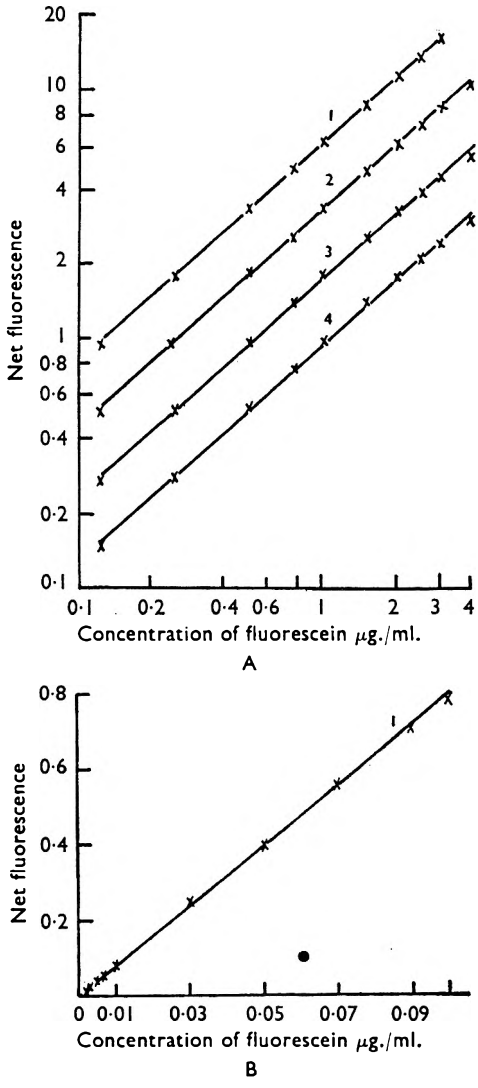


FIG. 2. The effect of concentration on the fluorescence of fluorescein solutions.

1. Standard solution containing 0·125 μg./ml.
2. ,, ,, ,, 0·25 ,,
3. ,, ,, ,, 0·50 ,,
4. ,, ,, ,, 1·0 ,,

Filter OG_1 was used for all subsequent work in preference to filter OB_2 as the cell blank was smaller indicating that only the fluorescent radiation was transmitted and light of unsuitable wavelength, such as that reflected from the cell lid and walls, was cut off.

Calibration of the Instrument

Experiments were carried out to obtain the calibration data and to determine the lowest concentration of fluorescein that could be estimated. A range of solutions were prepared, buffered to *p*H 6 and these were compared with four standard solutions containing 1, 0·5, 0·25 and 0·125 μg./ml. fluorescein in buffer of *p*H 6. The greatest accuracy in determining the concentration is considered to be obtained when the reading falls within the middle third of the scale[15,21].

The results are shown in Figs. 2A and 2B.

Fig. 2A represents the fluorescence-concentration relation plotted on a log-log scale for solutions 0·125 to 4·0 μg./ml. fluorescein using a 0·125, 0·25, 0·5 and 1·0 μg./ml. solutions of fluorescein as standards. It is seen that it follows a straight line suggesting the relation to be an exponential function of the formula $F = a\,c^n$

where a = value of F when c is unity which is 0·97, 1·78, 3·4 and 6·3 for standards 1, 0·5, 0·25 and 0·125 μg./ml. respectively.

c = concentration of fluorescein solution.

n = slope of the curve which was 0·88 in each case.

This value for n may apply only to the data obtained under our experimental conditions.

Fig. 2B shows that the data fall on a straight line for concentrations between 0·003–0·1 μg./ml.

Accuracy of the method

Test solutions of fluorescein buffered at *p*H 6 were prepared and their concentration was determined. For concentrations between 0·02–1·5 μg./ml. the error varied from −0·53 to −5·83 per cent. With more dilute solutions the error increased and for a solution containing 0·01 μg./ml. the error was −19 per cent.

The effect of heat on fluorescein solutions

Solutions containing 0·1, 0·01 and 0·001 per cent. w/v of fluorescein in distilled water were boiled under reflux for 80, 8 and 8 hours respectively. The fluorescence of the heated and unheated solutions suitably diluted at *p*H 6 was compared with standard solutions containing 0·5 and 1·0 μg./ml. of fluorescein.

Results are given in Table I.

TABLE I

THE EFFECT OF HEAT ON FLUORESCEIN SOLUTIONS

Concentration of standard	Nominal concentration of test dilution μg./ml.	Net fluorescence					
		0·1 per cent. w/v solution		0·01 per cent. w/v solution		0·001 per cent. w/v solution	
		Heated	Not heated	Heated	Not heated	Heated	Not heated
1·0 μg./ml.	0·25	0·278	0·267	0·283	0·288	0·277	0·288
	0·50	0·518	0·52	0·534	0·537	0·524	0·537
	1·0	1·02	1·0	0·99	0·99	0·97	0·99
	2·0	1·86	1·81	1·78	1·80	1·75	1·80
0·5 μg./ml.	0·25	0·51	0·50	0·525	0·532	0·515	0·532
	0·50	1·004	0·997	0·979	0·978	0·962	0·978
	1·0	1·90	1·83	1·81	1·81	1·77	1·81
	2·0	3·38	3·32	3·26	3·27	3·2	3·27

From these results it is evident that boiling has not affected the fluorescence of these solutions.

TABLE II

THE EFFECT OF LIGHT ON FLUORESCEIN SOLUTIONS

Concentration of standard	Nominal concentration of test dilution μg./ml.	Net fluorescence					
		0·1 per cent. w/v solution		0·01 per cent. w/v solution		0·001 per cent. w/v solution	
		Light	Dark	Light	Dark	Light	Dark
1·0 μg./ml.	0·25	0·282	0·291	0·279	0·278	0·276	0·278
	0·50	0·532	0·537	0·533	0·531	0·531	0·531
	1·0	0·985	0·989	0·986	0·99	0·982	0·99
	2·0	1·76	1·78	1·78	1·78	1·74	1·75
0·5 μg./ml.	0·25	0·531	0·556	0·524	0·523	0·516	0·524
	0·50	0·983	0·985	0·984	0·983	0·978	0·982
	1·0	1·82	1·82	1·81	1·83	1·8	1·82
	2·0	3·24	3·27	3·27	3·29	3·23	3·23

The effect of light on fluorescein solutions

Solutions containing 0·1, 0·01 and 0·001 per cent. w/v of fluorescein in distilled water were prepared and each divided into two portions. One portion was exposed to indirect sunlight for fourteen days and the other kept in the dark, both at room temperature. Dilutions buffered at *p*H 6 were prepared from each portion and their fluorescence was compared against standards containing 0·5 and 1·0 μg./ml. of fluorescein. Results are shown in Table II.

Exposure of the fluorescein solutions to indirect sunlight for 14 days in the presence of air did not materially affect their fluorescence.

Volatile fluorescent material in fluorescein

Two solutions in distilled water were prepared, solution A from fluorescein previously dried to constant weight at 105° C. and solution B from fluorescein which had not been dried. The concentration of each solution was 0·1 per cent. w/v of fluorescein calculated with respect to the dry weight. 100 ml. of the solutions were distilled at a very low rate using the same loading on the heating mantle in each case, the time to collect 25 ml. of the distillate being recorded. The concentration of fluorescein in the distillate was estimated by comparison with a standard containing 0·125 μg./ml. A quantity of distilled water, equal to the volume of the distillate collected was added to the still and the distillation repeated. Four such distillations were carried out with each solution. The results are recorded in Table III.

TABLE III

VOLATILITY IN STEAM OF THE DRIED AND UNDRIED FLUORESCEIN

	Solution A (fluorescein dried at 105° C.)				Solution B (fluorescein not dried)			
Successive runs using the same solution of fluorescein	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Time in minutes to collect 25 ml.	136	135	147	128	194	200	224	194
Fluorescein in the distillate μg./ml.	1st 0·00294		2nd 0·00306		1st 0·00813		2nd 0·00692	
	3rd 0·0029		4th 0·00298		3rd 0·00558		4th 0·0029	

The concentration of fluorescein in the distillate from solution A was constant for successive distillations and this may be due to the volatility of fluorescein in steam. For solution B the concentration of fluorescein in the distillate was initially higher than that obtained from solution A but it decreased with successive runs and in the 4th distillation the concentration was the same as that obtained from solution A. This suggests that the fluorescein originally contains some volatile fluorescent material which is removed by drying at 105° C. to constant weight.

DISCUSSION

From Fig. 1 a solution of fluorescein 1 μg./ml. shows a maximum fluorescence at *p*H 6 which differs from the results reported by Boutaric

and others[9,10] that fluorescein exhibited a maximum fluorescence at *p*H 8. They used a solution containing 0·5 g./l. (500 μg./ml.) and this is greatly in excess of the concentration at which concentration quenching occurs where the yield of fluorescence decreases with increasing concentration.

Under our experimental conditions the fluorescence of fluorescein solutions containing from 0·125 to 4 μg./ml. was found to be an exponential function of the concentration according to the expression $F = a\,c^{0.88}$ where a = 0·97, 1·78, 3·4 and 6·3 for standards 1, 0·5, 0·25 and 0·125 μg./ml. respectively. Cohen[12] obtained a linear relation for fluorescein solutions containing 0·125 to 6·0 μg./ml. using a fluorimeter in which the galvanometer deflection was taken as a measure of the fluorescence. Pyke[22] and Hennessy and Cerecedo[23] whose fluorimeters were similar to that of Cohen[12] obtained a linear relationship between galvanometer deflection and concentration for concentrations up to 50 mg. and 20 μg. of aneurine respectively. Whereas Williams and Wokes[18] obtained a linear relationship at concentrations of $\frac{5}{16}$–$2\frac{1}{2}$ μg./ml. of aneurine using a fluorimeter similar to that used in this work. However, at concentrations below 0·1 μg./ml. of fluorescein we found that the relationship was linear. Fluorescein could be estimated down to a concentration of 0·02 μg./ml. and could be detected down to 0·001 μg./ml., the error of the estimation becoming high as the drum readings approach the extremity of the scale and the galvanometer deflections are very small.

Summary

1. The Fluorescein Sodium B.P.C. used for this work contained some volatile fluorescent material which was volatile in steam but was removed by drying at 105° C. to constant weight.

2. The maximum intensity of the fluorescence of fluorescein solutions was found to be at *p*H 6.

3. The fluorescence of 0·1, 0·01 and 0·001 per cent. w/v fluorescein solutions was unaffected by boiling under reflux for 80, 8 and 8 hours respectively.

4. Exposure of the fluorescein solutions to indirect sunlight at room temperature for fourteen days did not affect their fluorescence.

5. Fluorescein solutions down to a concentration of 0·02 μg./ml. have been estimated with an error of less than 6 per cent. and fluorescein was detected down to a concentration of 0·001 μg./ml.

The authors wish to thank Dr. F. Wokes for his advice on the fluorimetric assays.

References

1. Shotton and Habeeb, *J. Pharm. Pharmacol.*, 1954, **6**, 1023.
2. Bouchard, *J. Chim. Phys.*, 1936, **33**, 232.
3. Jenness, *Phys. Rev.*, 1929, **34**, 1275.
4. Rollefson and Stoughton, *J. Amer. chem. Soc.*, 1941, **63**, 1517.
5. Bouchard, *C.R. Acad. Sci., Paris*, 1933, **196**, 485.
6. Jette and West, *Proc. Roy. Soc.*, 1928, A**121**, 299.
7. Pringsheim, *Fluorescence amd Phosphorescence*, 1949.
8. Chechan, *C.R. Acad. Sci., Paris*, 1946, **222**, 80.

9. Boutaric and Roy, *ibid.*, 1939, **209**, 162.
10. Boutaric and Maraux, *Bull. Soc. chim. Fr.*, 1948, 5e series, 952.
11. Volmar, *Chim. et Ind.*, 1937, **37**, 446.
12. Cohen, *Rec. Trav. chim. Pays-Bas.*, 1935, **54**, 133.
13. Mathur and Bhatnagar, *Indian J. Phys.*, 1928, **3**, 37.
14. Isbell, *Analyst*, 1949, **74**, 618.
15. Lothian, *J. sci. Instrum.*, 1941, **18**, 200.
16. Umberger and La Mer, *J. Amer. chem. Soc.*, 1945, **67**, 1099.
17. Wokes and Slaughter, *Analyst*, 1949, **74**, 624.
18. Williams and Wokes, *Quart. J. Pharm. Pharmcol.*, 1947, **20**, 240.
19. Desha, Sherrill and Harrison, *J. Amer. chem. Soc.*, 1926, **48**, 1493.
20. Betrand, *C.R. Acad. Sci., Paris*, 1945, **220**, 525.
21. Weissberger, *Physical Methods of Organic Chemistry*, Vol. II, 2nd Ed., Interscience Publishers N.Y., 1949, p. 1409.
22. Pyke, *Biochem. J.*, 1937, **31**, 1958.
23. Hennessy and Cerecedo, *J. Amer. chem. Soc.*, 1939, **61**, 179.

THE PHARMACOGNOSY OF THE ASPIDOSPERMA BARKS OF BRITISH GUIANA

PART I.

BY G. E. TREASE AND J. D. KULKARNI

From the Department of Pharmacy, University of Nottingham

Received March 18, 1955

IN 1949 the Forest Department of British Guiana supplied us with authenticated specimens of the barks of their native species of *Aspidosperma*, some of which, particularly *Aspidosperma excelsum*, were locally credited with medicinal properties[1]. The barks of five species were received and workers in this department have now examined them pharmacologically[2,3,4,5,6,7] and have by means of partition chromatograms obtained information regarding their alkaloidal constituents[8]. This work, which is being continued, is of interest in view of the growing importance of *Rauwolfia* species since the *Rauwolfias* and *Aspidospermas* are closely allied alkaloid-containing plants of the family Apocynaceæ. Woodson[9] recognises 52 species of *Aspidosperma*, many of which are large timber-producing trees. If, therefore, the barks or alkaloids from them prove to be of medicinal value, abundant and cheap raw material is available in many parts of tropical America.

The present paper describes the pharmacognostical macroscopical characters of the five barks used by Lewis and Banerjee[2,3,4,5,6,7] and by Palmer[8], together with the detailed histology of one of them. Further histological and quantitative microscopical work on the other four barks is in progress.

MATERIAL

Barks of five different species were obtained from the Conservator of Forests, British Guiana, and the Conservator of Forests, British Honduras. Different collections of each species are denoted by the letters A, B and C as follows:—

Species	Specimens		
Aspidosperma ulei Mgf., formerly known as *A. vargasii* A.DC, a tree 3 to 20 m. high found in British Guiana, Venezuela and Columbia	1 A	Collected British Guiana	1949
	1 B	,, ,,	1950
	1 C	,, ,,	1954
Aspidosperma album Vahl (R. Benoist), formerly known as *A. woodsonianum* Mgf., a tree 10 to 30 m. high found from north-eastern Columbia to the Amazon Valley	2 A	Collected British Guiana	1949
	2 B	,, ,,	1950
	2 C	,, ,,	1954
Aspidosperma megalocarpon Muell. Arg., formerly known as *A. desmanthum* Benth., a tree 7 to 30 m. high found from south-eastern Mexico to British Guiana	3 A	Collected British Guiana	1949
	3 B	,, British Honduras	1953
	3 C	,, British Guiana	1954
Aspidosperma excelsum Benth., a tree 15 to 35 m. high found in British Guiana and Dutch Guiana	4 A	Collected British Guiana	1949
	4 B	,, ,,	1950
	4 C	,, ,,	1954
Aspidosperma oblongum A.DC., a tree up to 35 m. high found in British, Dutch and French Guianas	5 A	Collected British Guiana	1949
	5 B	,, ,,	1950
	5 C	,, ,,	1954

The above barks have been compared with samples of the bark of *Aspidosperma quebracho-blanco* Schlecht, a drug formerly included in the U.S.P. and B.P.C. and described in the U.S. Dispensatory 1943[10]. This has been the subject of papers by Schlechtendal[11], Holmes[12], and Short[13].

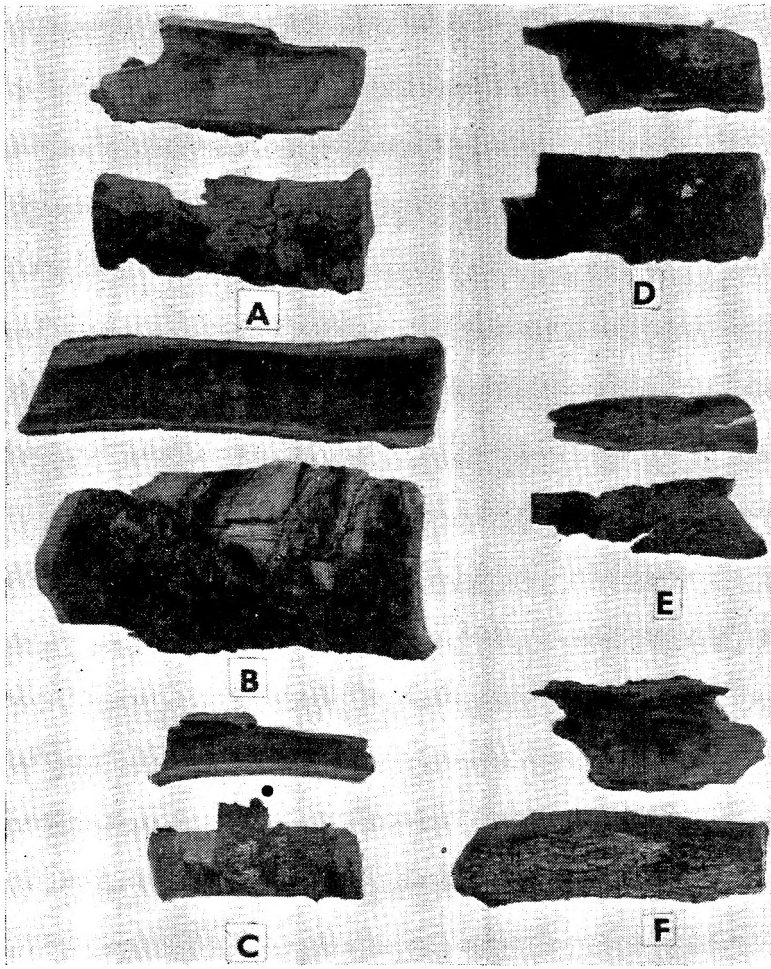


FIG. 1. Photographs of outer and inner surfaces of Aspidosperma barks × $\frac{1}{6}$. A, *A. quebracho-blanco*; B, *A. album*; C, *A. ulei*; D, *A. oblongum*; E, *A. excelsum*; F, *A. megalocarpon*.

The specimen A in Figure 1 was collected by Dr. Martin Cardenas in 1953 at Cochabamba in Bolivia.

EXTERNAL CHARACTERS

Aspidosperma ulei (Fig. 1C). Curved or channelled pieces up to 16 cm. long, 7 cm. wide and 10 mm. thick. Outer surface consisting of scaly cork, which readily exfoliates exposing a nut-hard yellow cortex. The

cork is light brown in colour and shows large transverse lenticels, transverse cracks and patches of greyish-green lichen. The lenticels are well-marked in the rossed bark and are seen to occur in rings at intervals varying from about 5 to 25 mm. Inner surface pale yellow to yellowish-brown, longitudinally ridged and striated. Difficult to fracture; transverse fracture yellowish, except in the cork, and consisting of an outer granular zone and an inner fibrous zone. Odour, indistinct; taste, bitter and aromatic.

Aspidosperma album (Fig. 1B). Specimen 2 B occurs in curved pieces up to 26 cm. long, 11 cm. wide and 10 mm. thick. Outer surface warty, with patches of thin, dark grey cork partly covered with lichen. The surface warts are up to 5 mm. in diameter, corky and easily powdered and from cinnamon-brown to dull red in colour. Transverse rings about 3 to 7 mm. apart of conspicuous lenticels and occasional deep transverse cracks several cm. in length. Inner surface yellowish to grey, uniformly coloured or patchy, surface usually striated longitudinally but in the larger pieces patches of the innermost zone of fibres tend to exfoliate. Fracture, short in the outer part which appears granular, fibrous in the inner part. Odour, indistinct; taste, bitter and aromatic.

Aspidosperma megalocarpon (Fig. 1F). Almost flat pieces having a tendency to curve lengthwise in the direction of the cork; up to 18 cm. long, 8 cm. wide and 8 mm. thick. Outer surface consisting of a grey to reddish-brown outer cork with a patchy or almost complete coat of whitish lichen. Outer cork with longitudinal fissures and transverse cracks, easily rubbed off exposing a thin, brick-red inner cork. Inner surface cinnamon-brown to grey, finely striated longitudinally. Fracture short in the outer part, fibrous in the inner part. Odour, indistinct; taste, bitter. The specimen from British Honduras (3 B) was indistinguishable from those from British Guiana.

Aspidosperma excelsum (Fig. 1E). Curved or channelled pieces up to about 16 cm. long, 4 to 6 cm. wide and 3 to 8 mm. thick. Outer surface finely furrowed, greyish or brownish-black, patches of epiphytes. Inner surface pale yellowish-brown or reddish-brown, finely striated longitudinally. Fracture short. Odour, indistinct; taste, bitter and aromatic.

Aspidosperma oblongum (Fig. 1D). In flat or slightly curved pieces up to 15 cm. long, 7 cm. wide and 3 to 4 mm. thick. Outer surface rough from numerous transverse and longitudinal cracks, greyish or brownish-black, with whitish or greenish patches of lichen. Cork not easily separated. Inner surface pale yellow to almost black, not furrowed but finely striated longitudinally. Fracture short and laminating, the surface showing, even with the naked eye, bands of sclerenchyma and projecting phloem fibres. Odour, indistinct; taste, bitter and aromatic.

Microscopy

Microscopical examination of sections and powders of the British Guiana barks shows that they, together with the bark of *Aspidosperma quebracho-blanco*, possess a number of characters of cell-structures and cell-contents in common, whilst differing in other histological details.

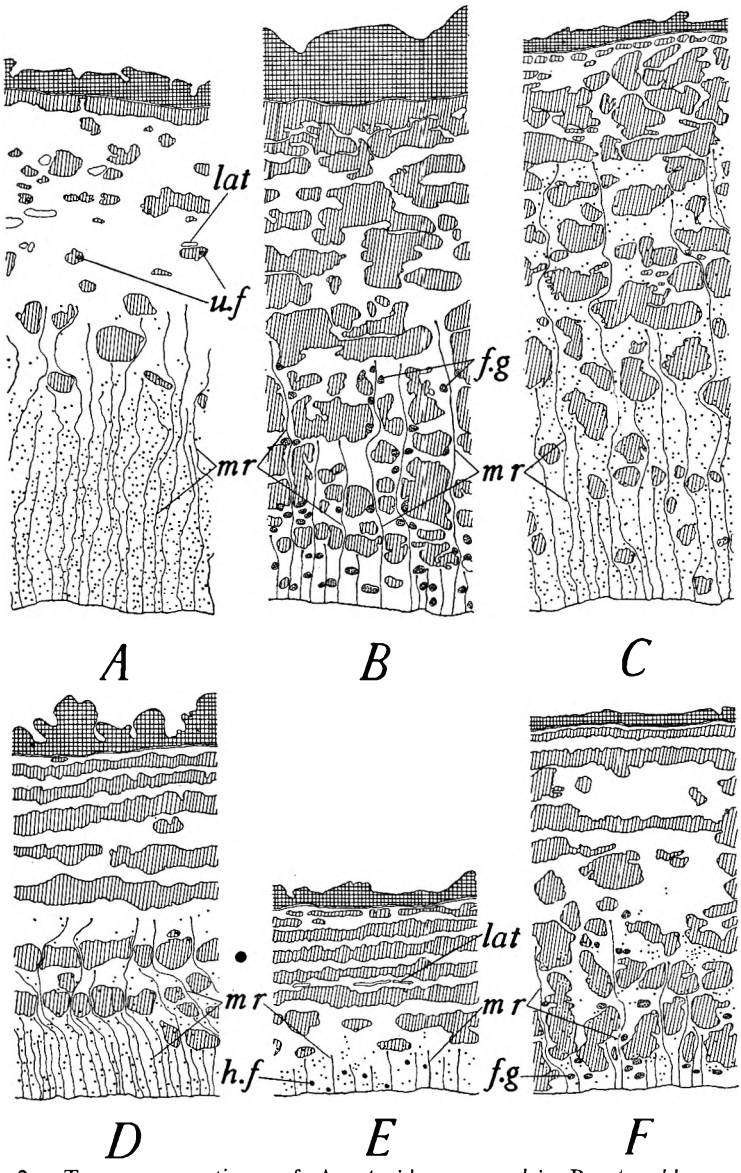


FIG. 2. Transverse sections of A, *Aspidosperma ulei*; B, *A. oblongum*; C, *A. quebracho-blanco*; D, *A. megalocarpon*; E, *A. excelsum*; F, *A. album*. All at × 8, cross-hatched area, cork; diagonally-hatched area, sclereids; *f.g*, fibres in groups; *h.f*, fibre with large cell-cavity; *lat*, latex canal; *m.r*, medullary ray; *u.f*, unlignified fibre.

The present paper describes the detailed investigation of one of them, *Aspidosperma ulei*.

Sclerenchyma Distribution in Transverse Sections

All the Aspidosperma barks examined contain sclereids and phloem fibres, but, as will be seen from Figure 2, the sclerenchyma arrangement

varies considerably in different species. In *A. ulei* (Fig. 2A), *A. album* (Fig. 2F), *A. quebracho-blanco* (Fig. 2C) and *A. oblongum* (Fig. 2B) the sclereids are mainly or entirely in isolated groups, whereas in *A. megalocarpon* (Fig. 2D) and *A. excelsum* (Fig. 2E) the sclereids are mainly arranged in bands. Phloem fibres, as shown by black dots in Figure 2, are present as isolated fibres in *A. ulei*, *A. quebracho-blanco*, *A. megalocarpon* and *A. excelsum* (Fig. 2, A, C, D and E), whereas in *A. oblongum* and *A. album* (Fig. 2, B and F) the fibres are arranged in groups containing two to several components. These isolated fibres and fibre-groups are sometimes enclosed within the sclereid groups.

Detailed Histology of A. ulei (Figs. 3 and 4)

Cork consisting of numerous layers of very much collapsed reddish-brown and tangentially-elongated cells, which, when expanded by treatment with 80 per cent. sulphuric acid (Fig. 3, A and B, *ck*, and Fig. 4, A, *ck*), are seen to be brick-shaped, walls suberised, non-lignified or only slightly lignified; R = 4 to **7** to 11 μ, T = 54 to **84** to 115 μ, H = 4 to **9** to 18 μ*; polygonal in surface view. Phellogen (Fig. 3, A, *ph*, and B, and Fig. 4, A, *ph*), 1 or 2 layers of thin-walled tangentially-elongated cells; R = 10 to **15** to 20 μ, T = 60 to **85** to 110 μ and H = 7 to **10** to 15 μ. Phelloderm a well-marked tissue within this phellogen (Fig. 3, A and B), of sclerenchymatous cells, together with some parenchyma; the greater amount of sclerenchyma consisting of large isodiametric cells 20 to 60 μ diameter, with small lumen, well-marked simple or branched pits, thick, stratified and lignified walls, arranged as a compact tissue, some 15 layers broad, interspersed with few isodiametric parenchyma cells, some of which contain prismatic calcium oxalate crystals, and extending to the cortex. In places, several layers of tangentially-elongated parenchymatous cells lie between the phellogen and the broad band of sclereids; also in this outer zone of phelloderm there may be one or several layers of tangentially-elongated sclereids (Fig. 3, A and B, and Fig. 4, A, *e.sc*), R = 15 to **20** to 25 μ, T = 62 to **78** to 108 μ, H = 11 to **15** to 18 μ, with small lumen, thick, lignified and stratified walls, traversed by well-marked pits; each of these elongated sclereids is surrounded by thin-walled, small parenchymatous cells, containing solitary prismatic calcium oxalate crystals (Fig. 3, B, and Fig. 4, A, *ox*). Cortex of large, thin-walled, tangentially-elongated parenchymatous cells containing starch (Fig. 3, C, and Fig. 4, B, C and D, *p*); together with large isodiametric or somewhat tangentially-elongated latex canals, the contents of which appear to be granular and are stained yellow with iodine solution. R = 85 to **125** to 180 μ, T = 240 to **500** to 1680 μ and H = 100 to **145** to 210 μ (Fig. 3, C, and Fig. 4, C, *lat*). Scattered throughout the cortex are large groups of isodiametric sclereids similar in structure to those found in the phelloderm, surrounded by

* R, T and H indicate the measurements made in the radial, tangential and longitudinal directions respectively; the use of these symbols is suggested by Moll and Janssonius in their *Botanical Pen Portraits* (1923). The symbol H indicates height, the measurement in the longitudinal direction, which has been used instead of L, as used by these workers.

thin-walled isodiametric parenchymatous cells each containing a prismatic calcium oxalate crystal. Towards the inner region of the cortex some of the groups of sclereids may be associated with single unlignified or only slightly lignified large fibres (Fig. 3, C, *uf*), with very small lumen, thick striated walls, traversed by a few simple pits along which splitting

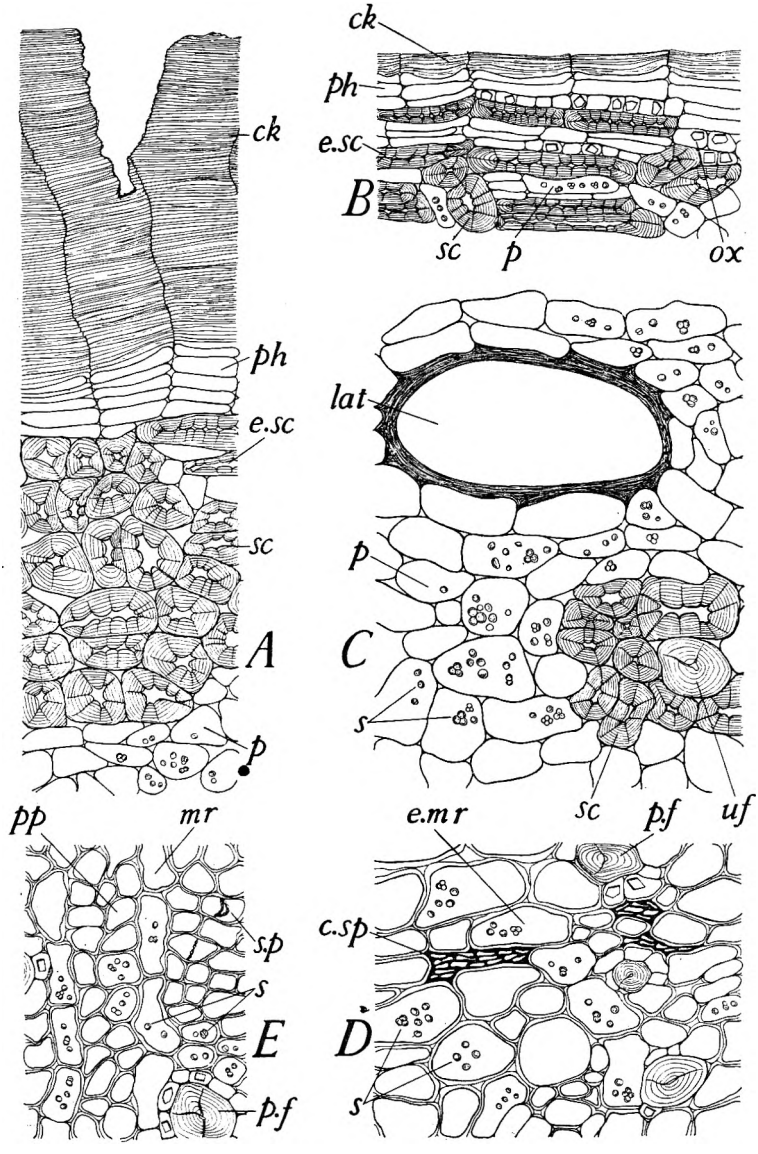


FIG. 3. T.S. *Aspidosperma ulei* × 200. *ck*, cork; *c.sp*, collapsed sieve tubes; *e.mr*, end of the medullary ray; *e.sc*, elongated sclereid; *lat*, latex canal; *mr*, medullary ray; *ox*, calcium oxalate; *p*, cortical parenchyma; *ph*, phellogen; *p.f*, phloem fibre lignified; *pp*, phloem parenchyma; *s*, starch; *sc*, sclereid; *s.p*, sieve plate; *uf*, unlignified fibre.

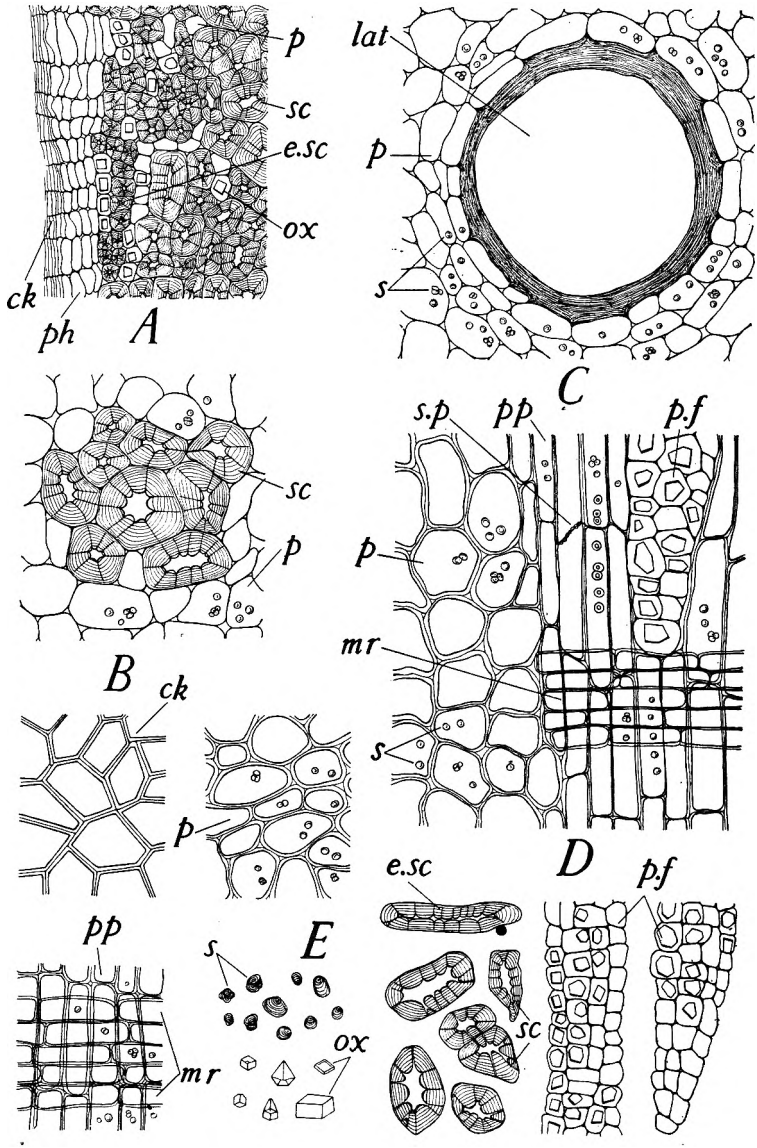


FIG. 4. L.S. and powder of *Aspidosperma ulei* × 200. *ck*, cork; *e.sc*, elongated sclereid; *lat*, latex canal; *mr*, medullary ray; *ox*, calcium oxalate; *p*, cortical parenchyma; *ph*, phellogen; *p.f*, phloem fibre; *pp*, phloem parenchyma; *s*, starch; *sc*, sclereid; *s.p*, sieve plate.

may have occurred, 35 to 56 to 70 μ diameter, and up to 1000 μ long, with bluntly pointed ends; calcium oxalate prism sheath absent. Phloem, which is up to 60 per cent. of the thickness of the bark, consists of sieve tissue, phloem parenchyma, sclereid groups, phloem fibres and medullary rays. Sieve tissue is very much collapsed (Fig. 3, D, *c.sp*), except in

the innermost region of phloem, where sieve tubes may be distinguished with oblique, compound sieve plates (Fig. 3, E, *s.p*, and Fig. 4, D, *s.p*). Phloem parenchyma (Fig. 3, E, and Fig. 4, D, *pp*) is thin-walled, with compound pits on the vertical walls, its cells contain starch; sclereid groups large, chiefly in the outer part of the phloem, of isodiametric cells similar to those found in the cortex, and surrounded by thin-walled isodiametric parenchymatous cells containing single prismatic calcium oxalate crystals; phloem fibres (Fig. 3, D and E, and Fig. 4, D, *p.f*) numerous, mostly isolated or very rarely in groups of 2, lignified, with very small lumen, thick striated walls, traversed by a few simple pits along which splitting may have occurred, 55 to **65** to 80 μ diameter, 2500 to **3650** to 5370 μ long, with bluntly pointed ends, similar to the lignified fibres found in the cortex, except that the phloem fibres are surrounded by thin-walled parenchymatous cells, each containing a prismatic calcium oxalate crystal (Fig. 3, D and E, *ox*, and Fig. 4, D, *ox*). Medullary rays (Fig. 3, E, and Fig. 4, D, *mr*), 1 to 2 cells wide and 5 to 7 cells in depth, very wavy and much displaced by the groups of sclereids through which the rays do not pass. The cells are R = 30 to **48** to 65 μ, T = 15 to **22** to 32 μ, H = 36 to **52** to 65 μ, and contain starch grains. Starch abundant in the cortical and phloem parenchyma, simple or 2- to 4-compound; individual grains, with excentric hilum, spherical, ovoid or plano-convex and up to 15 μ. Calcium oxalate as square, rectangular or obliquely rectangular prisms or as small cubes of various sizes, measuring up to 35 μ, are found associated with sclereid groups and around fibres in different zones of the bark; there is no relationship between crystal shapes and the region of bark in which they occur (Fig. 3, B, D and E; Fig. 4, A, *ox*).

Powder. Yellowish in colour; cork cells in surface view polygonal and reddish-brown in colour; sclereids in more or less intact groups with crystal sheath, or separated (Fig. 4, E, *e.sc* and *sc*); individual sclereids, which are isodiametric or elongated, have heavily lignified walls with simple or branching pits; portions of phloem fibres (Fig. 4, E, *p.f*) and occasional portion of unlignified fibre from the cortex as described previously; phloem parenchyma (Fig. 4, E, *pp*), with thin pitted walls may be associated with cells of medullary rays (Fig. 4, E, *mr*); cortical parenchyma of thin-walled cells which are tangentially-elongated (Fig. 4, E, *p*); starch abundant, simple or 2- to 4-compound, individual grains with excentric hilum, spherical, ovoid or plano-convex and up to 15 μ (Fig. 4, E, *s*); calcium oxalate prisms of various shapes and sizes up to 32 μ in maximum length (Fig. 4, E, *ox*), as described previously.

Microscopical Measurements of Five Aspidosperma Barks

A quantitative microscopical method for distinguishing between powdered barks of closely allied species such as these Aspidospermas, which is based on the ratio between their stone cells and fibres, will be the subject of a further communication. The following figures, each based on about 250 measurements, made from macerations of the bark and from powders, are recorded as an indication that such measurements

alone are insufficient to distinguish between the many different barks from this genus.

MEASUREMENTS IN μ

	Sample	Fibres		Sclereids		Cork cells	Starch
		Length	Width	Length	Width	Tangential	Grains
A. ulei	1 A	2530–5370	65–80	40–140	25–60	12–25	4–7–15
	1 C	2500–5340	55–75	35–130	30–60	12–25	4–7–15
A. album	2 A	835–2585	25–40	35–125	25–75	25–50	4–9–18
	2 C	795–2500	25–45	30–125	25–65	25–45	4–9–20
A. megalocarpon ..	3 A	695–1615	30–40	25–100	20–60	25–45	4–11–18
	3 C	630–1600	25–40	25–115	15–60	20–40	4–11–18
A. excelsum ..	4 A	1530–2780	35–75	20–70	15–35	15–35	4–7–15
	4 C	1500–2725	35–75	25–80	15–40	20–40	4–7–15
A. oblongum ..	5 A	1295–2640	35–60	40–110	20–55	25–60	4–10–15
	5 C	1335–2670	30–55	30–115	25–55	25–55	4–10–15

DISCUSSION

The 52 species of Aspidosperma have been classified by Woodson[9] and by Pichon[14]. Although we and our colleagues at Nottingham have examined only a few of these species, our findings, based on entirely different characters, support the accepted botanical groupings.

In *A. ulei* (Woodson's Series III):

1. The stone cells are in groups.
2. The alkaloids antagonise the pressor response to adrenaline in spinal cats[5].
3. The bark contains at least 9 alkaloids, no one of which predominates in amount[8]. This is also true of 2 other species belonging to this series, *A. tomentosum*[15] and *A. australe*[16].

In *A. excelsum* and *A. oblongum* (Woodson's Series VI):

1. Stone cells mainly in bands.
2. The barks resemble one another pharmacologically but differ from the barks of the other species examined. For example, the alkaloids have marked hypotensive and sympathetic properties. Some of them are indole derivatives[2–5].
3. *A. excelsum* bark has not yet been fully investigated but its alkaloids resemble those of *A. oblongum*[6]. Chromatograms from *A. oblongum* show 3 alkaloids; the major one being yohimbine[8].

In *A. album* and *A. megalocarpon* (Woodson's Series IX):

1. Stone cells mainly in bands, and in groups in the inner region of cortex and phloem; dimensions of cells and starch grains similar.
2. The barks resemble one another pharmacologically but differ from the barks of the other species examined[2–5].
3. The barks of both species contain 2 major alkaloids and other minor ones. The total alkaloids found are similar in amount, being 0·71 and 0·74 per cent. respectively[8].

The diagnostic characters of *A. ulei* are as follows:—

1. Presence of very much collapsed unlignified cork, consisting of reddish-brown and tangentially-elongated cells.

2. Phelloderm mainly consisting of sclerotic tissue.
3. Presence, in the outer zone of phelloderm, of one or several layers of tangentially-elongated sclereids.
4. Large isodiametric or somewhat tangentially-elongated latex canals.
5. Large groups of sclereids of isodiametric cells are present in cortex and phloem which are surrounded by thin-walled parenchymatous cells, some of which contain a single prism of calcium oxalate.
6. Unlignified or slightly lignified large single fibres associated with the groups of sclereids in the inner region of cortex.
7. Lignified large fibres, usually single, sometimes in groups of 2, may be associated with the groups of sclereids, but mainly without, surrounded by calcium oxalate crystal sheath, are present throughout the phloem region.
8. Starch is present in cortical parenchyma, phloem parenchyma and also in the cells of medullary ray.

Summary

1. The macroscopical characters of 5 *Aspidosperma* barks from British Guiana are described.
2. The diagnostic histological characters of the bark of *Aspidosperma ulei* have been illustrated and described.
3. The dimensions of cork cells, stone cells and fibres of 5 *Aspidosperma* barks are recorded.
4. Differences in sclerenchyma arrangement are recorded and illustrated. These appear to be correlated with the botanical classification of the *Aspidospermas* and with certain observations made on the pharmacology and alkaloidal constituents of these barks.

We desire to thank Dr. J. M. Rowson and Dr. T. E. Wallis for their interest and assistance in this work; the Conservators of Forests in British Guiana and British Honduras for the supply of material, and our Nottingham colleagues for keeping us informed on the progress of their pharmacological and chemical work on these barks.

References

1. *Br. Guiana Forest Bull.*, No. 2 (n.s.), 1948, 39.
2. Banerjee and Lewis, *Nature, Lond.*, 1953, **171**, 802.
3. Banerjee and Lewis, *J. Pharm. Pharmacol.*, 1954, **6**, 246.
4. Banerjee and Lewis, *ibid.*, 1954, 6, 466.
5. Banerjee, Lewis and Sharp, *ibid.*, 1954, 6, 660.
6. Banerjee, Ph.D. Thesis, Nottingham, 1954.
7. Banerjee and Lewis, *J. Pharm. Pharmacol.*, 1955, 7, 42.
8. Palmer, M.Pharm. Thesis, Nottingham, 1954.
9. Woodson, Jr., *Ann. Missouri Bot. Gard.*, 1951, **38**, 119.
10. *The Dispensatory of the U.S.A.*, 1943, p. 1490.
11. Schlechtendal, *Bot. Zeit.*, 1861, **XIX**, 137.
12. Holmes, *Pharmaceutical Journal and Pharmacist*, 1926, **62**, 4.
13. Short, *ibid.*, 1926, **62**, 103.
14. Pichon, *Bull. Mus. Nat. d'Hist. Nat.*, 1947, ser. 2, **19**, 362.
15. Floriani, *Rev. centro. estud. farm. bioquim*, 1935, **25**.
16. Orazi, *Ann. assc. quim. argentina*, 1946, **34**, 158.

A NOTE ON THE RESORPTION AND EXCRETION OF RIBOFLAVINE FROM ALUMINIUM MONOSTEARATE SUSPENSIONS IN THE RAT

By F. G. Sulman

Department of Applied Pharmacology,
The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

Received February 17, 1955

Nutritional studies in this country have shown that riboflavine deficiency is very frequent[1], and that its incidence is much greater than that of other vitamin deficiencies[2]. The clinical manifestations of this deficiency, such as glossitis, cheilosis and corneal vascularisation, are particularly common during pregnancy. Thus, reduced excretion of riboflavine (averaging 95μg./l. of urine instead of 360 μg./l. as in normal pregnant women) was found in 21 per cent. of 900 pregnant women[3]. Riboflavine deficiency is also associated with prematurity of the fœtus, a high incidence of antenatal death and post partum agalactia or hypogalactia[4]. Surprisingly enough, these clinical manifestations appeared not only in the poorer classes in which riboflavine intake was inadequate, but in well-nourished subjects as well. The latter finding is principally due to inadequate resorption of this vitamin. The frequent occurrence of intestinal and hepatic disorders such as amœbic dysentery, gastric achlorhydria and hypochlorhydria, and various types of chronic hepatitis in undoubtedly the most important etiologic factor in the production of deficiency symptoms in these subjects[5].

Riboflavine therapy generally produces striking improvement in cases in which the deficiency is due to insufficient intake. On the other hand, poor results usually follow oral administration in subjects suffering from deficiency due to inadequate resorption. In these patients even parenterally administered riboflavine is eliminated from the body within a few days[6].

In order to maintain a constant level of riboflavine in the tissues, we have attempted the implantation of pellets so as to afford continuous and adequate resorption of the vitamin. This method of supply seems to us particularly advantageous in patients who cannot be trusted to take medication regularly. For this purpose we prepared pellets containing 50 mg. of riboflavine fused with 50 mg. of cholesterol[7] which on implantation maintained a high level in man and animals for up to $1\frac{1}{2}$ months.

Recently we have simplified the treatment by developing a single injection technique. Two aspects of this type of therapy, the rate of excretion and the rate of recovery from the site of injection of a suspension of riboflavine aluminium monostearate, are reported in this paper.

Technique

Riboflavine suspensions were prepared with 2 per cent. aluminium monostearate*. They are stable when protected from light, easily

* We are indebted to Dr. G. Friedlaender of "Teva" Middle East Pharmaceutical & Chemical Works, Ltd., Jerusalem, Israel, for the preparation of the suspensions and solutions used.

tolerated, contain up to 50 mg./ml. and cause no local reaction on injection. Their effect was compared with that of buffered riboflavine solutions prepared by the same manufacturer.

Twelve male and 12 female rats (Hebrew University strain) weighing 150 ± 10 g. were used. They received standard Purina diet which gave a daily urinary excretion of 10–20 μg. of riboflavine per rat. The rats were placed in groups of 2–4 in metabolism cages with unlimited intake of food and water. The urine was collected once a day in 1 ml. glacial acetic acid; and every 24 hours the cages were rinsed with 100 ml. of tap water which was added to the urine. After a one week period of adaptation, the urine was assayed daily for the vitamins. The rats were then given a single injection of suspension (4 male and 4 female rats to each dose level of 20, 25 and 50 mg.) and the excretion of the vitamin in the urine of the 12 male rats was determined daily by the fluorometric method[8]. The urine was assayed at dilutions containing 0·1–0·2 μg./ml. The excretion was expressed in terms of μg. of riboflavine excreted per rat per day.

TABLE I

AVERAGE URINARY EXCRETION OF RIBOFLAVINE IN RATS INJECTED SUBCUTANEOUSLY WITH SUSPENSIONS OF RIBOFLAVINE ALUMINIUM MONOSTEARATE AND RECOVERY OF RIBOFLAVINE FROM THE SITE OF INJECTION

Days after injection	Total urinary excretion* versus recovery† from site of injection	Riboflavine suspension injected		
		20 mg.	25 mg.	50 mg.
		mg.	mg.	mg.
1–12	Excretion	10·4	14·2	17·8
	Recovery	8·6		28·4
13–24	Excretion	7·5	7·2	10·5
	Recovery	1·2	1·5	9·8
25–36	Excretion	1·1		3·1
	Recovery	0·9	0·6	2·6
37–48	Excretion	0·5		2·1
	Recovery	0·1	0·1	1·4

* Average value of excretion derived from 4 rats.
† Single value of recovery derived from 1 rat.

An additional group of 4 male rats received an injection of 20 mg. of a riboflavine solution and served for comparison with the 3 groups receiving the suspension.

The 12 female rats served mainly for the study of the resorption of the vitamin from the lumps which were removed from the site of injection. Occasionally, their urines were also checked for riboflavine excretion. There were no significant deviations from the excretion found in the male rats. The female animals were killed at intervals of 12 days. The organised riboflavine residues at the site of injection were removed, minced and extracted with 0·1N hydrochloric acid according to the method of the Association of Vitamin Chemists[8].

RESULTS

Riboflavine suspensions injected subcutaneously at dosage levels of 20, 25 and 50 mg. per rat gave average daily urinary excretion as shown in Figure 1. One injection of 20 mg. was enough to maintain an elevated excretion of the vitamin for 52 days. After injection of 50 mg. excretion was increased for 105 days.

For comparison, 4 male rats were injected subcutaneously with 20 mg.

of a riboflavine solution. Average excretion in their urine was increased only during the first 4 days. Seventy-five per cent. of the injected riboflavine was excreted in the urine during the first day and was accompanied by diuresis of 25 ml. as compared with 2·5–5 ml. on a control day. Larger doses of the solution proved to be toxic. The toxicity was not

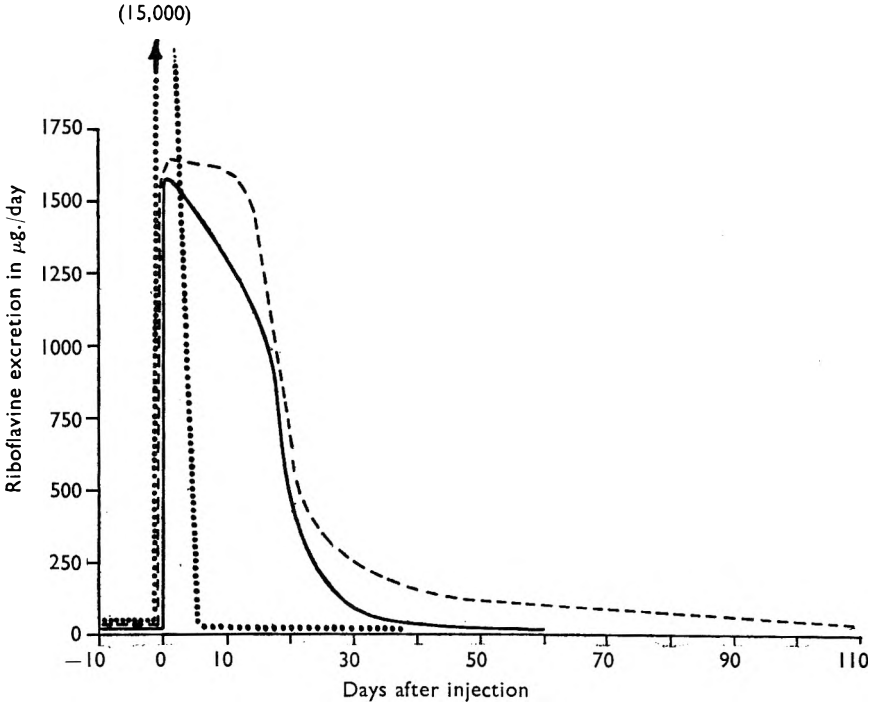


FIG. 1. The excretion of riboflavine in μg./day after injection of:—
20 mg. in suspension (——)
50 mg. ,, ,, (— ● —)
20 mg. in solution (· · · · ·)

due to the solvent, but rather to the high doses of the riboflavine itself. This question is under further study.

A comparison of the residual riboflavine content in the lumps removed from the site of injection is made with the total excretion in Table I. Riboflavine in the urine is derived from that injected and that contained in the diet. The amount recovered from the urine and the site of injection roughly equalled the amount injected and ingested.

DISCUSSION

The injection of suspensions described here is superior to the implantation method described by us earlier[7]. Whereas 50 mg. implanted provided riboflavine for 45 days only, the same quantity injected as an aluminium monostearate suspension lasted up to 105 days.

The results obtained with the suspension method are quite satisfactory.

The very slow resorption of the vitamin is not only due to slow liberation from the suspension but also to the poor solubility of the vitamin in body fluids, its solubility in an aqueous medium is only 1:10,000.

There exist at present 3 methods of injecting crystal suspensions:

(1) *Crystal formation at the site of injection*: The drug is injected as an aqueous solution with 2 per cent. urethane, the latter is removed by the body fluids, leaving a precipitate at the site of injection which is resorbed within 14–21 days[9].

(2) *Crystal formation in the syringe*: The drug is dissolved in an organic medium and when mixed with normal saline in the syringe, is precipitated as a fine crystalline suspension which becomes coarse after injection in the tissues and is resorbed within 14–21 days[10].

(3) *Crystal formation in the ampoule*: The drug is suspended with the aid of a suitable suspending agent, such as pectin, sodium oleate, magnesium stearate or, as described above, aluminium monostearate before packaging in ampoules.

It seems that the last method using 2 per cent. aluminium monostearate is the most promising. We have therefore begun an investigation on its use in man in collaboration with Drs. Bromberg and Brzezinski. Furthermore the application of this method to steroid hormone therapy will be studied.

Summary

1. The use of riboflavine suspensions with 2 per cent. aluminium monostearate for depot therapy has been studied in rats. A depot of 50 mg. takes up to 105 days to be resorbed and smaller quantities accordingly shorter periods (52 days for 20 mg.). This method of administration is superior to the implantation of pellets previously described by us.

2. The depots formed produced no local reactions and allowed slow and continuous resorption of the vitamin from the site of injection.

Our thanks are due to Drs. Y. M. Bromberg, A. Brzezinski, and E. Y. Diamant for helpful suggestions, and to Mr. E. Zawojski for his help.

References

1. Dostrovsky and Sagher, *Harefuah (Israel)*. 1942, **22**, 19; *Dermatologica*, 1942, **86**, 325.
2. Berger-Rabinowitz, Dissertation Hebrew University, Jerusalem, 1945.
3. Brzezinski, Bromberg and Braun, *J. Obstet. Gynæc., Brit. Emp.*, 1945, **52**, 43.
4. Brzezinski, Bromberg and Braun, *ibid.*, 1947, **54**, 182.
5. Brzezinski, Bromberg and Braun, *Harefuah (Israel)*, 1948, **34**, 131.
6. Brzezinski, Braun and Bromberg, *ibid.*, 1951, **40**, 124.
7. Bromberg, Brzezinski and Sulman, *Proc. Soc. exp. Biol. N.Y.*, 1947, **64**, 354.
8. Ass. Vit. Chem., Methods of Vitamin Assay 2nd Ed. Interscience Publishers, New York, 1951, p. 166.
9. Rappaport and Lass, *Lancet*, 1947, **2**, 296.
10. Zondek and Rozin, *J. clin. Endocrin.*, 1948, **8**, 406.

LOCAL ANÆSTHETIC ACTIVITY OF 4-ALKOXYBENZAMIDES

By P. Kolosy, P. Teyssié and H. Vanderhaeghe
From Recherche et Industrie Thérapeutiques, Genval, Belgium

Received February 14, 1955

Considering the large number of esters whose local anæsthetic properties have been reported, it is surprising that only a few amides appear to have been tested.

Among the local anæsthetics used clinically, cinchocaine is the most powerful when tested with laboratory methods.[1] This product was selected from a series of 2-alkoxyquinolin-4-carboxamides[2]. Sievers and McIntyre[3] have examined diethylaminoethylamides of α-substituted cinnamic acids. These substances do possess moderate activity but are locally irritating. More recently, Büchi and collaborators[4] have compared a number of dialkylaminoethyl-esters and amides of 3-butoxy-4-aminobenzoic acid. Both groups were found to have nearly the same anæsthetic potency so far as nerve conduction is concerned, but the surface anæsthesia is lower and the tissue irritation is increased with the amides. Several esters and amides of 2:6-dialkoxypyridine-4-carboxylic acid were also investigated by the same group[5,6]. In these series the amides were rather more active than the esters, but all substances were very irritating locally. Having investigated previously a group of esters of 4-alkoxybenzoic acid[7], we decided to examine the corresponding amides, but limiting the study to the diethylaminoethylamides, since in the ester group, variations in the side-chain produced only small quantitative differences in the pharmacological properties[7,8].

Pharmacological Activity

Surface and infiltration anæsthesia, tissue irritation and hæmolytic activity were determined by the methods described previously[7]. All results are summarised in Table I.

These results show that the amides are less active than the corresponding esters. The maximum surface anæsthetic activity is obtained with the butoxybenzamide (IV), whereas with the esters there is a regular increase of activity up to the hexylderivative (XII). The infiltration anæsthesia is greatly reduced in the amides, whereas the local irritation remains of the same order of magnitude as with the esters. It may be concluded that the compounds of these series offer no prospect of therapeutic significance.

Preparation

NN-*Diethylethylenediamine*. A simple modification of a method described in the literature[9,10] was used to prepare this amine.

Diethylaminoethanol was transformed into diethylaminoethyl chloride in 80 per cent. over-all yield[11,12]. 35 g. (0·19 mole) of potassium phthalimide was added to a solution of 18·5 g. (0·14 mole) of diethylaminoethyl chloride in 100 ml. of anhydrous xylene, and the mixture

was refluxed for 16 hours. After cooling the solution was filtered, and the filtrate was extracted with 150 ml. of 5 N hydrochloric acid. The aqueous solution was refluxed for 2 hours, and, after cooling, the phthalic acid was filtered. The filtrate was evaporated to dryness, and the residue treated with an ice-cold concentrated solution of potassium hydroxide and extracted with ether. The ether solution was dried with potassium

TABLE I

A COMPARISON OF THE PHARMACOLOGICAL ACTIVITIES OF THE AMIDE AND ESTER DERIVATIVES

		Corneal anæsthesia				Infiltration anæsthesia in guinea-pigs		Tissue toxicity		
		Duration with 1 per cent. solution				Duration with 0·1 per cent. solution		Rating* and diameter (mm.) of reaction zone		
		Rabbits		Guinea-pigs						
	R	Minutes	Potency ratio cocaine = 1	Minutes	Potency ratio cocaine = 1	Minutes	Potency ratio procaine = 1	0·5 per cent. solution	1 per cent. solution	Hæmolytic concentration
R-C$_6$H$_4$-CONH·CH$_2$·CH$_2$N(C$_2$H$_5$)HCl										
I.	CH_3O	0	—	0	—	0	—	+ (2)	+ (3)	>1/100
II.	C_2H_5O	0	—	0	—	0	—	+ (2)	+ (4)	>1/100
III.	n-C_3H_7O	15	2·1	18	1·0	6	0·4	+ (2)	+ (3)	1/200
IV.	n-C_4H_9O	21	3·0	24	1·3	8	0·5	+ (2)	+ (3)	1/400
V.	n-$C_5H_{11}O$	6†	0·9	15	0·8	9	0·6	+++ (5)	+++ (9)	1/2000
VI.	n-$C_6H_{13}O$	9†	1·3	21	1·1	12	0·8	+++ (6)	+++ (10)	1/3500
R-C$_6$H$_4$-COO·CH$_2$CH$_2$·N·(C$_2$H$_5$)$_2$HCl										
VII.	CH_3O	0	—	0	—	0	—	+ (3)	++ (4)	>1/100
VIII.	C_2H_5	9	1·3	24	1·3	33	2·2	0	0	>1/100
IX.	n-C_3H_7O	12	1·7	26	1·4	24	1·6	+ (4)	++ (7)	1/200
X.	n-C_4H_9O	19	2·7	42	2·3	28	1·9	0	++ (6)	1/200
XI.	n-$C_5H_{11}O$	25	3·6	45	2·5	37	2·5	++ (5)	+++ (8)	1/2000
XII.	n-$C_6H_{13}O$	44	6·3	95	3·3	56	3·7	+++ (9)	+++ (10)	1/3000
	Procaine	0	—	0	—	15	1·0	0	+ (3)	>1/100
	Cocaine	7	1·0	18	1·0	27	1·8	+ (2)	+ (5)	—

* + Erythema.
++ Erythema with petechiæ.
+++ Erythema with ulceration.

† These products were irritating for the eye.

carbonate, and after removing the solvent, the product distilled. Yield: 10·5 g. (67 per cent.). B.pt. 144 to 147° C.

p-*Alkoxybenzoyl-diethylaminoethylamides*. No reaction was observed between methyl *p*-ethoxybenzoate and *NN*-diethylethylenediamine in methanol or water. For this reason all products were prepared by the following method. 0·04 mole of *NN*-diethylethylenediamine and 0·04

TABLE II

	M.pt. ° C.*	Per cent. nitrogen†		
		Calculated	Found	
I.	110 to 111°	9·77	9·78	9·75
II.	151 to 152°	9·31	9·31	9·30
III.	128 to 130°	8·90	8·88	8·91
IV.	112 to 113°	8·52	8·54	8·54
V.	98 to 99°	8·17	8·19	8·17
VI.	93 to 94°	7·85	7·84	7·84

* Most products are hygroscopic.
† The elementary analyses were made by Dr. A Konovalov in the Laboratory of General Chemistry, University of Louvain.

mole of the acid chloride were dissolved separately each in 20 ml. of benzene. The two solutions were mixed with cooling, and subsequently heated for 4 hours on the water bath. When the product did crystallise on cooling, it was filtered and recrystallised. Otherwise, the solution was evaporated to dryness, *in vacuo*, and the residue was crystallised from acetone or methylethylketone.

Table II lists the melting points and elementary analyses of these preparations.

Summary

The diethylaminoethylamides of several *p*-alkoxybenzoic acids were prepared. The study of their pharmacological properties has shown that they possess a smaller anæsthetic activity, but nearly the same toxicity as the corresponding esters.

References

1. Hamilton, Westfall and Fergusson, *J. Pharmacol.*, 1948, **94**, 299.
2. Miescher, *Helv. Chim. Acta*, 1932, **15**, 163.
3. Sievers and McIntyre, *J. Pharmacol.*, 1938, **62**, 252.
4. Büchi, Stünzi, Flury, Hirt, Labhart and Ragaz, *Helv. Chim. Acta*, 1951, **34**, 1002.
5. Labhart, Thesis, Zürich (1946), cited by Büchi, *Arzneimit.-Forsch.*, 1952, **2**, 34.
6. Büchi, Labhart and Ragaz, *Helv. Chim. Acta*, 1947, **30**, 507.
7. Vanderhaeghe, Kolosy and Claesen, *J. Pharm. Pharmacol.*, 1954, **6**, 119.
8. Carney in Suter *Medicinal Chemistry*, Vol. I, Wiley, 1951, p. 300.
9. Schinzel and Benoit, *Bull. Soc. chim., Fr.*, 1939, [5], **6**, 501.
10. Koji and Miura, *J. pharm. Soc. Japan*, 1942, **62**, 217, through *Chem. Abstr.* (1950), 10161E.
11. Slotta and Behnisch, *Ber. dtsch. Chem. Ges.*, 1935, **68**, 754.
12. Doering and Rhoads, *J. Amer. chem. Soc.*, 1951, **73**, 3082.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Canescine and Pseudoyohimbine from the Roots of *Rauwolfia canescens* L. A. Stoll and A. Hofmann. (*J. Amer. chem. Soc.*, 1955, **77**, 820.) In addition to the 4 alkaloids, α-yohimbine (rauwolscine), yohimbine, serpentine and reserpine which have been isolated from the roots of *Rauwolfia canescens* L., 2 further alkaloids have now been isolated from this source. These latter were isolated from the methanol mother liquor of reserpine by means of chromatography with aluminium oxide and fractional crystallisation. One of the alkaloids, m.pt. 265 to 278° C. (cor., in vacuum tube) with decomposition, crystallised from methanol in hexagonal plates, and was found to be identical with pseudoyohimbine which was originally found in yohimbé bark. The second alkaloid, which was not identical with any known compound, was called canescine. It crystallises from 15 parts of methanol in thick pentagonal and hexagonal plates, m.pt. 230 to 234° C. (cor.) with decomposition, $[\alpha]_D - 163 \pm 2°$ (*c.*, 0·5 in pyridine). The values obtained on analysis indicate the empirical formula $C_{32}H_{38}O_8N_2$. The hydrochloride crystallised from aqueous acetone in thin rectangular plates, m.pt. 247 to 253° C. (cor.) with decomposition. It yields an equivalent of trimethoxybenzoic acid upon alkaline hydrolysis; boiling with sodium methylate in methanol gives canescinic acid methyl ester and trimethoxybenzoic acid methyl ester. From the analytical and ultra-violet data and from biogenetic considerations, the structure, 11-desmethoxyreserpine was suggested for canescine. It possesses pharmacological properties similar to those of reserpine (see p. 493); above all, it produces a marked and prolonged fall in blood pressure. A. H. B.

ANALYTICAL

Ergot Alkaloids, Paper Chromatographic Separation of. J. Tuzson and G. Vastagh. (*Pharm. Acta Helvet.*, 1954, **29**, 357.) A rapid method, which does not require specially treated paper, is as follows. The alkaloids (about 10 to 12 μg.), in the form of the free bases, are applied to the paper from an alcoholic solution, and the solvent used is composed of toluene or benzene, light petroleum and methanol (25:25:10). The paper is dried, and the spots are observed under ultra-violet light. The R_F values are as follows (using Whatman No. 1 paper): Ergotinine, 0·89; ergotoxine, 0·70; ergotamine, 0·43; ergotaminine, 0·71; ergosine, 0·48; ergosinine, 0·74; ergometrine, 0·16; lysergic acid, 0·028. G. M.

Kjeldahl Nitrogen Determination, Potassium Permanganate in the. A. E. Beet. (*Nature, Lond.*, 1955, **175**, 513.) A method for the Kjeldahl digestion process is described for semimicro (1 to 2 mg.) and micro (0·2 to 1 mg.) quantities of nitrogen. The substance is heated with sulphuric acid for about 5 minutes and after slight cooling, potassium permanganate is added in successive small amounts with shaking until the digest becomes char-free. After boiling for one minute, cooling, and adding sufficient permanganate to produce a dirty sage-green colour, digestion for a further minute completes the conversion; the ammonia is determined by steam distillation after making alkaline. About

90 per cent. of the nitrogen of coals has been converted at the char-free stage, the remaining 10 per cent. being converted by the final permanganate addition and the short after-boiling; with resistant non-charring substances, all the conversion occurs during the last period. Loss of nitrogen (or ammonia) does not occur during the digestion unless the temperature rises above 330° C. On the semimicro scale coals, cereals, feeding stuffs, leather and various alkaloids of low nitrogen content have been satisfactorily examined, while the same success, using micro-amounts, has been achieved with pyridine carboxylic acids, tryptophan, and all the alkaloids so far examined. R. E. S.

Penicillin Fermentations, Colorimetric Determination of o-Hydroxyphenylacetic Acid. S. C. Pan. (*Analyt. Chem.*, 1955, **27**, 65.) A method was developed based on the conversion of phenols into *p*-nitrosophenol which gives a yellow colour with ammonia. The reactions were performed entirely in an aqueous medium, giving a more sensitive assay; as little as 3μg. of *o*-hydroxyphenylacetic acid could be accurately determined by the procedure described. The *o*-hydroxyphenylacetic acid was separated from other ingredients of a cornsteep liquor medium by extraction with amyl acetate. With 2·5 volumes of amyl acetate per volume of aqueous solution, the extraction efficiency varied, within the range of 50 to 500 *o*-hydroxylphenylacetic acid per ml., between 84 and 89 per cent. An average value of 86 per cent. was therefore used as a correction factor and the recovery values, calculated on this assumption, ranged from 93·1 to 101·5 per cent., which was considered satisfactory.

R. E. S.

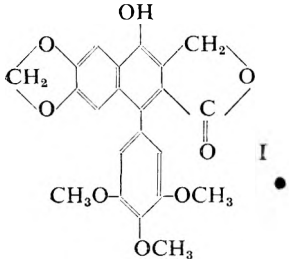
GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Purpurea Glycosides A and B, Decomposition of, by Digipurpidase. K. B. Jensen. (*Acta pharm. tox., Kbh.*, 1954, **10**, 347.) Digipurpidase, an enzyme present in the leaves of *Digitalis purpurea*, hydrolyses purpurea glycosides A and B into digitoxin and gitoxin. A method is described for estimating its activity. The enzyme preparation is the dried powdered digitalis leaf after extraction of the glycosides with ethanol. The substrate is a relatively pure preparation of purpurea glycosides A and B (Sandoz). For the estimation, a volume of a methanol solution of the substrate, corresponding to 70 to 90 μg. of the glycosides, is transferred to a 2-ml. test tube using a micrometer syringe. After evaporation of the methanol, 25 mg. of the enzyme preparation is added and 1·0 ml. of a phosphate buffer solution at *p*H 5·9. The tube is shaken in a water bath (different times and temperatures were used in the investigation), 0·4 ml. of methanol is then added and the tube incubated at 20° C. in water. The enzymatic hydrolysis is followed by fluorimetric determination of the primary glycosides after paper chromatographic separation of the split products. A mixture of acetone, chloroform and formamide is used for development of the chromatograms. The results show that increasing temperature accelerates the glycoside-cleaving action of digipurpidase reaching a maximal at 60 to 65° C., but it is accompanied by an increasing inactivation of the enzyme. Previous heating of the enzyme preparation showed incomplete inactivation at 80° C. for 4 hours and little inactivation at 50 to 60° C. It is concluded that in the drying of digitalis leaves extensive cleavage of the glycosides will occur unless a temperature of 80° C. is rapidly attained throughout the leaf mass. The leaf will not be stabilised if drying is stopped as soon as the leaf is dry enough for powdering. Enzymatic activity of digipurpidase is considerably inhibited by 20 per cent. v/v of ethanol or methanol. During the extraction of digitalis leaves with 70 per cent. ethanol little hydrolysis should occur. G. F. S.

***Syrenia angustifolia*, Cardiac Glycosides of.** N. P. Maksyutina and D. G. Koleskikov. (*Aptechnoe Delo*, 1954, **3**, 18.) A preparation containing the total glycosides of *Syrenia angustifolia* was obtained by extracting the whole ground herb with ethanol, evaporating the extract to a syrup, extracting with water then with chloroform-ethanol and finally chromatographing on an alumina column. After removal of chloroform-soluble impurities, a bright yellow powder, termed "Corglisan," was obtained (4–5 g. from 10 kg. of herb). The biological activity on cats was 0·27 mg./kg. It gave positive Liebermann, Keller-Kiliani and Legal reactions. On acid hydrolysis, 15 g. of "Corglisan" yielded 7 g. of an aglycone fraction. This was dissolved in benzene and chromatographed on alumina, the column being eluted successively with benzene, benzene-chloroform, chloroform, chloroform containing increasing proportions of ethanol and finally with pure ethanol. Forty fractions were collected and 3 crystalline compounds were obtained: (i) m.pt. 185–6° C., crystallising in needles from methanol, (ii) m.pt. 198–200° C. and (iii) m.pt. 174–5° C. Paper-chromatography showed one of the compounds to be identical with strophanthidin. Two sugars were obtained from the hydrolysis liquor; one of these was soluble in acetone and gave a positive Keller-Kiliani reaction. E. H.

PLANT ANALYSIS

Dehydropodophyllotoxin, a New Compound Isolated from *Podophyllum peltatum* L. H. Kofod and C. Jorgensen. (*Acta. chem. scand.*, 1954, **8**, 1296.) During chromatography of podophyllin using sorbed formamide as a stationary phase and benzene as eluant, an intense blue fluorescence of certain fractions of the eluate between those containing podophyllotoxin and those containing α-peltatin was observed. Paper-chromatographic analysis of these eluates revealed five and possibly six different spots detectable by their blue fluorescence in ultra-violet radiation. A substance m.pt. 272 to 274° C. was isolated from the largest spot and the yield corresponded to 0·1 per cent. from podophyllin. Formula I is proposed for the substance which has been named dehydropodophyllotoxin. A. H. B.



I

***Podophyllum emodi* Wall. *var. hexandrum* (Royle), Chemical Examination of.** S. C. Caakravarti and D. P. Chakraborty. (*J. Amer. pharm. Ass., Sci. Ed.*, 1954, **43**, 614.) Powdered roots and rhizomes were extracted with light petroleum in a Soxhlet apparatus, and crystalline podophyllotoxin was obtained on cooling the extract in a freezing mixture. A viscous brown oil with a pungent odour was obtained from the mother liquor; yield 0·75 per cent. of the weight of material extracted. Resin was obtained by the method described in the B.P. 1948 for podophyllin (yield 7·3 per cent.). It contained 3·68 per cent. of the pungent oil. After purification by the removal of tar, the resin was submitted to chromatographic analysis on an activated alumina column, using a mixture of equal volumes of benzene and dehydrated ethanol as developing solvent. Podophyllotoxin (32 per cent.), picropodophyllin (0·33 per cent.) and the yellow pigment quercetin (6 per cent.) were isolated from the resin. G. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Angiotonin (Hypertensin), Purification of. C. A. Kuether and M. E. Haney. (*Science*, 1955, **121**, 65.) A method is described for preparing preparations of angiotonin of a higher purity than previously reported. The highly purified preparation was unstable and lost pressor activity, rapidly deteriorating to a residual activity of about 500 AU/mg. G. F. S.

Kynurenine, Investigation on the Excretion of, in Humans. M. Špaček. (*Canada J. Biochem. Physiol.*, 1955, **33**, 14.) Kynurenine is a derivative of tryptophane which appears to be present in human urine. 24-hour specimens of urine from physically well psychotics, old people and college students, were analysed. Over a period of 4 days values for one person were fairly constant unless changes occurred in the patients condition or diet. Kynurenine excretion varied independently of the excretion of creatinine. Unusually high or low concentrations of kynurenine were independent of the *p*H of the urine. The indican reaction was often positive in urines containing large amounts of kynurenine. Elimination of tryptophane from the diet made little difference where there was no tendency to excrete kynurenine, but in cases with abnormal concentrations on a mixed diet the excretion tended to return to normal values. Comparatively large doses of tryptophane were needed to affect urinary kynurenine. Of the vitamins, pyridoxine had no consistent effect, but there was a decrease after nicotinamide. No sex differences were found, but there were increasing concentrations of kynurenine with increasing age. G. F. S.

BIOCHEMICAL ANALYSIS

Isoniazid in Blood Serum and Cerebrospinal Fluid, Estimation of. G. Hunter. (*Brit. med. J.*, 1955, **1**, 585.) The method is a modification of that of Cuthbertson *et al.* applicable to 1·25 ml. of sample. With either material protein is coagulated and removed by warming with acetic acid and centrifuging. The supernatant liquid is heated in a boiling water bath with picryl chloride solution, and the mixture is shaken with acetic acid and butanol. After centrifuging again, the butanol layer is separated and its optical density at 500 mμ is determined. A calibration curve is constructed from aqueous solutions of known strengths of isoniazid. A control determination is conducted at the same time and the value deducted from the observed value. This method makes it possible to determine isoniazid added to serum within about 70 to 95 per cent. of the amount added. H. T. B.

Morphine, Rapid Method for the Estimation of. J. M. Fujimoto, E. L. Way and C. H. Hine. (*J. Lab. clin. Med.*, 1954, **44**, 627.) A method is described for the estimation of morphine in body fluids. For total morphine (bound plus free) acidify 15 ml. of urine with 1·5 ml. of concentrated hydrochloric acid in a 50 ml. glass-stoppered centrifuge tube. Stopper and autoclave for 30 minutes at 15 lb. pressure. Cool, add 10 ml. of 16 N potassium hydroxide and 20 ml. of *n*-butanol. Shake for 15 minutes, centrifuge and transfer a 15 ml. aliquot of the butanol layer to a centrifuge tube containing 22 ml. of N sulphuric acid. Shake for 3 minutes and centrifuge. Remove the butanol layer by aspiration and transfer 20 ml. of the acid layer to another extraction tube containing a drop of phenolphthalein indicator. While

agitating the tube, add concentrated potassium hydroxide until pink. Add approximately 1 g. of sodium bicarbonate and shake until dissolved. Add 21 ml. of chloroform and shake for 5 minutes. Centrifuge and aspirate the upper aqueous layer. Shake a 20 ml. aliquot of the chloroform layer in a centrifuge tube with 7 ml. of phosphate buffer, *p*H 5·8. Centrifuge and transfer 5 ml. of the final buffer solution of morphine to a 10 ml. volumetric flask. To this aliquot add 2 ml. of silicomolybdic acid reagent and 2 ml. of concentrated ammonium hydroxide. Stopper immediately, shake and bring up to volume with distilled water. Allow 15 minutes for full colour development and determine the optical density at 675 mμ on a spectrophotometer. After correcting for reagent blank the amount of morphine is obtained from a standard curve for morphine. For free morphine omit the hydrolysis step. The final buffer extract of morphine can also be used in ultra-violet absorption studies, as a highly specific test for morphine, since therapeutic agents possessing structural features common to morphine may interfere with its determination.

G. F. S.

Poliomyelitis Virus and Antibody, a Simplified Colorimetric Test for. M. M. Lipton and A. J. Steigman. (*Proc. Soc. exp. Biol. N.Y.*, 1955, **88**, 114.) A simplified method is described for the titration of poliomyelitis viruses and their type-specific antibodies in animal and human sera. The results are indicated by colour changes in prepared test tubes containing dispersed HeLa cells, which produce acid from glucose and change the colour of phenol red to yellow. In the presence of unneutralised poliomyelitis virus the HeLa cells are destroyed and the indicator remains red. There was mostly good agreement between the colour and the activity of cytopathogenic viruses *in vitro* determined by the microscopic examination for friable cells. G. F. S.

CHEMOTHERAPY

Cephalosporin C, a New Antibiotic containing Sulphur and D-α-Aminoadipic Acid. G. G. F. Newton and E. P. Abraham. (*Nature, Lond.*, 1955, **175**, 548.) Cephalosporin C has been isolated from a species of *Cephalosporium* in the form of its sodium salt, $[\alpha]_D^{20} + 103°$; $\lambda_{max.}$ 260 mμ $\epsilon_{max.}$ 9500; equivalent weight 480 ± 15 (titration); 470 ± 15 (X-ray measurements), and is probably $C_{16}H_{20}O_8N_3SNa,2H_2O$. In the infra-red, a band shown at 5·61 μ is characteristic of the common penicillins (and of cephalosporin N), and in the former has been attributed to C=O of the fused β-lactamthiazolidine ring system. A band at 5·77 μ could be due to an ester or lactone grouping. Cephalosporin C gives a positive ninhydrin reaction and has been shown to be a monoamino-dicarboxylic acid, having two acidic groups with pK values of 3·1 and < 2·6 respectively, and a basic group with a pK of 9·8. It is stable in aqueous solution at *p*H 2·5 but is inactivated and degraded at pH 12. It is not inactivated by penicillinase from *B. subtilis*, strain 569, but loses activity in the presence of penicillinase from *B. cereus* (NRRL 569). Acid hydrolysis yields 1 mole of carbon dioxide and D-α-aminoadipic acid. Treatment with 1-fluoro-2:4-dinitro-benzene and subsequent hydrolysis yields 2:4-dinitrophenyl-α-aminoadipic acid, indicating that the α-amino group is unsubstituted. The pK value of this group suggests that the α-carboxyl group is also free. Acid hydrolysis yields little if any penicillamine, though isolation of valine indicates that the carbon skeleton of penicillamine is present. Cephalosporin C shows a level of activity similar to that of cephalosporin N against *E. coli*, and an activity of 8 to 10 units/mg. against *Staph. aureus* and *Salm. typhi*. J. B. S.

PHARMACY

NOTES AND FORMULÆ

Aneurine, Stability of Solutions of. E. Pongratz. (*Pharm. Acta Helvet.*, 1954, **29**, 352.) The stability of solutions of aneurine has has been determined by using for the assay the biological method of Schopfer, which is based on the specific sensitivity of *Phycomyces blakesleeanus* for this compound. No loss of strength could be detected in a plain solution of aneurine (5 per cent.), nor in one containing, in addition, calcium glucolævulinate, ascorbic acid and cysteine. G. M.

Barbituric Acids, Decomposition of Solutions of. H. Nuppenau. (*Dansk Tidsskr. farm.*, 1954, **28**, 261.) Aqueous solutions (about 10 per cent.) of various barbituric acids (as sodium derivatives) were kept at definite temperatures for varying periods. The total amount of decomposition was determined by the cobaltamine method, and in addition the carbon dioxide produced was determined, and the final *p*H of the solutions. A selection of the results is given in the table below:—

Compound	Temperature	Time	Per cent. decomposition	Final *p*H
Allobarbitone sodium ..	20° C.	105 days	2·8	10·43
	20° C.	524 days	11·3	10·04
	30° C.	105 days	6·0	10·22
	30° C.	524 days	23·5	9·21
	90° C.	2 hours	2·7	10·43
	90° C.	10 hours	10·9	9·73
	100° C.	2 hours	5·6	10·09
Barbitone sodium	20° C.	20 days	3·2	10·46
	20° C.	100 days	18·2	10·03
	30° C.	20 days	11·4	10·33
	30° C.	100 days	43·2	9·61
	90° C.	½ hour	5·2	10·79
	90° C.	2 hours	28·1	10·16
	100° C.	½ hour	11·0	10·56
Hexobarbitone sodium ..	20° C.	24 hours	4·2	10·69
	20° C.	72 hours	10·1	10·55
	30° C.	24 hours	7·3	10·50
	30° C.	72 hours	13·9	10·50
Phenobarbitone sodium ..	20° C.	10 days	3·0	9·72
	20° C.	50 days	10·7	9·31
	30° C.	10 days	7·0	9·52
	30° C.	50 days	20·7	9·07
	90° C.	10 minutes	5·4	9·58
	90° C.	60 minutes	10·4	9·34
	100° C.	10 minutes	6·5	9·54
	100° C.	60 minutes	17·0	9·35
Amylobarbitone sodium	20° C.	14 days	3·7	10·26
	20° C.	90 days	15·1	10·04
	30° C.	14 days	8·0	10·10
	90° C.	10 minutes	2·0	9·94
	90° C.	60 minutes	8·2	9·92
	100° C.	10 minutes	4·2	9·94
	100° C.	60 minutes	17·9	9·85

The decomposition follows the curve of a monomolecular process, and the velocity constants and temperature coefficients are given. G. M.

Diphemanil Methylsulphate (Prantal Methylsulphate). (*New and Nonofficial Remedies: J. Amer. med. Ass.*, 1955, **157**, 342.) Diphemanil methylsulphate is 4-diphenylmethylene-1 : 1-dimethylpiperidinium methylsulphate and occurs as a white or nearly white, bitter, crystalline substance, with a faint characteristic

odour, m.pt. 189° to 196° C., very slightly soluble in ether, and soluble, at 25° C., in about 33 parts of water, ethanol, and chloroform. It is stable to heat and light but somewhat hygroscopic. A 1 per cent. solution has *p*H 4·0 to 6·0, and yields a brown precipitate with potassium permanganate. The picrate has a melting point of 194° to 200° C., after being dried *in vacuo* for 5 hours. When dried at 105° C. for 4 hours, diphemanil methylsulphate loses not more than 0·5 per cent. of its weight; it yields not more than 0·1 per cent. of sulphated ash. It contains 97 to 103 per cent. of anhydrous diphemanil methylsulphate, when determined by precipitation as the reineckate, and 98 to 102 per cent. when determined by hydrolysis with potassium hydroxide in propylene glycol, and precipitation of the liberated sulphate with barium hydroxide. It is a quaternary parasympatholytic agent. G. R. K.

PHARMACOGNOSY

Ergot Sclerotia, Alkaloid Formation in. Y. H. Loo and R. W. Lewis. (*Science*, 1955, **121**, 367.) The time required for alkaloids to appear in the sclerotia of cultured ergot has been investigated. A plot of tetraploid Rosen rye was inoculated over two days by spraying the flowers with a sugar-spore suspension, the spores being produced in shake cultures on a medium of 40 per cent. commercial sucrose in potato broth prepared by boiling 400 g. of sliced potatoes in sufficient water to produce 1 l. of broth when decanted. Samples each consisting of about 200 heads were collected from the plot at random 8, 10, 12, 15, 17, 19 and 26 days after the last inoculation, and the heads were dried for 2 days at 60 to 80° C., after which the sclerotia were removed and weighed. The average weights of sclerotia in mg. for daily samples were 4·9, 6·2, 7·3, 10·2, 23·2, 38·0 and 55·6 mg. respectively. Pigmentation was not complete until after the 12th day, when the sclerotia were found to be heavily pigmented. The amount and nature of the alkaloids produced was then determined. Dried pulverised samples were extracted with ammoniacal ethanol, the ethanol was removed and the alkaloids at *p*H 8 in the water layer were extracted into chloroform after which they were returned to aqueous maleic acid solution. The percentage yield of alkaloids was then determined colorimetrically by a modification of the Van Urk method with the following results: 0, 0, 0·005, 0·012, 0·05, 0·14 and 0·12 per cent. respectively. A visible absorption curve (400 to 800 mμ) of the blue reaction-product formed with the samples collected from the 12th to 26th day was identical with lysergic acid. An ergonovine type of activity was demonstrated by pharmacological assays, and paper chromatography using a butanol–acetic–water system identified ergonovine as the main component in extracts exhibiting a blue fluorescence in ultra-violet light. The authors conclude from the results that the alkaloids are synthesised in the fungus during the later stages of development. J. R. F.

***Rauwolfia sellowii*, Alkaloidal Content of.** T. A. Neubern de Toledo and R. Wasicky. (*Scientia Pharm.*, 1954, **22**, 217.) The alkaloids in *Rauwolfia sellowii* were determined by the method of Hörhammer and Rao (*Arch. Pharm. Berl.*, 1954, **287**, 75.) The content of different parts of the plant was as follows: Bark of thicker roots, 8·3 per cent.; bark of thinner roots, 3·5 per cent.; bark of twigs, 1·19 per cent.; bark of stem, 2·04 per cent.; shoots without leaves, 0·72 per cent.; leaves, 2·1 per cent. The content in the wood was practically nil. Thus the alkaloidal content decreases from the root to the ends of the twigs, though present only in the peripheral parts. The leaves should prove a useful source of the alkaloids. G. M.

PHARMACOLOGY AND THERAPEUTICS

Acetazoleamide (Diamox) Diuresis. A. Ruskin. (*Arch. intern. Med.*, 1955, **95**, 24.) Acetazoleamide, or 2-acetylamino-1 : 3 : 4-thiadiazole-5-sulphonamide, is a diuretic agent. This study is concerned with the kind, degree and length of diuresis, comparison of single and multiple dosage, possible hæmodynamic mechanisms, and possible effects on enzymes other than carbonic anhydrase. In 15 patients with cardiac failure given 3 to 6 g. of acetazoleamide by mouth in divided doses over 24 hours, the urine volume generally more than doubled in 24 hours, with marked rises in sodium and potassium excretion. While the diuresis slackened the day after the drug was given electrolytes continued to be excreted in large amounts. While diuresis was a constant effect, the urinary sodium and potassium concentration actually fell slightly in 5 and 2 of the 15 cases respectively on the first, but not on the second day. The toxic effects were mild parasthesias and drowsiness, nausea and vomiting in 3 cases, and a reversible psychosis in 1 case of nephrosclerosis with uræmia. In 3 cases of congestive failure refractory to mercurials, acetazoleamide produced a satisfactory diuresis. In many of the patients diuresis and weight loss continued for many days, up to 3 weeks, without further use of diuretics. In 12 patients with congestive heart failure a comparison of the effects of 500 mg. of acetazoleamide administered by mouth in a single dose or of 4 such doses given over 24 hours showed that diuresis was only slightly more effective in the second and third 8-hour periods after multiple doses. Acetazoleamide diuresis was found by the author to be associated with carbonic anhydrase inhibition in the renal tubules, producing an alkaline urine, excessive distal tubular excretion of potassium, and decreased tubular reabsorption of sodium and other cations, bicarbonate, and, consequently, water. Lack of toxicity in the heart and kidney was evidenced by failure to inhibit the activity of succinic dehydrogenase and adenosine triphosphatase in those organs. Such hæmodynamic alterations as may occur after acetazoleamide administration do not contribute to its diuretic effect. S. L. W.

Adrenaline and Noradrenaline, Intrathecal Injections of. S. O. Liljedahl. (*Acta physiol. scand.*, 1955, **33**, 19.) The effect of adrenaline and noradrenaline on the blood glucose and blood pressure of the cat is studied. The injection is given either into the cisterna magna or the third ventricle of the brain or as a continuous intravenous infusion. Adrenaline, when given intracisternally, produces a marked increase in the blood sugar level without raising the blood pressure. Noradrenaline, given by the same route, again has no effect on the blood pressure and has less effect than adrenaline on the blood sugar level. Injections into the third ventricle produce similar results. Continuous intravenous infusion of suitable doses of either amine causes a similar rise of blood sugar as when given intracisternally, adrenaline being 5 to 10 times more potent than noradrenaline. Again there is no effect on the blood pressure. These experiments suggest that the hyperglycæmic effect of either adrenaline or of noradrenaline, when injected into the cisterna magna or the third ventricle, is brought about by absorption of small quantities from the dural space. M. M.

Anticholinesterases and Muscle Relaxants. B. G. B. Lucas and S. Miles. (*Brit. med. J.*, 1955, **1**, 579.) Gallamine and tubocurarine act by competing with acetylcholine at the motor end-plate, while succinylcholine and decamethonium iodide act in the same way as an accumulation of acetylcholine, namely by persistently polarising the motor end-plate and rendering it insensitive to acetylcholine. Succinylcholine is hydrolysed in the same way as acetylcholine

by cholinesterase but decamethonium iodide does not depend on cholinesterase for its removal. The amount of cholinesterase in the body may therefore alter the duration of action of some relaxants, so that exposure to anti-cholinesterase substances may necessitate modification of the usual dosage of the relaxants. To test the hypothesis a comparison was made of the duration of respiratory paralysis following intravenous injection of comparable dosages of tubocurarine, gallamine, decamethonium iodide and succinylcholine into rhesus monkeys before and after a standard dose of the anti-cholinesterase sarin (*iso*propylmethyl phosphonofluoridate). The sarin was given subcutaneously in doses equal to two-thirds of the LD50, which produced miosis, salivation, respiratory distress and muscular fibrillation. The relaxants were investigated in pairs, the time being taken from the injection to the re-establishment of effective natural respiration. Blood was withdrawn for plasma cholinesterase determination at the beginning and end of the period. With tubocararine and gallamine there was a significant decrease in the period of respiratory paralysis after injection of sarin; with succinylcholine there was an increase. Sarin had no appreciable effect on the duration of action of decamethonium iodide. With the increasing use of anticholinesterases as insecticides caution is needed to avoid the possibility of poisoning by muscle relaxants other than decamethonium iodide and the same would apply to war casualties exposed to sarin. H. T. B.

Antihistamines as Adrenaline Sensitisers. K. Kuriaki and T. Uchida. (*J. Pharmacol.*, 1955, **113**, 228.) The three antihistamines used are diphenhydramine, methaphenilene and *N*-dimethylaminoethyl phenothiazine hydrochloride (Anergan). The actions of these drugs on adrenaline responses, sympathetic and vagal stimulation and on the activity of amine oxidase are studied. The preparations used are the isolated heart of the toad, the blood vessels of the hind limb of the toad, the isolated intestine of the rabbit, the blood pressure and respiration of the rabbit and the nictitating membrane of the cat. Anergan, and particularly diphenhydramine, sensitise receptors to the effects of adrenaline and sympathetic stimulation but block the effects of vagal stimulation. Methaphenilene has variable effects, sometimes increasing the response to adrenaline and sometimes decreasing it. Consistent with their ability to act as adrenaline sensitisers, diphenhydramine and Anergan inhibit the activity of amine oxidase, whereas methaphenilene produces no inhibition. Thus it is assumed that the adrenaline sensitising effect of these antihistamines is related to their anti-amine oxidase activity. M. M.

Azaserine, Effect of, on the Growth of Mouse and Rat Tumours. K. Sugiura and C. C. Stock. (*Proc. Soc. exp. Biol., N.Y.*, 1955, **88**, 127.) The effects of crude culture filtrates of *Streptomyces fragilis* and partially purified and crystalline preparations of azaserine (*O*-diazoacetyl-L-serine) have been tested against 17 mouse tumours, 5 rat tumours and 3 ascite tumours of the mouse. The effects of the filtrates were generally similar to crystalline azaserine. Azaserine had a marked inhibitory effect against 1-day-old implants of Sarcoma 180, a moderate effect on Adenocarcinoma E0771, Patterson lymphosarcoma and Mecca lymphosarcoma and on Sarcoma 180 ascites tumour, Ehrlich ascites carcinoma and Krebs 2 ascites carcinoma. There was no inhibition of any of the tumours, except Sarcoma 180, when tests were made with 7-day-old growths. Daily doses of 5 mg./kg./day inhibited Walker carcino-sarcoma 256, Sarcoma R39, Jensen sarcoma and Murphy–Sturm lymphosarcoma, but only a slight inhibitory effect on Flexner–Jobling carcinoma. G. F. S.

Benzathine Penicillin, Rectal Absorption in the Rabbit. S. Carvalho and A. Santos. (*Rev. Portugesa Farm.*, 1954, **4**, 237.) Serum levels of benzathine penicillin have been studied in the rabbit after rectal administration of 150,000 U. in suppositories with a water soluble (polyethylene glycols 6000–75 per cent. and 1500–15 per cent.) and cocoa butter base. Penicillin was detectable, using the F.D.A. method, after 10 minutes; the highest concentrations appearing between 20 minutes and 3 hours after administration of the water soluble suppository and between 30 minutes and 7 hours after the cocoa butter preparation. These were 0·35 U./ml. and 0·12 U./ml., respectively. J. R. F.

1-*n*-Butylamino-3-*p*-toluidino-2-propanol (W181), the Analeptic Action of. F. M. Berger and T. E. Lynes. (*J. Pharmacol.*, 1954, **112**, 399.) 1-*n*-Butylamino-3-*p*-toluidino-2-propanol has been shown to relieve paralysis caused by mephenesin (Berger, *J. Pharmacol.*, 1953, **107**, 250). It also has high analeptic activity in mice against phenobarbitone and benzimidazole, arousal being obtained with non-convulsant doses of the analeptic. Activity is somewhat less against hexobarbitone, pentobarbitone, thiopentone and chloral hydrate, doses of the order of twice the LD50 being necessary. Convulsions caused by W181 were prevented or lessened in severity by thiopentone, chloral hydrate, mephenesin, phenobarbitone and pentobarbitone, in that order of effectiveness. Benzimidazole or hexobarbitone in the doses used had no anticonvulsant effect against single LD50 doses. G. P.

Chlorpromazine in Psychiatric Conditions. H. E. Lehmann. (*Canad. med. Ass. J.*, 1955, **72**, 91.) This is a report on the results of chlorpromazine therapy in 238 neuropsychiatric patients. In general, the most promising application seems to lie in the treatment of the manic phase of manic-depressive psychosis, in which the drug brought about a complete remission within 40 days in 48 per cent. of cases. In conditions of chronic manic excitement which have resisted other therapeutic measures it often produces favourable results, though the drug may have to be administered for 2 or 3 months in these cases. Of 98 cases of schizophrenia complete recovery was obtained within 40 days of commencement of treatment in 28 per cent. of those patients whose symptoms had been present for 1 month or less. If much improved patients are included, 39 per cent. of 54 acute schizophrenics improved in less than 2 months sufficiently to be discharged from hospital. No recoveries and only 1 case of considerable improvement occurred among the 44 subacute and chronic schizophrenic patients, but symptomatic improvement and control was obtained in a large proportion of cases. The lowering of blood pressure calls for continued medical supervision and nursing care so long as the patient is receiving large doses of the drug. 5 per cent. of patients receiving the drug for more than a week developed allergic conditions, such as urticaria or angioneurotic œdema. 3 per cent. of patients showed gastro-intestinal symptoms, and 4 to 6 per cent. developed an extrapyramidal syndrome resembling Parkinsonism when the drug was given in large doses over a long period. Several patients with a history of epileptiform seizures developed convulsions while receiving the drug. 8 patients developed jaundice during treatment but responded well to discontinuation of the drug and supportive treatment. No untoward effects on bone marrow or kidney function were observed. Chlorpromazine differs from other short-acting sedatives because of its more selective effect on mesencephalic–diencephalic structures, thus providing a new therapeutic approach to certain troublesome psychiatric conditions. In acute psychotic breakdowns associated with affective disturbances, more specifically psychomotor excitement and

emotional tension, it may shorten or prevent full development of an attack and may be preferable to electroshock therapy. S. L. W.

Chlorpromazine in the Treatment of Intractable Hiccups. C. E. Friedgood and C. B. Ripstein. (*J. Amer. med. Ass.*, 1955, **157**, 309.) 46 men and 2 women aged from 26 to 80 years of age and suffering from intractable hiccups were treated with chlorpromazine. Symptoms had persisted from days to weeks and had not responded to heavy sedation, carbon dioxide inhalations, or any other therapy including, in 5 patients, phrenic nerve crush. A dose of 50 mg. was given intravenously and was usually sufficient to stop the hiccups; when necessary a second dose was given within 2 to 4 hours. In several of the older and debilitated patients, 25 mg. was given intravenously as the initial dose and 25 mg. intramuscularly. Of the 50 patients treated, 41 were relieved almost immediately without recurrence of symptoms. 5 patients had recurrence after at least 6 hours relief. In these, further administration reduced the intensity and frequency of the hiccups but did not effect a cure. 4 patients showed no response at all because the causal factors were not treated, such as a subphrenic abcess, or failure of a colostomy stoma to open. Good results were also obtained in patients with a milder type of hiccups by oral administration although the effect was often delayed up to 24 hours. G. R. K.

Dromoran, Fate of, in the Dog. P. Shore, J. Axelrod, C. Hogben and B. B. Brodie. (*J. Pharmacol.*, 1955, **113**, 192.) This paper describes a sensitive method for the estimation of Dromoran (3-hydroxy-*N*-methylmorphinan) in biological material and, using this method, a comparison is made of the fate of the (−)-isomer, levorphan and the (+)-isomer, dextrorphan in the body. The drug is extracted from alkaline biological material with benzene and estimated by forming a methyl orange complex. It is found that the fate of both the isomers in the dog is almost identical. About 60 per cent. appears in the urine in a conjugated form. The fate of the remainder is unknown. This transformation is rapid with a biological half-life for both isomers of less than 1 hour. This is considerably faster than that for morphine. In spite of this rapid disappearance of the drug the animal remains narcotised for a considerably longer time, suggesting that the activity of the compound might be mediated through a metabolic product. After parental administration, the drug is found in high concentration in the gastric juice. Neither levorphan nor dextrorphan is demethylated to the corresponding nor-isomer. M. M.

Erythromycin, Treatment of Neonatal Staphylococcal Infection with. J. O. Forfar, A. F. Maccabe, C. L. Balf, H. A. Wright and J. C. Gould. (*Lancet*, 1955, **268**, 584.) A trial designed to test the efficiency of erythromycin in neonatal staphylococcal infections and to determine whether resistance would appear quickly under control conditions was carried out in the maternity units of 2 hospitals. In one unit 140 cases were treated with erythromycin, in the other 80 cases were treated with erythromycin plus streptomycin to determine whether combined therapy would reduce the risk of resistant strains emerging. Treatment ranged over 2 to 9 days and the combination proved no more effective than erythromycin alone. There were no failures in the treatment of deep and superficial skin sepsis, but 8 per cent. of conjunctivitis cases did not respond. It was found by phage-typing that a limited number of strains were responsible for the cases of clinical infection. No strain of *Staph. pyogenes* developed resistance to erythromycin during the trial, the antibiotic did not interfere with the normal growth of *E. coli* in the gut, and no case of fungus or staphyloccus infection of the alimentary tract was found.

A free interchange of staphylococci between staff and infants took place and cross infection was common. The increased use of streptomycin appeared to increase the proportion of streptomycin-resistant organisms among staphylococci isolated from staff nasal carriers. J. R. F.

Hydrastine, Hydrastinine and Sparteine, Toxicity of. C. F. Poe and C. C. Johnson. (*Acta pharm. tox., Kbh.*, 1954, **10**, 338.) A comparison has been made of the toxicities of these alkaloids to albino rats and the results compared with their toxic action to micro-organisms. By the intraperitoneal route the estimates of LD50 for rats 6 weeks old, were hydrastine 104 mg./kg. and sparteine 42 mg./kg. The LD50 of sparteine was about 65 per cent. greater by the subcutaneous route. Lethal doses of hydrastine caused nervous excitation with tetanic spasms. Tested against the normal fermentative action of *Escherichia* and *Ærobacter* and on their growth, the toxicities of hydrastine, hydrastinine and sparteine were very low. G. F. S.

4-Hydroxy*iso*phthalic Acid, Analgesic and Antipyretic Activities of. G. B. Chesher, H. O. J. Collier, F. A. Robinson, E. P. Taylor, S. E. Hunt, J. I. Jones and A. S. Lindsey. (*Nature, Lond.*, 1955, **175**, 206.) 4-hydroxy-*iso*phthalic acid is a byproduct of the manufacture of salicylic acid to which it has a structural similarity. Pharmacological tests show that it has analgesic properties. In young rats, by the tail pressure method, the median effective dose was 303 (limits 261–353) mg./kg. and it had 4·1 per cent. of the activity of codeine. The LD 50 to rats was 1·071 mg./kg. (limits 968–1185). It was more effective and less toxic than aspirin. Chronic toxicity in mice was low and of the same order as aspirin. Excretion tests in rats showed 40 per cent. of a 10 mg. dose by mouth was excreted unchanged in the urine and 25 per cent. in the fæces. Antipyretic tests in rabbits showed it to be as effective as aspirin in counteracting fevers caused by a preparation of pyrogen from *Proteus vulgaris*. Clinical trials of the drug are in progress. G. F. S.

Mephenesin in the Treatment of Progressive Myoclonic Epilepsy. R. E. Kelly and D. R. Laurence. (*Brit. med. J.*, 1955, **1**, 456.) The main disability in progressive myoclonic epilepsy is myoclonus, the disease progressing until the patients exist in "status myoclonicus" and die of exhaustion and inanition within 4 to 5 years of the onset. The disease soon becomes resistant to the action of anticonvulsants. It has now been found that the myoclonus can be controlled by massive dosage of mephenesin. 5 cases are reported, of whom 4 helpless patients were restored temporarily to activity by the intravenous injection of a 1 per cent. solution of mephenesin in saline. One less serious case obtained considerable benefit from oral mephenesin, and treatment by mouth appeared to produce significant improvement in all, but in 3 cases it is too early to assess results. The drug is rapidly metabolised and the clinical effect of an intravenous injection may pass off in as little as 30 minutes, while the effect of an oral dose may last for only 1 to 1½ hours. Up to 48·5 g. of mephenesin per day has been required. When given by mouth the drug should be taken on a full stomach, to diminish the side effects, namely dizziness, drowsiness and vomiting, and may be taken partly as an elixir and partly as tablets, although the latter are absorbed less readily and probably less completely than the elixir. If the patient sips the elixir, the drug may anæsthetise the throat and this may lead to inhalation of the preparation. Of two cases reported fully, one is maintaining freedom from myoclonus on 15 g. of mephenesin carbamate per day. The second is taking a total of 30 g. per day of which 8 to 10 g. is in the form of an elixir and 20 to 22 g. as tablets. H. T. B.

***N*-Methyl-α-phenylsuccinimide (Milontin), Some Effects of, on the Central Nervous System.** W. H. Funderburk and R. T. Woodcock. (*J. Pharmacol.*, 1954, **112**, 404.) The action of *N*-methyl-α-phenylsuccinimide, an anticonvulsant drug, was studied at various levels of the cerebrospinal axis in the cat, and compared with that of trimethadione. Neither drug had much effect on the patellar reflex, or on the modification of this reflex by contralateral or ipsilateral sciatic stimulation. The electroencephalogram of the curarised cat under artificial respiration showed marked wave-slowing and sleep spindles with both depressants. Arousal responses to sensory stimulation were readily obtained with doses up to 100 mg./kg. The EEG arousal response induced by stimulation of the reticular formation activating centre was reduced with doses of 100 mg./kg. There were no changes in EEG spindle activity in mesencephalic-sectioned cats (*cerveau isolé* preparation) with doses of Milontin up to 150 mg./kg. or of trimethadione up to 400 mg./kg. The only difference between the two drugs lay in their ability to suppress cortical after-discharge, Milontin being the more potent. G. P.

2-Methyl-2-*n*-propyl-1:3-propanediol Dicarbamate (Miltown), a New Interneuronal Blocking Agent. F. M. Berger. (*J. Pharmacol.*, 1954, **112**, 413.) Miltown, an analogue of mephenesin, had a more prolonged sedative and paralysing effect than mephenesin on monkeys, cats, rabbits, rats and mice. As with mephenesin the paralysing action is located in the spinal cord, Miltown readily depressing the polysynaptic flexor reflex in the cat, when the monosynaptic knee jerk was relatively unaffected. In non-paralysing doses it antagonised strychnine- and leptazol-induced convulsions and deaths in mice. The drug also prolonged hexobarbitone sleep time and prevented the tonic extensor phase of electroshock seizures. Rats receiving 2 per cent. of Miltown in their food over a period of 15 months were lighter than controls, but otherwise showed no ill effects. The drug was active orally and did not cause nausea or emesis. There were no significant effects on respiration, heart rate or other autonomic functions. Detoxication is apparently by conjugation as the glucuronide; duration of action is about 8 times longer than that of mephenesin. G. P.

Noradrenaline and Isoprenaline, Effects of, on Neuromuscular Transmission during Partial Curarisation. F. Dybing. (*Acta pharm. tox., Kbh.*, 1954, **10**, 364.) Experiments have been carried out in rabbits recording the contractions of the flexor digitorum longus muscle obtained on stimulation of the sciatic nerve and by direct stimulation of the muscle. Constant curarisation was obtained by a continuous infusion intravenously of a solution of tubocurarine. When the muscular contractions had remained constant for 20 minutes, adrenaline, noradrenaline, or isoprenaline (*iso*propylnoradrenaline) was injected intravenously. Noradrenaline, like adrenaline, had a short anticurare effect followed by a weak augmentation of the partial neuromuscular block. Isoprenaline caused only an augmentation of the neuromuscular block with no initial anticurare effect. The directly stimulated muscle showed no decrease in contraction on the injection of isoprenaline during partial curarisation. The results indicate that the neuromuscular effects of the sympathomimetic amines do not depend upon a reduction in blood flow. G. F. S.

Pentobarbitone Anæsthesia, Potentiation of, in Mice by Isoniazid and Related Compounds. A. Goldin, D. Dennis, J. M. Venditti and S. R. Humphreys. (*Science*, 1955, **121**, 364.) The authors, while investigating enzymatically

catalysed exchange reactions, observed that several of the congeners of nicotinic acid employed in antitubercular studies prolonged the anæsthetic action of pentobarbitone in mice. The 10 to 12-week-old male mice (weighing 20 to 25 g.) used, were given the barbiturate by the intraperitoneal route and the other compounds subcutaneously. The extent of the anæsthesia produced by the barbiturate when administered 15 minutes after isoniazid was found to increase with increasing doses of the latter. Prolongation of anæsthesia was achieved with as little as 50 mg./kg. of isoniazid with doses of 60 mg./kg. of pentobarbitone. The barbiturate afforded protection against the acute toxicity of isoniazid even when administered in the initial stage of convulsive seizure. The extent of potentiation and protection appear to depend on the relative doses of the pentobarbitone and isoniazid employed. 1-*iso*Nicotinyl-2-*iso*propyl hydrazine phosphate (Marsilid), *iso*nicotinic acid amide, nicotinic acid hydrazide, 3-acetyl pyridine, hydrazine hydrate and glycine, also prolonged the anæsthesia. Although both isoniazid and Marsilid when administered simultaneously with, or 4 hours prior to, pentobarbitone were found to prolong anæsthesia, they do not appear to act in a similar manner. Marsilid 250 mg./kg. caused a 40 to 50 per cent. reduction in the dose of pentobarbitone required to induce anæsthesia in 50 per cent. of the animals (ED50). With 250 mg./kg. of isoniazid reduction of the ED50 was not significant. With a sub-anæsthetic dose of the barbiturate (30 mg./kg.), 250 mg./kg. of Marsilid induced anæsthesia, while 50 to 400 mg./kg. of isoniazid did not. The barbiturate did not protect against the toxicity of 3-acetyl pyridine, while nicotinamide did so without reducing the potentiating effect of the 3-acetyl pyridine on the pentobarbitone anæsthesia. As an inhibition of diphosphopyridine nucleotidase activity by pentobarbitone has been observed, the relationship of enzymatic transformations, involving diphosphopyridine nucleotidases, to the potentiation of barbiturate anæsthesia is under investigation. J. R. F.

Recanescine, An Alkaloid from *Rauwolfia canescens*, Pharmacological Properties of. I. H. Slater, R. C. Rathbun, F. G. Henderson and N. Neuss. (*Proc. Soc. exp. Biol., N.Y.*, 1955, **88**, 293.) The authors have isolated and characterised a new sedative alkaloid from *Rauwolfia canescens* to which they have given the name recanescine. The physical and analytical data indicate that this is 11-desmethoxyreserpine and, in a footnote to the paper, attention is drawn to a report of an alkaloid canescine from the same source by Stoll and Hoffmann, which is probably identical (see p. 480). Recanescine, as an ethyl acetate solvate, is readily soluble in glacial acetic acid and when diluted suitably, was injected intravenously into mice, rats, rabbits, cats and monkeys, which were then subjected to a number of pharmacological experiments. Where possible, similar experiments were carried out with the same dose of reserpine. From the results it was concluded that the new alkaloid retains the characteristic pharmacological activity of reserpine and therefore the methoxyl group of reserpine is not essential for the sedative and hypnotic action. J. R. F.

Reserpine, Antagonism of, to Morphine Analgesia in Mice. J. A. Schneider. (*Proc. Soc. Biol., N.Y.*, 1954, **87**, 614.) Reserpine has been found to antagonise the analgesic effect of morphine in mice, while chlorpromazine prolonged it. White mice of both sexes were subjected to a beam of heat focussed on the tip of the tail of each animal according to the method of Gross (*Helv. physiol. Acta*, 1947, **5**, C31.) The intensity of the pain stimulus was set to obtain an average reaction time of 4 seconds under control conditions. No stimulus was applied for longer than 10 seconds, each animal being stimulated twice

during the tests. The results indicate that reserpine in doses up to 10 mg./kg. s.c. did not alter the pain threshold, but when given 2 hours before a dose of morphine (10 mg./kg. s.c.) the analgesic action of the latter was reduced in proportion to the dose of reserpine. A 10 mg./kg. dose of chlorpromazine produces a slight analgesic effect, and in combination with morphine (10 mg./kg.) prolonged the analgesic effect of the latter. The author suggests that the reserpine/morphine relationship may be the result of competitive action at various sites of the central nervous system, and points out that the results show that the modes of action of chlorpromazine and reserpine differ, although they both appear to have a similar clinical effect on the central nervous system.

J. R. F.

Rheumatoid Arthritis, Treatment of, by Stimulation of the Adrenal Cortex. H. F. West and G. R. Newns (*Lancet*, 1955, **268**, 578.) Mild to moderate adrenal stimulation with corticotrophin in 11 rheumatoid patients, over 1 to 2 years, has been found so far to influence favourably the course of moderately severe rheumatoid disease. Various brands of long acting corticotrophin were used in a dosage designed to give a daily urinary output of 15 to 40 mg. of 17-ketogenic steroids. The doses, given 12 hourly, varied from 7·5 to 40 units per day, the unit being 1/3 of a U.S.P. unit. To control the level of stimulation, 24-hour urine specimens were assayed every 5 to 7 days until a suitable maintenance level had been found, and then monthly. In the 6 males, the disease had lasted from 3 months to 13 years and in the 5 females from 2 to 20 years. The former were treated over 12 to 17 months, the latter over 12 to 24 months. The average results show a general improvement. The E.S.R. fell from an average of 38 mm. to 19 mm., Hb increased from 12·3 to 13·3 g./100 ml., the white cell count increased from 8600 to 10,900, and weight from 130 to 147 lb. All patients felt better in themselves, the hand grip became stronger, and there was an improvement in physical ability. In 4 patients there was a rise in blood pressure, in 2 pigmentation appeared, and 2 showed an increase in bone erosion. A leucocytosis usually accompanied severe stimulation. A comparison with 27 patients treated with cortisone acetate reveals that the stimulation method produces better results with fewer side effects. In conclusion the authors recommend an extension of the clinical trial and express the need for a more satisfactory adrenal stimulant for long-term use than the present preparations of corticotrophin. J. R. F.

Sodium Nitrate Poisoning Treated by Exchange Transfusion. N. G. Kirby. (*Lancet*, 1955, **268**, 594.) Machine oil containing sodium nitrite is being increasingly used and is widely obtainable. Such a preparation which after analysis was found to contain 36·5 per cent. of sodium nitrite, 7·5 per cent. of an emulsifying agent and 56 per cent. of water, was responsible for poisoning an 11-year old girl who had taken a mouthful. She was admitted to hospital 50 minutes later, and methæmoglobinæmia diagnosed. While oxygen was administered and a gastric lavage performed, an i.v. transfusion of group-O Rh-negative blood was started, a total of 1700 ml. being given. Following 48 hours in an oxygen tent, pyrexia and increased pulse rate lasting 4 days, and an affected blood picture, the patient improved sufficiently in 14 days to be discharged, with a blood picture within normal limits, and which remained so after 16 weeks. The author stresses the need for prompt action, in severe cases, preferably by exchange transfusion, and also the necessity of following up cases because of the danger of neutropenia and agranulocytosis. J. R. F.

Streptomycin, Rectal Absorption in Man. S. Carvalho and P. da Silva. (*Rev. Portugesa Farm.*, 1954, **4**, 225.) Following previous work, in which the

rectal absorption of streptomycin in rabbits was studied, the authors have administered 500 mg. of the antibiotic, as sulphate, to humans in a suppository base consisting of polyethylene glycols 1500–15 parts and 6000–75 parts. The antibiotic was detectable in the blood stream 15 minutes after administration, reaching a maximum concentration of 2 μg./ml. of serum in 1 hour and remained detectable up to 7 hours. The maximum level is considerably below that obtained by the intramuscular injection of the same quantity of antibiotic.

J. R. F.

Tricyclamol Chloride, Action of. P. Aylett and A. H. Douthwaite. (*Brit. med. J.*, 1955, **1**, 691.) This is a preliminary report on the effects of the new parasympathetic blocking drug, tricyclamol chloride (DL-*N*-methyl-3-*cyclo*-hexyl-3-hydroxy-3-phenylpropyl pyrrolidinium chloride). It is an odourless, crystalline substance, freely soluble in water, and may be given by mouth or intramuscular injection. The doses employed in the experiments varied from 25 to 100 mg. The drug was shown to have a marked effect in reducing the motility of the stomach and duodenum, and to a greater extent than was the case with hyoscyamine. This action may be of value in controlling the pain of peptic ulceration which is probably, at least in part, due to spasm. In a patient with gastroenterostomy and stomal ulcer quite severe pain was relieved after repeated oral doses of 50 mg. Tricyclamol had a less marked effect in reducing acidity of the gastric contents, but it prolonged the reduction of gastric acidity obtained by a dose of alkali (aluminium hydroxide gel). It is suggested that a combination of 100 mg. of tricyclamol chloride by mouth with a full dose of antacid might go some way towards overcoming the difficulty hitherto experienced in controlling nocturnal secretion in the treatment of duodenal ulcer. Side effects, including dryness of the mouth, dilated pupils, and blurring of print on reading, were observed in most of the patients; these effects seemed more pronounced after intramuscular injection than after oral use. S. L. W.

Uracil and Related Oxypyrimidines, Central Depressant Properties of. D. G. Wenzel and M. L. Keplinger. (*J. Amer. pharm. Ass., Sci. Ed.*, 1955, **44**, 56.) Uracil, thymine, 6-methyluracil and thiouracil were administered orally to mice in a dose of 2 millimole/kg. Thirty minutes later, 100 mg./kg. of hexobarbitone sodium was given intraperitoneally. Although these oxypyrimidines are devoid of hypnotic activity, they increased the sleeping time due to hexobarbitone. Similar results were obtained when the substances were injected intraperitoneally (1millimole/kg.) followed after 5 minutes by 100 mg./kg. of hexobarbitone sodium. Administered to mice in doses of 6 and 12 millimole/kg., followed after 40 minutes by 100 mg./kg. of leptazol (pentetrazol), the substances were ineffective in preventing the convulsions induced by leptazol. The compounds were however capable of preventing maximal electroshock seizures. The oral toxicity in mice was low, the LD50 varying from 3500 mg./kg. for thymine to more than 7500 for uracil and 6-methyluracil.

G. B.

Valmid, a Non-barbiturate Central Nervous System Depressant, a Study of the Effects of, in Humans. C. M. Gruber, K. G. Kohlstaedt, R. B. Moore and F. B. Peck, Jr. (*J. Pharmacol.*, 1954, **112**, 480.) Valmid (1-ethinyl:1-carbamyl *cyclo*hexane), administered in doses of 0·4 to 1·5 g. to patients in hospital, was an active hypnotic agent. The sedative action of 500 mg. was equal to that of 100 mg. of quinalbarbitone sodium, but the duration of action was about half of that of the barbiturate. No prolongation or intensification of the action of Valmid was observed in 2 patients with uræmia and cirrhosis.

In hypnotic doses the drug had no effect on respiration, heart rate or blood pressure in normal patients. G. P.

Veratrum Alkaloids, Mechanism of Vasomotor Action of. S. C. Wang, S. H. Ngai and R. G. Grossman. (*J. Pharmacol.*, 1955, **113**, 100.) A study has been made of the effects of veratrum alkaloids—Veriloid, protoveratrine, germitrine, neogermitrine, germerine, veratridine and veratramine—on the blood pressure of anæsthetised cats and dogs, to determine the extravagal sites of action. The alkaloids were injected intravenously, intra-arterially, or applied locally to the carotid sinus. To determine changes in the excitability of the medulla oblongata the central vasomotor mechanism was stimulated directly. In vagotomised animals moderate to severe hypotensive reactions were obtained with Veriloid, protoveratrine, germitrine, neogermitrine and germerine. During the hypotensive response the carotid sinus reflex was depressed or eliminated and the excitability of the medullary vasomotor centre was increased. The hypotensive response was not dependent on the activity of the carotid body, and it is suggested to be due to increased repetitive firing of the carotid sinus baroreceptors. Veratridine and veratramine depress the vasomotor centre and here it is believed that the carotid sinus baroreceptors play a secondary role in the hypotensive reaction. In all cases hypotension is mediated through inhibition of the sympathetic nervous system, no appreciable change in blood pressure being observed in chronically sympathectomised, cervical spinal animals or animals in which the sympathetic nerves have been blocked with Hydergine. Veratrum alkaloids had practically no direct action on the blood vessels. Veratramine caused a bradycardia on the denervated heart. Repeated doses of veratrum alkaloids only cause tachyphylaxis when the receptors are still under the influence of the alkaloids. G. F. S.

Vitamin K_1 and Water-soluble Vitamin K, Clinical Comparison of. J. R. Gamble, E. W. Dennis, W. W. Coon, P. Hodgson, P. W. Willis, J. A. MaCris and I. F. Duff. (*Arch. intern. Med.*, 1955, **95**, 52.) The prothrombin responses to water-soluble and to oil-soluble vitamin K preparations in patients under treatment with anticoagulants is compared. It is shown that oil-soluble vitamin K_1 (Mephyton) is more effective than any other agent now available in combating drug-induced hypoprothrombinæmia. In contrast, the water-soluble vitamin K preparations are unreliable and inconstant in effect. In most cases oil-soluble vitamin K_1 in doses as low as 1 to 5 mg. orally produces as satisfactory a response, in as short a time as 4 hours, as the large intravenous doses of water-soluble vitamin K usually recommended. This low dose has the advantage of permitting an early resumption of anticoagulant therapy, and no refractoriness was observed after using these small doses to the subsequent administration of anticoagulants administered orally. In the case of severe bleeding due to oral anticoagulants, intravenous vitamin K_1 in a dose of 10 to 50 mg. is recommended, in addition to whole blood or plasma transfusions, if the latter are necessary to combat shock; the smaller dose should be adequate when bleeding is moderate and resumption of therapy is planned. In hypoprothrombinæmia due to absorptive difficulties water-soluble vitamin K preparations or the oil-soluble vitamin K_1 appear to be equally effective, the latter in amounts as small as 5 mg. intravenously. In patients with jaundice of unknown ætiology a 50 mg. intravenous dose is recommended, and where there is poor initial response this dose may be repeated on successive days to produce a rise to safe prothrombin levels. No untoward effects have been observed from the use of this preparation. S. L. W.

PRINTED BY W. HEFFER & SONS LTD., CAMBRIDGE, ENGLAND